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Full Length Research Paper

## Pharmacokinetic study of vinorelbine in Chinese patients with non-small-cell lung cancer by high-performance liquid chromatography (HPLC) with fluorescence detection

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To investigate the pharmacokinetics of vinorelbine in Chinese subjects with non-small-cell lung cancer (NSCLC), a high-performance liquid chromatographic method using fluorescence detection was established to determine the concentrations of vinorelbine in human blood and plasma. Samples were collected from 10 Chinese patients with NSCLC after intravenous infusion of 40 mg vinorelbine. Pharmacokinetic parameters were calculated using 3P87 software. The pharmacokinetics of vinorelbine in Chinese patients fitted a two-compartment model. The pharmacokinetic parameters calculated from plasma and blood drug concentrations were: AUC (530.99 ± 88.56) ng·ml<sup>-1</sup>·h and (904.91 ± 194.97) ng·ml<sup>-1</sup>·h, C<sub>max</sub> (861.78 ± 247.25) ng·ml<sup>-1</sup> and (1,053.85 ± 295.98) ng·ml<sup>-1</sup>, and t<sub>1/2β</sub> (33.70 ± 1.58) h and (40.40 ± 21.30) h, respectively. The pharmacokinetic profiles of vinorelbine in Chinese NSCLC patients were similar to those reported for non-Chinese NSCLC patients.

**Key words:** Vinorelbine, plasma drug concentration, blood drug concentration, pharmacokinetics, high-performance liquid chromatography.

### INTRODUCTION

Also known as vinorelbine and Navelbine®, 5'-nor-anhydrovinblastine is a first-line, semi-synthetic anti-cancer agent. It has similar actions to vindesine and vincristine, but lower toxicity than those agents. It has been used widely in China (Liu et al., 2001) and many

other countries (Provencio et al., 2011) because of its significant activity in the treatment of advanced non-small-cell lung cancer (NSCLC) and metastatic breast cancer. Many studies have been reported; its pharmacokinetics in cancer patients after single administration (Wargin and

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#Yuchao Dong and Hailiang Xin made equal contributions to this article.

Lucas, 1994; Levêque and Jehl, 1996; Gebbia and Puozzo, 2005; Robieux et al., 1996) as well as combination with other chemotherapy (Airoldi et al., 2003; Tokudome et al., 2008). Pharmacokinetic data were obtained with high-performance liquid chromatographic (HPLC) techniques coupled with fluorescence detection (Gauvin et al., 2000; Robieux et al., 1996), ultraviolet detection (Campone et al., 2001; Puozzo et al., 2007) and electrospray ionization mass spectrometry/mass spectrometry (ESI-MS/MS) (Qian et al., 2011).

Body weight and platelet count were confirmed as influencing blood clearance of vinorelbine. Also, pharmacokinetic interaction occurred between vinorelbine and other drugs probably due to their inhibition of CYP450-3A4 (Rezai et al., 2011). The results by Robieux et al. (1996) showed lower clearance rate of vinorelbine in patients with severe liver failure but not with moderate secondary liver involvement and supported correspondingly vinorelbine dose reduction. Moreover, association does not alter the pharmacokinetic profile of both vinorelbine and other combined drugs (Airoldi et al., 2003). However, only one Chinese pharmacokinetics study of vinorelbine has been reported (Qian et al., 2011), which using a LC/MS/MS determination method. Therefore, more and further study on the pharmacokinetics of vinorelbine in Chinese would be beneficial for its dose individualization and reduce the side effect.

Here we describe a study of the pharmacokinetics of vinorelbine in Chinese NSCLC patients using a modified HPLC method with fluorescence detection. This HPLC method could fulfill the requirements for a sufficiently simple, accurate and precise assay to carry out pharmacokinetic studies of vinorelbine. The pharmacokinetic results would be the reference for the vinorelbine individual administration and lead to more clinical safety and efficiency.

## MATERIALS AND METHODS

### Reagents

Vinorelbine ditartrate was from Pierre Fabre Corporation (Castres, France). Methanol (BDH, Poole, UK), tetrahydrofuran (Merck, Darmstadt, Germany) and diethyl ether (Merck) were of HPLC grade. Phosphoric acid and potassium dihydrogen phosphate were analytically pure reagents. The water used in all experiments was redistilled.

### Equipments

The chromatographic system consisted of a Shimadzu 10AT pump (Shimadzu, Kyoto, Japan), a Shimadzu RF-10AXL fluorescence detector, a Rheodyne 7725i loading valve fitted with a 100  $\mu$ l sample loop (Rheodyne, Rohnert Park, CA, USA), and a Shimadzu C-R8A integrator. An 80-2 precipitator, a XW-80A vortex mixer, a pH meter (Orion, Beverly, CA, USA), a JL-120DT ultrasonic cleaner (Shanghai Jili Scientific Instruments, Shanghai, China) and liquid-

liquid extraction equipment were used. The excitation wavelength was 280 nm and the emission wavelength was 360 nm. The HPLC analytical column (4.0 mm  $\times$  150 mm) was packed with 5  $\mu$ m diameter Hypersil-C<sub>18</sub> particles (Dalian Elite Analytical Instruments, Dalian, China).

### Chromatographic conditions

The mobile phase was a solution of methanol-phosphate buffer-tetrahydrofuran (45:52.5:2.5). The buffer was prepared using 50 mM potassium dihydrogen phosphate, and was adjusted to pH 4.0 employing phosphoric acid. Before use, the mobile phase was degassed ultrasonically. The flow rate was 1 ml·min<sup>-1</sup>. The injection volume was 50  $\mu$ l. Chromatography was undertaken at room temperature ( $\approx$ 15°C).

### Extraction procedure

A 5 ml aliquot of diethyl ether was added to 1 ml of plasma sample (or 0.5 ml whole-blood sample diluted with 0.5 ml of water) in a 10 ml ground-glass stoppered glass centrifuge tube. It was vortex-mixed for 3 min, and then centrifuged at 4,000 rpm for 10 min at room temperature. The supernatant organic phase (4 ml) was transferred to a new glass tube and evaporated to 1 ml at 30°C. Then, 200  $\mu$ l of potassium dihydrogen phosphate buffer at pH 4.0 was added to it. After vortex-mixing for 2 min and centrifugation at 4,000 rpm for 10 min at room temperature, 50  $\mu$ l of the acidic aqueous phase was injected into the chromatographic system.

### Precision and extraction recovery

The chromatogram peak area of vinorelbine was determined for known concentrations of vinorelbine in plasma or blood. Calibration graphs of plasma and blood samples from 1 to 1000 ng·ml<sup>-1</sup> (n=3) were prepared in duplicate by spiking plasma or blood with increasing amounts of vinorelbine to determine the concentration of unknown samples.

Intra-assay precision was determined by analyzing (n=5) plasma or blood samples spiked with vinorelbine at 2, 50 and 250 ng·ml<sup>-1</sup>. Inter-assay precision was tested by analyzing samples of the three concentrations on five days.

The extraction recovery was determined three times at 2, 50 and 250 ng·ml<sup>-1</sup>. The peak areas obtained after extraction were compared with peaks resulting from standard solutions at the same concentrations.

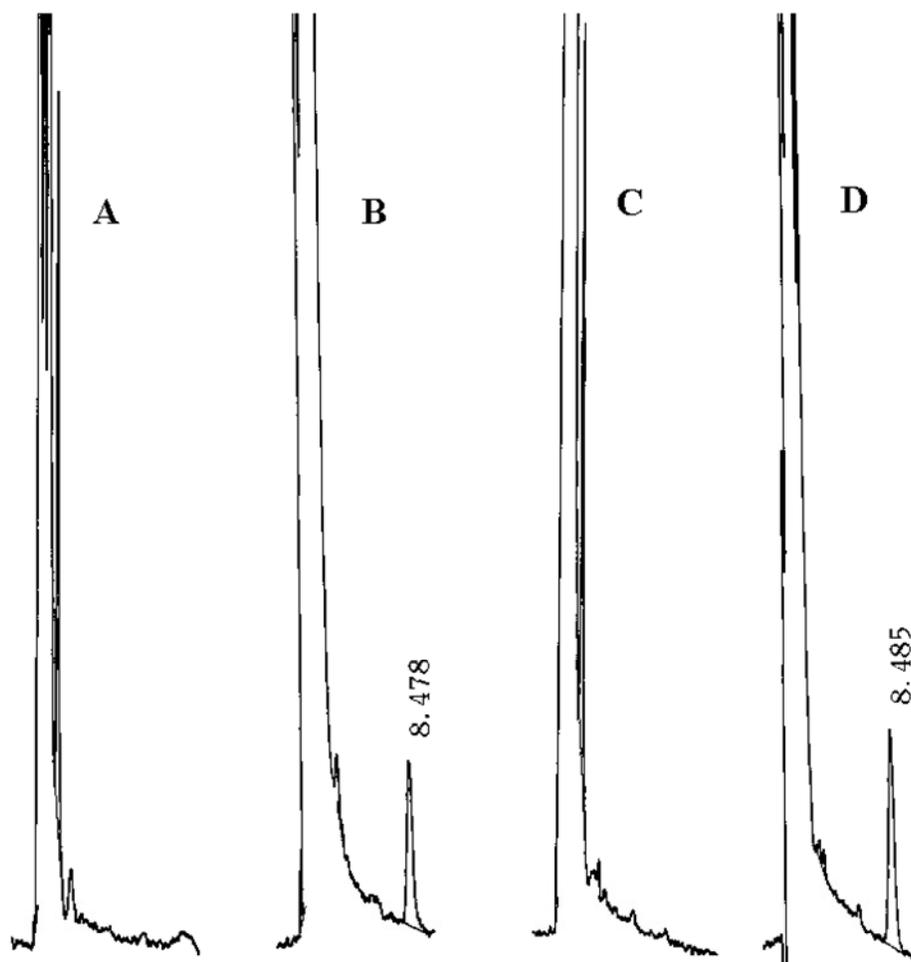
## RESULTS

### Chromatograms

Chromatograms of vinorelbine in different samples are shown in Figure 1. The retention time of vinorelbine was 8.5 min. There was no obvious interference in plasma or blood samples.

### Linearity

Calibration graphs were obtained by plotting the peak area of vinorelbine against its concentrations in plasma or



**Figure 1.** Chromatograms of vinorelbine. A: blank plasma; B: plasma sample 1 h after administration of 40 mg vinorelbine; C: blank blood; D: blood sample 1 h after administration of 40 mg vinorelbine.

blood. Calibration graphs were set up three times for plasma concentrations of 1, 2, 5, 20, 50, 100, 250 and 1,000  $\text{ng}\cdot\text{ml}^{-1}$  and for blood concentrations of 2, 4, 10, 40, 100, 500 and 2,000  $\text{ng}\cdot\text{ml}^{-1}$ . They were described by the equations of  $C = 2.76 \times 10^{-4}A + 0.8025$  ( $r=0.9994$ ) for plasma samples and  $C = 5.34 \times 10^{-4}A + 0.4384$  ( $r=0.9998$ ) for blood samples, where C is the concentration of vinorelbine spiked in blank plasma or blood, and A is the peak area of vinorelbine.

#### Limit of quantification (LOQ) and limit of detection (LOD)

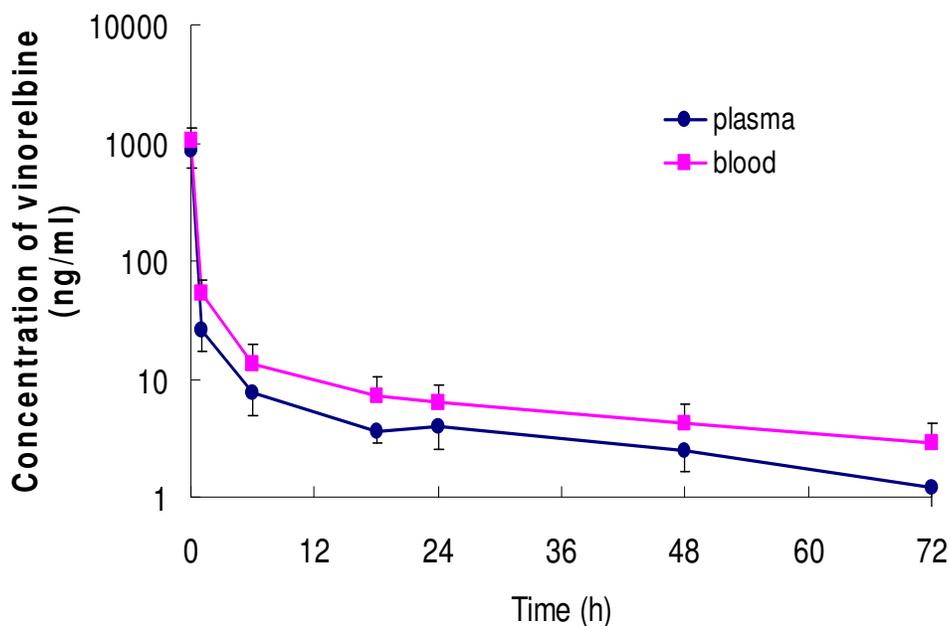
The LOQs were 1  $\text{ng}\cdot\text{ml}^{-1}$  in plasma and 2  $\text{ng}\cdot\text{ml}^{-1}$  in blood, respectively. The LODs were 0.5 and 1  $\text{ng}\cdot\text{ml}^{-1}$  (signal-to-noise ratio (S/N)  $\geq 3$ ), respectively.

#### Precision and accuracy

The results for the accuracy of the determination assay, relative recovery, and extraction recovery are presented in Table 1.

#### Pharmacokinetic study

The analytical procedure described above was used to determine the concentrations of vinorelbine in plasma and blood samples from 10 Chinese patients (7 males; age  $56 \pm 9$  years) with NSCLC. The chemotherapeutic protocol comprised vinorelbine and cisplatin. Vinorelbine (40 mg) was administered as a 10 min continuous intravenous infusion. Serial blood samples were collected before administration and 1, 6, 18, 24, 48 and 72 h after infusion. Three milliliters of blood was collected in a



**Figure 2.** Concentration–time curve of vinorelbine (40 mg) in 10 Chinese NSCLC patients after rapid intravenous infusion (n=10).

**Table 1.** Accuracy and recovery of vinorelbine in human plasma and blood (n=5).

Concentration (ng·ml <sup>-1</sup> )	RSD Intra-day (%)	RSD Inter-day (%)	Relative recovery (%) ± SD	Extraction recovery (%) ± SD
<b>Plasma</b>				
250	2.59	4.38	103.82 ± 2.69	77.52 ± 3.39
50	6.53	6.72	98.77 ± 6.45	70.92 ± 4.63
2	7.91	10.79	101.69 ± 8.04	74.41 ± 8.03
<b>Blood</b>				
250	3.21	4.57	100.03 ± 3.21	75.37 ± 2.42
50	6.24	7.21	102.82 ± 6.42	71.28 ± 4.45
2	8.54	9.91	97.11 ± 8.29	91.77 ± 7.84

RSD: relative standard deviation

heparinized glass tube. A total of 0.5 ml was taken for a blood sample; the rest was prepared for a 1 ml plasma sample after centrifugation at 4,000 rpm for 5 min at room temperature.

Figure 2 shows the mean plasma and blood concentration *versus* time profiles of vinorelbine in 10 NSCLC patients. Pharmacokinetic parameters (Table 2) were estimated using 3P87 pharmacokinetic software (Chinese Pharmacological Society, Beijing, China). The pharmacokinetic characteristics of vinorelbine in Chinese NSCLC patients afforded a two-compartment model calculated by using plasma or blood concentration data. The elimination half-life of vinorelbine was  $33.70 \pm 1.58$  h

for plasma and  $40.40 \pm 21.30$  h for blood samples. We used values of the area under the curve (AUC) to calculate the ratio  $AUC_{\text{blood}}/AUC_{\text{plasma}}$ , which was 1.7. It was comparable with the ratio of blood concentration /plasma concentration in these 10 patients, remaining almost constant ( $\approx 1.7$ ) during the 72 h of the study.

## DISCUSSIONS

The pharmacokinetic information of vinorelbine as first-line chemotherapy in Chinese NSCLC patients is too few (Qian et al., 2011). In this paper, we modified a HPLC

**Table 2.** Pharmacokinetic parameters obtained from plasma and blood concentrations of 10 Chinese NSCLC patients after rapid intravenous infusion of 40 mg vinorelbine (mean  $\pm$  S.D., n = 10).

Parameter	Unit	Plasma	Blood
A	ng·ml <sup>-1</sup>	855.53 $\pm$ 248.04	1041.95 $\pm$ 299.92
$\alpha$	h <sup>-1</sup>	3.82 $\pm$ 0.2	3.25 $\pm$ 0.15
B	ng·ml <sup>-1</sup>	6.24 $\pm$ 1.6	11.89 $\pm$ 7.64
$\beta$	h <sup>-1</sup>	0.02 $\pm$ 0.00	0.02 $\pm$ 0.01
V <sub>d</sub>	L	49.15 $\pm$ 12.86	39.86 $\pm$ 9.09
t <sub>1/2<math>\alpha</math></sub>	h	0.18 $\pm$ 0.01	0.21 $\pm$ 0.01
t <sub>1/2<math>\beta</math></sub>	h	33.7 $\pm$ 1.58	40.4 $\pm$ 21.3
K <sub>12</sub>	h <sup>-1</sup>	2.16 $\pm$ 0.53	2.00 $\pm$ 0.45
K <sub>21</sub>	h <sup>-1</sup>	0.05 $\pm$ 0.01	0.06 $\pm$ 0.04
K <sub>10</sub>	h <sup>-1</sup>	1.63 $\pm$ 0.38	1.21 $\pm$ 0.41
AUC	ng·ml <sup>-1</sup> ·h	530.99 $\pm$ 88.56	904.91 $\pm$ 194.97
CLs	L·h <sup>-1</sup>	77.25 $\pm$ 15.37	46.14 $\pm$ 12.07

A, coefficient of exponential functions of distribution phase;  $\alpha$ , absorption rate constant; B, coefficient of exponential functions of elimination phase;  $\beta$ , elimination rate constant; V<sub>d</sub>, volume of distribution; t<sub>1/2 $\alpha$</sub> , half-life of absorption; t<sub>1/2 $\beta$</sub> , half-life of elimination; K<sub>12</sub>, rate constant from central-compartment to peripheral-compartment; K<sub>21</sub>, rate constant from peripheral-compartment to central-compartment; K<sub>10</sub>, elimination rate constant from central-compartment; AUC, area under the concentration–time curve; CLs, clearance.

method (Gauvin et al., 2000; Robieux et al., 1996) by using a less expensive mobile phase (comprising mainly methanol and phosphate buffer) and sensitive fluorescence detection to determine the vinorelbine concentration in both plasma and blood. Fluorescence detection is certainly more routinely usable detection mode for drugs monitoring. An *in-vitro* study (Urien et al., 1993) reported that 84% of vinorelbine is bound to blood cells, mainly to platelets (78%). Hence, we also determined the blood concentration in patients to compare with the literature (Gauvin et al., 2000). It was reported that vinorelbine concentration in blood may remain constant, but that in plasma would vary depending on differences in the centrifuge conditions (Puozzo et al., 2007). So determination of the concentrations in both blood and plasma meanwhile is more significant.

The present method was relatively easy and feasible to perform and allowed to determine vinorelbine in blood and plasma at the nanogram level. Also, we employed two steps of careful vortex-mixing of overall duration 5 min instead of two steps of speed agitation for 60 min as commonly adopted procedure (Gauvin et al., 2000). Thus the whole procedure of sample extraction could save 55min, which made the determination more rapid. Although we did not use an internal standard to simplify the pretreatment procedure, the results showed relatively sensitivity and accuracy and were successfully applied to pharmacokinetic studies.

The pharmacokinetic profile of vinorelbine is often described as a 3-compartment model characterised by a long terminal half-life (t<sub>1/2</sub>) that varies between 20 and 40

h, a large apparent volume of distribution (V<sub>d</sub>) of around 70 L/kg and a high plasma clearance (CL) between 72.54 and 89.46 L/h when determined by HPLC method (Wargin and Lucas, 1994; Levêque and Jehl, 1996).

The disposition of the alkaloid is not altered by concurrent co-administration of cisplatin (Wargin and Lucas, 1994; Levêque and Jehl, 1996; Delord et al., 2009). However, Gauvin et al. (2000) reported the pharmacokinetic profiles exhibiting a three-compartment model with a mean elimination half-life of 42 h. The calculated pharmacokinetic parameters (Table 2) in our study were consistent with these previous study (Wargin and Lucas, 1994; Levêque and Jehl, 1996), with the mean elimination half-life was 33.70  $\pm$  1.58 h for plasma and 40.40  $\pm$  21.30 h for blood, in spite of using a simple HPLC method.

## Conclusion

The pharmacokinetic characteristics of vinorelbine in Chinese NSCLC patients were similar to those of non-Chinese patients and non-NSCLC patients described in the literature. Based on our and previous findings, dose modifications of vinorelbine of first use in Chinese patients may need not be under consideration generally. Our study may provide the reference for the vinorelbine individual administration and bring more clinical safety and efficiency to Chinese patients. However, more and further study may need to confirm our results.

## ACKNOWLEDGMENTS

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## REFERENCES

- Airoldi M, Cattel L, Cortesina G, Giordano C, Passera R, Pedani F, Novello S, Bumma C, Gabriele P (2003). Gemcitabine and vinorelbine in recurrent head and neck cancer: pharmacokinetic and clinical results. *Anticancer Res.* 23:2845-2852.
- Campono M, Fumoleau P, Delecroix V, Deporte-Fety R, Perrocheau G, Vernillet L, Borg-Olivier O, Louboutin JP, Bissery MC, Riva A, Azli N (2001). Phase I dose-finding and pharmacokinetic study of docetaxel and vinorelbine as first-line chemotherapy for metastatic breast cancer. *Ann. Oncol.* 12:909-918.
- Delord JP, Puozzo C, Lefresne F, Bugat R (2009). Combination chemotherapy of vinorelbine and cisplatin: a phase I pharmacokinetic study in patients with metastatic solid tumors. *Anticancer Res.* 29:553-560.
- Gauvin A, Pinguet F, Poujol S, Astre C, Bressolle F (2000). High-performance liquid chromatographic determination of vinorelbine in human plasma and blood: application to a pharmacokinetic study. *J. Chromatogr. B.* 748:389-399.
- Gebbia V, Puozzo C (2005). Oral versus intravenous vinorelbine: clinical safety profile. *Expert. Opin. Drug Saf.* 4:915-928.
- Levéque D, Jehl F (1996). Clinical pharmacokinetics of vinorelbine. *Clin. Pharmacokinet.* 31:184-197.
- Liu CY, Sun YH (2001). Present situation of second-line chemotherapy for non-small cell lung cancer. *Shi Yong Ai Zheng Za Zhi. pract. J. cancer (in Chinese)* 16:329-336.
- Provencio M, Isla D, Sánchez A, Cantos B (2011). Inoperable stage III non-small cell lung cancer: Current treatment and role of vinorelbine. *J. Thorac. Dis.* 3:197-204.
- Puozzo C, Ung HL, Zorza G (2007). A high performance liquid chromatography method for vinorelbine and 4-O-deacetyl vinorelbine: a decade of routine analysis in human blood. *J. Pharm. Biomed. Anal.* 44:144-149.
- Qian J, Wang Y, Chang J, Zhang J, Wang J, Hu X (2011). Rapid and sensitive determination of vinorelbine in human plasma by liquid. *J. Chromatogr. B.* 879:662-668.
- Rezai K, Urien S, Isambert N, Roche H, Dieras V, Berille J, Bonnetterre J, Brain E, Lokiec F (2011). Pharmacokinetic evaluation of the vinorelbine-lapatinib combination in the treatment of breast cancer patients. *Cancer Chemother. Pharmacol.* 68:1529-1536.
- Robieux I, Sorio R, Borsatti E, Cannizzaro R, Vitali V, Aita P, Freschi A, Galligioni E, Monfardini S (1996). Pharmacokinetics of vinorelbine in patients with liver metastases. *Clin. Pharmacol. Ther.* 59:32-40.
- Robieux I, Vitali V, Aita P, Freschi A, Lazzarini R, Sorio R (1996). Sensitive high-performance liquid chromatographic method with fluorescence detection for measurement of vinorelbine plasma concentrations. *J. Chromatogr. B.* 675:183-187.
- Tokudome N, Ito Y, Hatake K, Toi M, Sano M, Iwata H, Sato Y, Saeki T, Aogi K, Takashima S (2008). Trastuzumab and vinorelbine as first-line therapy for HER2-overexpressing metastatic breast cancer: multicenter phase II and pharmacokinetic study in Japan. *Anticancer Drugs* 19:753-759.
- Urien S, Bree F, Breillout F, Bastian G, Krikorian A, Tillement JP (1993). Vinorelbine high-affinity binding to human platelets and lymphocytes: distribution in human blood. *Cancer Chemother. Pharmacol.* 32:231-234.
- Wargin WA, Lucas VS (1994). The clinical pharmacokinetics of vinorelbine (Navelbine). *Semin. Oncol.* 21:21-27.

Full Length Research Paper

## Hepatoprotective effect of Barrisal (herbal drug) on carbon tetrachloride induced hepatic damage in rats

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Barrisal is an herbal preparation of Hamdard Laboratories (Waqf) Pakistan that has been used as hepatoprotective agent. In this study, the protective effects of Barrisal against liver damage were evaluated in carbon tetrachloride (CCl<sub>4</sub>)-induced chronic hepatotoxicity in rats. Four groups (I, II, III, IV) of Sprague-Dawley (SD) rats were used and Groups I and II were orally fed with saline and Barrisal (10 ml/kg), respectively for 14 consecutive days while Groups III and IV were orally fed with saline, Barrisal (10 ml/kg) and then a single dose of CCl<sub>4</sub> (0.3 ml/kg body weight in a 20% olive oil) was injected intraperitoneally 30 min after the last dose of Barrisal and the animals were starved for 24 h. The degree of protection was measured using biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein and albumin. Data obtained from results were compared using student's t-test (paired and unpaired) and one-way analysis of variance (ANOVA), significant differences were determined (Tukey honestly significant difference (HSD) test) using the Minitab 13 computing program. Values of P<0.05, were considered to be significant. The results showed that the treatment with Barrisal significantly lowered the CCl<sub>4</sub>-induced serum levels of hepatic enzyme markers (GOT, GPT, ALP, and total bilirubin, total cholesterol, total protein) indicated hepatoprotective effect of the Barrisal. Histopathological examination of liver sections confirmed that, pre-treatment with Barrisal reduced the hepatic damage induced by CCl<sub>4</sub>. Barrisal reduced the incidence of liver lesions including hepatic cells cloudy swelling, lymphocytes infiltration, hepatic necrosis, and fibrous connective tissue proliferation induced by CCl<sub>4</sub> in rats. Therefore, the results of this study suggest that Barrisal could protect liver against CCl<sub>4</sub>-induced oxidative damage in rats, and this hepatoprotective effect might be because of its antioxidant and free radical scavenger effects.

**Key words:** Barrisal, hepatoprotective, carbon tetrachloride (CCl<sub>4</sub>), hepatic enzymes marker, liver, kidney.

### INTRODUCTION

Barrisal syrup prepared by Hamdard Laboratories (Waqf) Pakistan is composed of only *Aloe vera* syn. *Aloe barbadensis* Mill. (*A. barbadensis* Linn. Family; Liliaceae).

Barrisal is widely prescribed in hepatic and spleen

disorders; it is claimed that Barrisal is an immune modulating drug having adaptogenic activity. The medicinal plant *Aloe vera* is used traditionally. It is a xerophytic plant which includes trees (*Aloe ferox*), shrubs and herbs

and herbs (*A. barbadensis*). It has large fleshy leaves, carrying spines at the margin and resemble to some extent the agave or century plant (*Agave Americana* Linne) (Capasso et al., 1998). Pharmacological activities reported so far for its gel, polysaccharides, polypeptides, anthrones and chromones include antibacterial (Tian et al., 2003), anti-inflammatory (Hutter et al., 1996), and immunoprotective (Strickland et al., 2004) activities.

*A. vera* has been recognized as a good medicine for the treatment of asthma, gastrointestinal ulcer, constipation, otalgia, cancer, burns, wounds, herpes simplex virus, diabetes and inflammation (Khan, 1997).

Different compounds like cinnamoyl, p-coumaroyl, feruloyl, and caffeoyl aloesin isolated from *Aloe* species possess antiinflammatory and antioxidative properties (Yagi and Takeo, 2003). However, the biological effects of Barrisal on liver injury have not been studied yet. This study investigated the protective ability of Barrisal on liver injury induced by carbon tetrachloride ( $\text{CCl}_4$ ) in rats.

## MATERIALS AND METHODS

### Barrisal

Barrisal (herbal syrup) used in the hepatoprotective and toxicological studies was obtained from Hamdard Laboratories (Waqf) Pakistan.

### Animals

Adult Sprague Dawley rats ( $n=40$ ) of both sex (200 to 225 g) were used for hepatoprotective, toxicological and histopathological studies, while NMR-1 mice ( $n=36$ : weight 20-30gm) were used only for toxicological studies. They were obtained from Animal House of Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences (Dr. HMIIPHS) and were housed in groups of 6 per cage for seven days prior to experimentation with free access to standard feed and tap water *ad libitum* and kept on a 12 h light/dark cycle. All animals were housed in an air-conditioned room at  $23\pm 1^\circ\text{C}$  during the quarantine period.

### Toxicological studies of Barrisal in mice

Different groups of mice with either sex (twelve animals per group) were used to measure the acute toxicity of Barrisal by oral route of administration.

- (1) Group-I treated as control, was given saline (0.5 ml/mouse)
- (2) Group-II was treated with 5 ml Barrisal/kg/day.
- (3) Group-III was treated with 10 ml Barrisal/kg/day.

All the animals were treated daily for fourteen consecutive days (Zaoui et al., 2002) and were kept under constant observation for 2 h after dosing to observe any change in general behavior or other physiological activities and weighed daily to monitor any change in body weight.

### Toxicological and hepatoprotective activity of Barrisal in rats

In the present study, the adult Sprague-Dawley rats of both sexes (200 to 225 g) were divided into four groups. Each group consisted of ten animals. The animals were treated as follows.

- (1) Group I was treated with saline.
- (2) Group II was treated with 10 ml/kg/day of Barrisal syrup administered orally for 14 consecutive days.
- (3) Group III animals served as reference control, which received a single dose of  $\text{CCl}_4$  (0.3 ml/kg body weight in a 20% olive oil) intraperitoneally and the animals were starved for 24 h (Fatima, 1993).
- (4) Group IV was treated with 10 ml/kg/day of Barrisal syrup for 14 consecutive days as of Group II and then a single dose of  $\text{CCl}_4$  (0.3 ml/kg body weight in a 20% olive oil) was injected intraperitoneally 30 min after the last dose of Barrisal and the animals were starved for 24 h.

### Autopsy

At the end of 14th day, all surviving mice and rats were anaesthetized with pentothal sodium (40 mg/kg) intraperitoneally.

### Estimation of different biochemical parameters

The blood samples approximately (4 to 8 ml) were drawn directly from cardiac puncture before dissecting the animals with sterile disposable syringe from all treated and control rats. Blood samples were left at room temperature for 20 min. Then incubated at  $37^\circ\text{C}$  for 30 min and centrifuged separately in BHG Hermle Z230 (Germany) at the speed of 3000 rpm for 20 min. Supernatants (serum) were separated out and the residue was discarded.

Serum obtained (1 to 4 ml) was subjected for the study of the following parameters: total protein, bilirubin, cholesterol, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP).

All tests were performed using commercial assay kits. All these kits were purchased from Diagnostica Merck (Germany). Spectrophotometer U-2000 (Hitachi) was used to measure the absorbance of light.

### Histology

At the end of toxicological studies, all vital organs of rats (heart, liver, spleen and kidneys) were subjected to microscopic examination and compared with control animals. They were fixed in 10% formalin. After usual processes of dehydration, clearing and infiltration, tissues were embedded in paraffin wax and sectioned into 7- $\mu\text{m}$  slices through Leica RM 2145-Rotation Microtom. The tissues were stained with hematoxylin and eosin. The slides were studied and photographed through Nikon Advance Trinocular Research Microscope OPTIPHOT Model X2T-21E equipped with Nikon Microphotography system; Model UFX-DX-35 and phase contrast N plan.

### Statistical analysis

Data obtained from results regarding serum biochemical estimations and body weight were compared using student's t-test (paired and unpaired) and one-way analysis of variance (ANOVA), significant differences were determined (Tukey HSD test) using the

MINITAB 13 Computing Program. Values of  $P < 0.05$  were considered to be significant.

## RESULTS

### Toxicological and hepatoprotective activity of Barrisal

Toxicological and hepatoprotective effects of Barrisal have been studied on four groups of rats and mice as already described in materials and methods.

In these studies, the administration of Barrisal (10 ml/kg) for fourteen consecutive days did not show any mortality in rats and mice. Further, this treatment with Barrisal (10 ml/kg) also did not produce any significant change in the physical activity (Table 1), food and water consumption, and body weight of these animals, as shown in Figure 1a and b for mice and rats, respectively. In addition, no apparent changes in lungs, heart, liver and kidneys were observed on autopsy at 14 days of this treatment.

### Biochemical parameters

The results obtained from rat blood serum analysis are presented in Table 2. This demonstrates that in the four groups of rats that were administered saline (being control, Group I), Barrisal (Group II), Saline+CCl<sub>4</sub> (Group III) and Barrisal+CCl<sub>4</sub> (Group IV), respectively, the serum level for all the parameters, that is, total protein, cholesterol, bilirubin, SGPT, SGOT and alkaline phosphatase, changed with respect to the control group (saline).

#### *Barrisal treatment*

The Barrisal treatment for 14 consecutive days to the 2nd group of rats was found to reduce the serum total protein, bilirubin, SGPT, and SGOT in comparison with the control group. However, ANOVA showed that this reduction in the aforementioned parameters was statistically non-significant ( $P > 0.05$ ) for total protein, ( $P > 0.05$ ) for bilirubin, ( $P > 0.05$ ) for SGPT and ( $P > 0.05$ ) for SGOT. However, a non-significant rise was observed in serum alkaline phosphatase ( $P > 0.05$ ), as shown in Table 2. Serum cholesterol level after Barrisal administration has been found to reduce significantly ( $P < 0.05$ ), with respect to its control (saline treated group).

#### *CCl<sub>4</sub> treatment*

In the 3rd group of rats, treatment with saline for 14 consecutive days was followed by the treatment with CCl<sub>4</sub>

for a period of 24 h. This treatment has been found to produce acute hepatotoxicity for which bilirubin, SGPT, SGOT and alkaline phosphatase used as marker. These results presented in Table 2, demonstrates that after CCl<sub>4</sub> treatment, the serum level for all the parameters, that is, total protein, bilirubin, SGOT, SGPT and alkaline phosphatase have been found to increase except cholesterol with respect to control. A statistical comparison of these results through one-way ANOVA followed by Tukey showed that the level of total protein was found to increase non-significantly ( $P > 0.05$ ), while the administration of CCl<sub>4</sub> has been found to increase the serum alkaline phosphatase ( $P < 0.05$ ), bilirubin ( $P < 0.05$ ), SGPT ( $P < 0.05$ ) and SGOT ( $P < 0.05$ ) being statistically significant. However, cholesterol level has shown non-significant fall ( $P > 0.05$ ).

#### *Treatment with Barrisal followed by CCl<sub>4</sub>*

In the fourth group, Barrisal (10 ml/kg) treated (14 days) rats were used for additional treatment with CCl<sub>4</sub> for a period of 24 h. Such treatment was found to reduce the serum transaminase activity as shown in Table 2. This activity was assessed due to significant reduction in serum alkaline phosphatase ( $P < 0.05$ ), bilirubin ( $P < 0.05$ ), SGPT ( $P < 0.05$ ), SGOT ( $P < 0.05$ ), compared to CCl<sub>4</sub> treated 3rd group, while similar comparison showed no statistically significant change in serum cholesterol level ( $P > 0.05$ ). In addition, this treatment has also reduced total protein, non-significantly ( $P > 0.05$ ).

### Hepatoprotective effect of Barrisal on liver histology

The normal histological pictures of tissues obtained from control rats have been presented in Figure 2a as well as the histological pictures obtained after treatment with Barrisal were not significantly different from those in the control group (Figure 2b). The liver sections showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and central vein.

Intraperitoneal administration of CCl<sub>4</sub> showed complete disorganization in the structure of liver, the lesion in liver sections, liver cirrhosis with destruction of normal structure, severely damaged hepatocytes and massive ascites retention. Further, various stages of necrosis, numerous vacuoles in the cytoplasm, multifocal necrosis, moderate portal edema and infiltration of numerous inflammatory cells into portal triads were observed in CCl<sub>4</sub> treated rats. Bile ductule epithelial cells were swollen, vacuolated and had foci of cell necrosis with hypertrophy in the hepatocytes as shown in Figure 2c. Hypertrophy was more pronounced around central vein and peripheral region.

**Table 1.** Toxicological study of different extracts and drugs in mice and rats.

S/No.	Drug	Animal	Dose (ml/kg)	Route of administration	No. of animals	Days	Mortality	Symptom
1	<i>Barrisal</i>	Mice	Saline	Oral	6 Male, 6 Female	14	Nil	Decreased motor activity and corner sitting
			5	Oral	6 Male, 6 Female	14	Nil	
			10	Oral	6 Male, 6 Female	14	Nil	
2	<i>Barrisal</i>	Rats	Saline	Oral	5 Male, 5 Female	14	Nil	Nil
			10	Oral	5 Male, 5 Female	14	Nil	Nil

**Table 2.** Toxicological and hepatoprotective effect of *Barrisal* (10 ml/kg) in rats.

Compound	Total protein	Cholesterol	Bilirubin	SGPT	SGOT	AP
Saline	11.93±5.7 (12)	113.3±23.6 (12)	1.03±0.39 (12)	39.66±27.4 (12)	46.14±17.8 (12)	69.02±14.46 (12)
<i>Barrisal</i>	10.67±4.7 (12)	76.79±38 <sup>a*</sup> (12)	0.89±0.76 (12)	34.75±13.15 (12)	34.75±13.15 (12)	79.04±14.33 (12)
Saline+CCl <sub>4</sub>	11.10±3.7 (12)	95.05±9.12 (12)	2.16±1.5 <sup>a*</sup> (12)	200.63±31.65 <sup>a***</sup> (12)	169.78±36.7 <sup>a***</sup> (12)	110.8±32.08 <sup>a***</sup> (12)
<i>Barrisal</i> +CCl <sub>4</sub>	10.45±1.2 (12)	95.78±16.7 (12)	1.51±0.49 <sup>b*</sup> (12)	154.67±14.23 <sup>b*</sup> (12)	93.85±6.75 <sup>b**</sup> (12)	65.98±16.07 <sup>b**</sup> (12)

All values are expressed as mean±SD (n). a = p < 0.05 compared to control groups b = p < 0.05 compared to Saline + CCl<sub>4</sub> groups \*p < 0.05, \*\*p < 0.001, \*\*\*p < 0.00001

A hepatoprotective effect of *Barrisal* has prevented CCl<sub>4</sub> to induce liver damage. The hepatic pictures of rats treated with *Barrisal* + CCl<sub>4</sub> demonstrated a prevalence of morphologically normal hepatocytes and resulted in a significant reduction of all morphological alterations caused by CCl<sub>4</sub> intoxication. Only some hepatocytes with early necrotic lesions were encountered (Figure 2d).

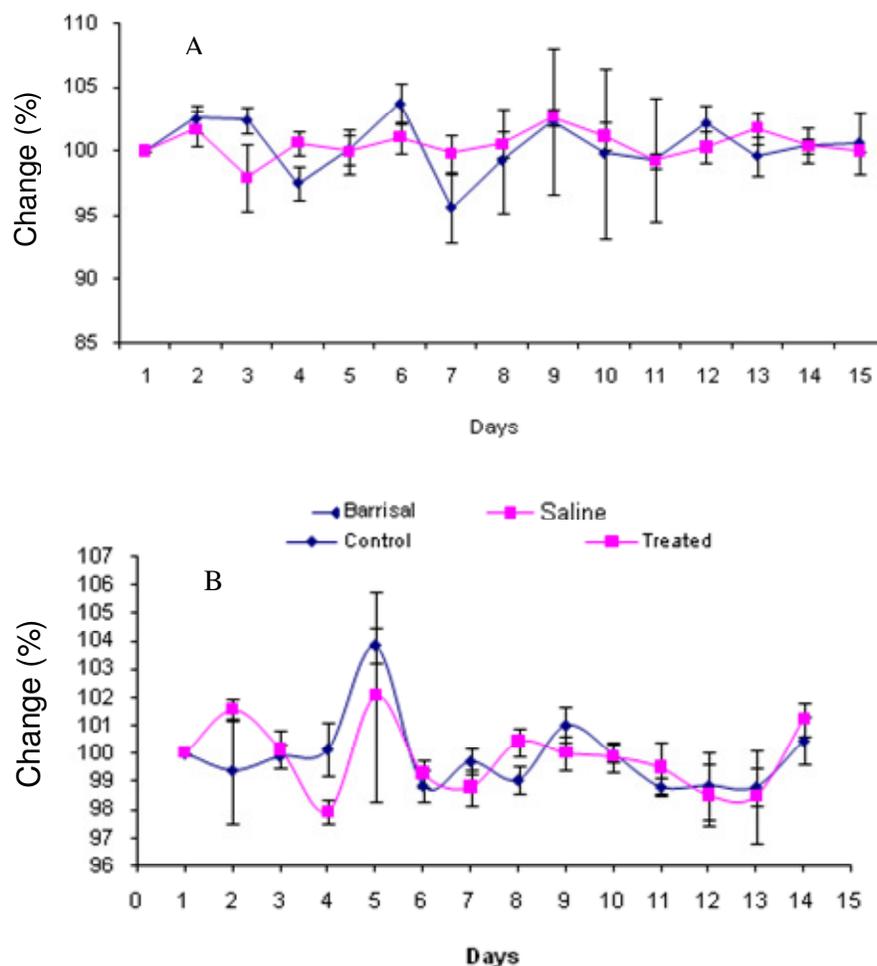
#### Hepatoprotective effect of *Barrisal* on heart histology

No effects were observed in the heart. They are

comparable with each other and they did not show any significant histological abnormality. The myofibrils within each cell are well displayed. In control and treated animals, branching of fiber is evident. Histological pictures of cardiac muscle, demonstrated that the fiber is dividing, then recombining and then spreading again. Each muscle cell possesses centrally located oval nuclei. Occasional muscle cells possess two nuclei. Intercalated disc indicated intracellular junction between two cardiac muscle cells. The intercellular areas are richly supplied by capillaries as shown in Figure 3b. They are comparable with their control (Figure 3a, b, c and d).

#### Hepatoprotective effect of *Barrisal* on kidney histology

The histological features of the control and *Barrisal* treated rats showed normal features (Figure 4a and b). Histopathological examination of the kidney of the rats intoxicated with CCl<sub>4</sub> revealed some renal disturbances and shrinkage in the cortical region and Bowman's capsule. Some cortical tubules showed disorganization while glomeruli shrunken and due to glomeruli atrophy it showed mild dilatation of Bowman's space while capillary loops exhibited congestion with an adhesion between visceral and parietal layers of



**Figure 1.** Effect of barrisal on body weight of (a) mice and (b) rats.

Bowman's capsule. Deterioration in some Bowman's capsule and tubular cell were observed. Renal tubules were dilated and their epithelial cells tend to be vacuolated. Interstitial inflammatory cell infiltration and fibrosis were clearly apparent after  $\text{CCl}_4$  treatment (Figure 4c).

The kidneys of the rats treated with Barrisal +  $\text{CCl}_4$  demonstrated a prevalence of morphologically normal glomeruli, Bowman's capsule and tubular cell. Histological appearance of the glomeruli and tubules were normal and rare vascular congestion was present in both the cortical and medullar region (Figure 4d).

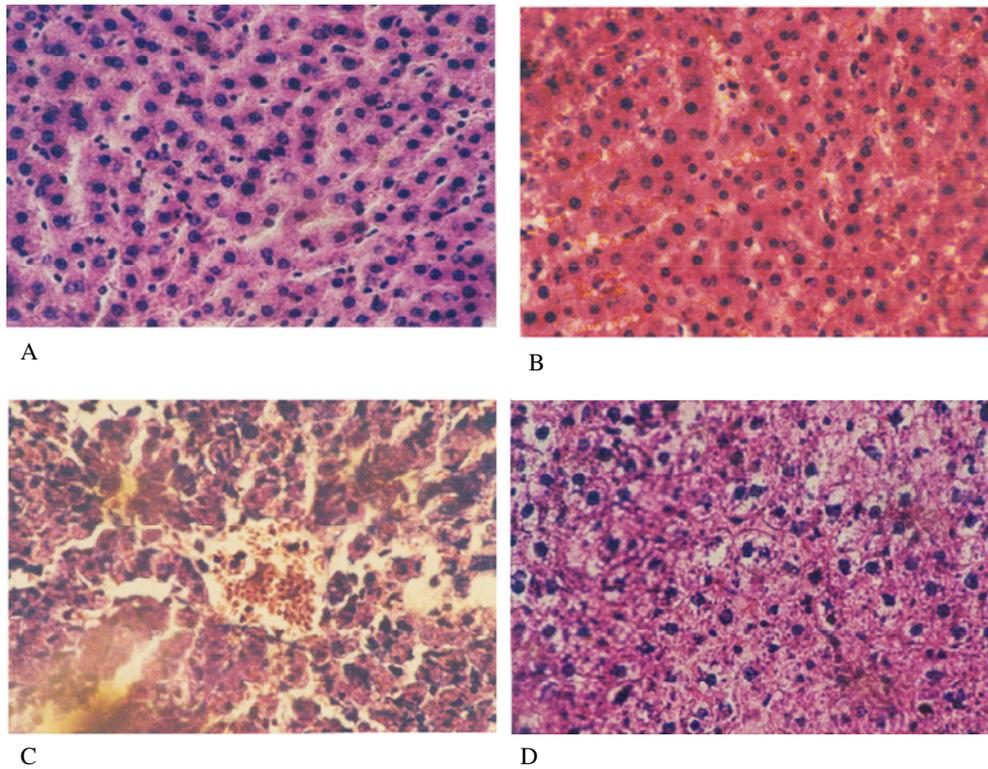
## DISCUSSION

The presented results for Barrisal treatment demonstrated no mortality in mice and rats. Similarly, no biochemical or histological changes have been detected as an evidence for the damage in the liver and other body

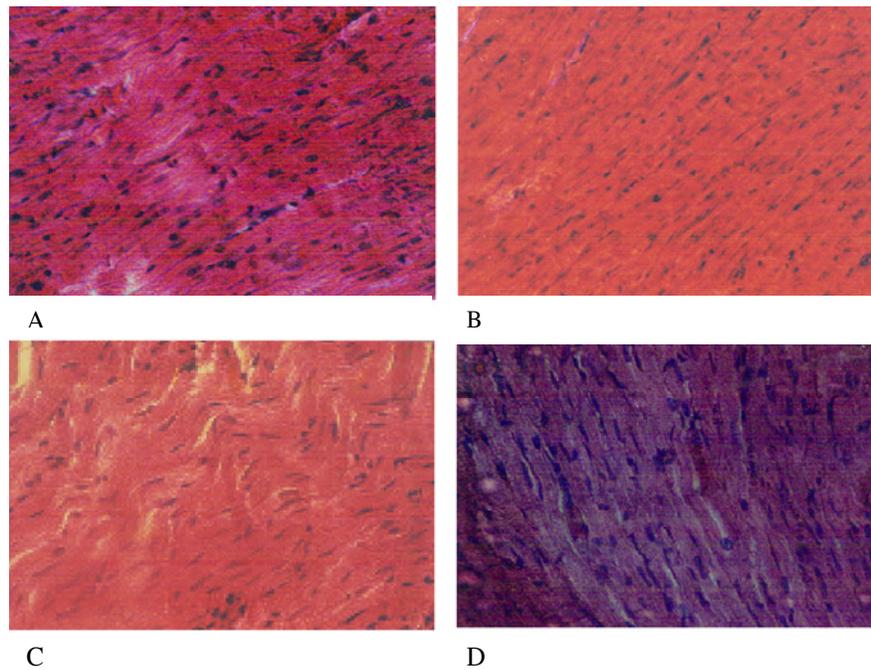
organs for the rats (Table 1). A non-significant change in body weight (Figure 1a and b) was observed during the treatment period which indicates that administration of Barrisal did not result in growth arrest (Figure 1a and b). Liver profile showed decrease bilirubin, SGPT and SGOT level, with an increase in alkaline phosphatase level, which was statistically non-significant. These results indicate that liver, heart and kidney are working normally. However, statistically non-significant changes in biochemical and histological parameters exhibit normal biological variability in rats.

In this study, the administration of Barrisal decrease the serum cholesterol level significantly ( $P < 0.05$ ). It is also reported earlier that '*A. vera*' caused a marked reduction in total serum cholesterol (Agarwal, 1985). This decrease in serum cholesterol after Barrisal administration has added advantage, because of the putative link between serum cholesterol and the incidence of atherosclerosis (Dixit et al., 1992).

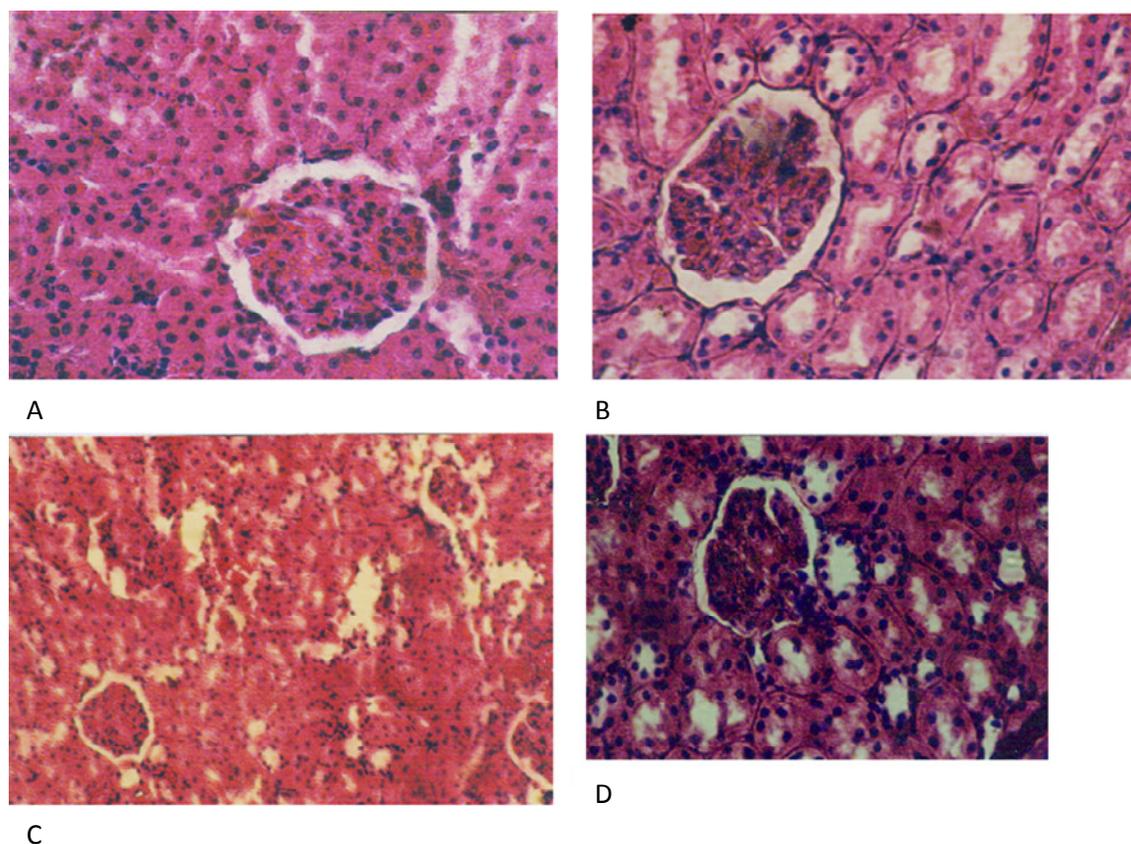
Hepatoprotective activity of Barrisal was carried out by



**Figure 2.** (A) Histological appearance of liver histology of control rats. (B) Liver histology of rats treated with Barrisal (10 ml/kg). (C) Liver histology of rats received CCl<sub>4</sub> olive oil. (D) Liver histology of rats received Barrisal+CCl<sub>4</sub> olive oil.



**Figure 3.** (A) Normal histological appearance of heart tissue of histology of control rats. (B) Histological appearance of heart tissue of Barrisal (10 ml/kg) treated rats. (C) Histological appearance of heart tissue of CCl<sub>4</sub> treated rats. (D) Histological appearance of heart tissue of Barrisal (10 ml/kg)+CCl<sub>4</sub> treated rats.



**Figure 4.** (A) Normal histological appearance of kidney tissue control rats. (B) Histological appearance of kidney tissue of Barrisal (10 ml/kg) treated rats. (C) Histological appearance of kidney tissue of CCl<sub>4</sub> treated rats. (D) Histological appearance of kidney tissue of Barrisal (10 ml/kg)+CCl<sub>4</sub> treated rats.

assessing the effect of Barrisal on the activities of plasma transaminases after CCl<sub>4</sub> treatment in rats. For this purpose, liver function tests for transaminases (SGOT, SGPT), bilirubin, protein, alkaline phosphatase were performed by considering them as useful markers to detect the extent of liver damage (Amin et al., 1996). The hepatotoxic effects of CCl<sub>4</sub> have been reported extensively. However, they depend upon the route of administration, amount employed, and duration of treatment (Lin et al., 1994). In the present study, the rats treated with a single dose of CCl<sub>4</sub> (Group III) developed hepatic damage, causing a substantial increase in the serum levels of transaminases: SGOT ( $P < 0.00001$ ), SGPT ( $P < 0.001$ ), bilirubin ( $P < 0.05$ ) and alkaline phosphatase ( $P < 0.001$ ) as shown in Table 2. Such rise in transaminases indicates the cellular leakage and loss of functional integrity of cell membrane in liver (Sango et al., 1998).

Further, liver injury is also attributed to the production of free radicals (Toshikazu et al., 1982). The mechanism of free radical production was reported by Durk and Frank (1984). They have suggested that bioactivation of

CCl<sub>4</sub> results in the production of trichloromethyl radical (initial metabolite) and trichloromethyl peroxy radical induced lipid peroxidation. Trichloromethyl free radical, initiates the biochemical events and attack membrane lipid/protein, thus ultimately culminate in liver cell necrosis (Pohl et al., 1984) leading to increased level of transaminases (SGOT, SGPT) and functional and structural disruption of liver cells as well as that observed in the present study (Table 2). Further, a non-significant decrease in serum cholesterol level in the present study after CCl<sub>4</sub> administration suggests that trichloromethyl radical can form covalent adducts with lipids, interact with O<sub>2</sub> to form a trichloromethyl peroxy radical (Pohl et al., 1983) causing a substantial decrease in cholesterol. CCl<sub>4</sub> treatment also causes significant increase in bilirubin level (Hyperbilirubinemia), because CCl<sub>4</sub> is also one of the most useful model to the severity of necrosis (Zimmerman, 1973). While there was no change in total protein level, these findings were in accordance with the observation that protein in general and albumin level in particular remains unchanged in acute liver damage (Anand et al., 1994). This brief period might not be sufficient

to demonstrate any rise in serum protein levels. Szymonik-Lesiuk et al. (2003) have shown that CCl<sub>4</sub> intoxication can lead to alteration in kidney; these findings are in accordance with the present observation. In the present study, an increased frequency of glomerulosclerosis and tubulointerstitial alterations was found in rats with reduced renal mass on CCl<sub>4</sub> administration (Figure 4c), thereby indicating nephrotoxicity on CCl<sub>4</sub> administration in rats. However, the pathogenesis of CCl<sub>4</sub> induced renal injury has not been clarified. While Rincon et al. (1999) showed that the effects of CCl<sub>4</sub> on kidney structure and function are dependent on the functional state of the liver. It is also suggested that kidney has high affinity for CCl<sub>4</sub> (Abraham et al., 1990), because cortex contain cytochrome P450 predominantly (Ronis et al., 1998) and cytochrome P450 lead towards CCl<sub>4</sub> activation, that is why the mechanism of CCl<sub>4</sub> nephrotoxicity is probably the same as that of liver.

Administration of Barrisal before CCl<sub>4</sub> exposure significantly reduced all the biochemical and histological alterations. In this study, Barrisal was found to significantly reduce the elevations in serum transaminases like SGOT (P<0.001), SGPT (P<0.05), bilirubin (P<0.05) and alkaline phosphatase (P<0.001) level against CCl<sub>4</sub> induced toxicity in rats. Histopathological evaluation of Barrisal in CCl<sub>4</sub> induced liver injury suggests a significant reduction of ballooned cells in liver of Barrisal treated rats when compared with only CCl<sub>4</sub> treated group. It was also found that multifocal necrotic changes induced by CCl<sub>4</sub> minimized or erased completely in Barrisal pretreated rats. These results indicate that Barrisal may improve general injury and suppress enzyme leakage from cellular membranes; it was also found that Barrisal showed an improvement in centrilobular necrosis around central vein. This study also revealed that Barrisal substantially protects against CCl<sub>4</sub> induce tubular and glomerular changes in the kidneys having nephroprotective effect.

In this study, we hypothesized that administration of Barrisal would effectively protect liver and kidney against CCl<sub>4</sub> induced injury and this effect was verified by both biochemical and histopathological observation (Figure 2 to 4). Numerous experimental studies have demonstrated that antioxidant treatment has beneficial effects on CCl<sub>4</sub> induced tissue injury (Banskota et al., 2001). Barrisal is widely prescribed in hepatic and spleen disorders, it is also claimed that Barrisal is an immune modulating drug having adaptogenic activity. Barrisal is a herbal formulation having *A. vera* syn. *A. barbadensis* Mill as an active ingredient contain cinnamoyl, p-coumaroyl, feruloyl, and caffeoyl aloesin, which have been reported to have antiinflammatory and antioxidative (Yagi and Takeo, 2003) properties and it also possess inhibitory effect (antilipoxygenase activity) in the acute inflammation process (Bezakova et al., 2005). That is why protective effects of Barrisal on the increase in plasma transaminase activities induced by CCl<sub>4</sub> treatment is due

to the presence of antioxidative compound cinnamoyl, p-coumaroyl, feruloyl, and caffeoyl aloesin.

Since a cellular membrane defect is a terminal feature of liver cell damage/necrosis in CCl<sub>4</sub> intoxication, and which is reduced under the influence of Barrisal. Therefore, it is possible that this preparation having the active ingredient of *A. vera* possesses the following character, on the basis of its possible cellular effect.

- (1) Hepatoprotective effect due to stabilization or strengthening of cell membrane.
- (2) Hepatoprotective effect may also be due to its antioxidative and anti-inflammatory property, which may prevent inflammatory hepatic damage, immunomodulating property and anti-oxidant property, thereby reducing the oxidative stress imposed by the CCl<sub>4</sub>.
- (3) Further, Barrisal did not allow CCl<sub>4</sub> to exert its harmful actions and therefore, it can protect liver and kidney functions. Thus, this study provides a rationale for the use of Barrisal as a suitable herbal treatment for the management of liver disorders.

## REFERENCES

- Abraham P, Wilfred G, Cathrine SP (1990). Oxidative damage to the lipids and proteins of the lungs, testis and kidney of rats during Carbon tetrachloride intoxication. *Clin. Chim. Acta* 289:177-179
- Agarwal OP (1985). Prevention of atheromatous heart disease. *Angiology* 36(8):485-92.
- Amin A, Nanji A, David Z (1996). In: David Z, Thomas B (eds.), *Hepatology. A textbook of liver disease*. WB Saunders Co., London. P 891.
- Anand KK, Singh B, Saxena AK, Chandan BK, Gupta VN (1994). Hepatoprotective studies of a Fraction from the fruit of *Terminalia bellerica* Roxb. On Experimental liver injury in rodents. *Phytother. Res.* 8:287-292.
- Banskota AH, Tezuka Y, Kadota S (2001). Recent progress in pharmacological research of propolis. *Phytother. Res.* 15:561-571.
- Bezakova L, Oblozinsky M, Sykorova M, Paulikova I, Kostalova D (2005). Antilipoxygenase activity and the trace elements content of *Aloe vera* in relation to the therapeutic effect. *Ceska Slov. Fam.* 54(1):43-6.
- Capasso F, Borrelli R, Capasso G, DiCarlo AA, Izzo I, Pinto N, Mascolo S, Castaldo R, Longo (1998). *Aloe* and its Therapeutic Use. *Phytother. Res.* 12:124-127.
- Dixit VP, Varma M, Mathur NT, Mathur R, Sharma S (1992). Hypocholesterolemic and antiatherosclerotic effects of solasodine (C27H42O2N) in cholesterol-fed rabbits. *Phytother. Res.* 6:270-273.
- Durk H, Frank H (1984). Carbon tetrachloride metabolism in vivo and exhalation of volatile alkanes: dependence upon oxygen partial pressure. *Toxicology* 30:249-257.
- Fatima N (1993). Hepatoprotective activity of *Icterene*. *Hamdard Med.* 36(3):56-62.
- Hutter JA, Salman M, Stavinoha WB, Satsangi N, Williams RF, Streeper RT, Weintraub ST (1996). Antiinflammatory C-glucosyl chromone from *Aloe barbadensis*. *J. Nat. Prod.* 59:541-543.
- Khan U (1997). *Indusynic Medicine Traditional Medicine of Herbal, Animal and Mineral Origin in Pakistan*, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. pp. 269-270, 325-326, 397-398.
- Lin SC, Lin CC, Lin Y, Chen CH (1994). Protective effect s of *Ban-zhilian* on hepatotoxin-induced liver injuries. *Am. J. Chin. Med.* 22(1):29-42.

- Pohl L, Schulick R, George J (1984). Reductive oxygenation mechanism of metabolism of carbon tetrachloride to phosgene by cytochrome p. 450. *Mol. Pharmacol.* 25:318-324.
- Pohl L, Schulick R, Highet R, George J (1983). Identification of dichloromethyl carbene as a metabolite of carbon tetrachloride. *Biochem. Biophys. Res. Commun.* 117:367-372.
- Rincon AR, Covarrubias A, Pedraza-Chaverri J, Poo JI, Armendariz-Borunda J, Panduro A (1999). Differential effect of CCL4 on renal function in cirrhotic and non cirrhotic rats. *Exp. Toxicol. Pathol.* 51:199-205.
- Ronis MJ, Huang J, Longo V, Tinderberg N, Ingelman-Sundberg M, Badger TM (1998). Expression and distribution of cytochrome P450 enzymes in male rat kidney: effect of ethanol, acetone and dietary conditions. *Biochem. Pharmacol.* 55:123-129.
- Sango R, Germano MP, Angelo V D, Guglielmo M, De Pasquale R (1998). Antihepatotoxic properties of *Entada africana* (Mimosaceae). *Phytother. Res.* 12:S157-S159.
- Strickland FM, Kuchel JM, Halliday GM (2004). Natural products as aids for protecting the skin's immune system against UV damage. *Cutis* 74(5):24-8.
- Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Slomka M, Madro A, Celinski K, Wielosz M (2003). Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. *J. Hepatobiliary Pancreat. Surg.* 10:309-315.
- Tian B, Hua YJ, Ma XQ, Wang GL (2003). Relationship between antibacterial activity of *Aloe* and its anthraquinone compounds *Zhongguo Zhong Yao Za Zhi* 28(11):1034-7.
- Toshikazu N, Fong KL, Edward KL, Steve SA, King MM, Larry O, Poyer JL, Paul BM (1982). Specificity of a Phenobarbital induced cytochrome P-450 for metabolism of Carbon tetrachloride to the trichloromethyl radical. *Biochem. Pharmacol.* 31(5):615-624.
- Yagi A, Takeo S (2003). Anti-inflammatory constituents, *Aloesin* and *Albemannan* in *Aloe* species and effects of tanshinon VI in *Salvia miltiorrhiza* on heart. *Yakugaku Zasshi* 123(7):517-32.
- Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M (2002). Acute and chronic toxicity of *Nigella sativa* fixed oil. *Phytomedicine* 9:69-74.
- Zimmerman HJ (1973). Hepatic Failure. In: Gall EA, Mostofi FK (eds.), *The Liver*. William and Wilkins, Baltimore. pp. 384-405.

Full Length Research Paper

## The biochemical changes in rats' blood serum levels exposed to different gamma radiation doses

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This study aimed to address the different gamma radiations doses effect on the liver and kidney function of rats: *in vivo*. A total of 60 healthy male Wistar-Kyoto rats were whole body gamma irradiated with Co 60 source with 0.883 cG/sec dose rate at the beginning of the experiment. The rats were randomly divided into 4 gamma-irradiation groups (25, 50, 75 and 100 Gy) The serum levels of activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma- glutamyltransferase (GGT), UREA, creatinine (CREA) and uric acid (UAC) were measured using automated biochemical analyzer. The ALT, GGT, ALP values significantly decreased with the different gamma radiation doses compared with the control. The AST and UREA values significantly decreased after irradiation with 25 and 50 Gy gamma radiation doses compared with the control while it significantly increased with 75 and 100 Gy gamma radiation doses. The levels of CREA values decreased with no significant manner after the irradiation with gamma radiation doses compared with the control. The levels of UAC values significantly increased with 50, 75 and 100 Gy gamma radiation doses. The serum ALT and AST levels are common markers for hepatic toxicity: A lower amount of ALP indicates liver problems. The decreased CREA and the increased UAC levels might indicate development of nephritis and renal dysfunction. The excess UAC might be converted to crystals depositing in the tiny tubes of the kidney and causing acute kidney damage. It is proposed that oxidative stress is linked to the organ damage following exposure to ionizing radiation, and after the onset of oxidative stress, antioxidant treatment should be applied to delay or prevent the progression of damage.

**Key words:** Gamma radiation, different radiation doses, liver function, kidney function, rats.

### INTRODUCTION

Serious problems are generally encountered after acute exposure to high doses of ionizing radiations (Yousri et al., 1991). Ionizing radiations interact with biological systems through free radicals generated by water radiolysis. This indirect action plays an important role in the induction of oxidative stress leading to cellular damage and organ dysfunction (Berrouid et al., 1996). Exposure of

mammals to ionizing radiations, leads to the development of a complex, dose-dependent series of changes, including injury to different organs, causing changes in the structure and function of cellular components, and resulting in tissue damage and death. Thus, radiation-induced damage might result in adverse health effects within hours to weeks or delayed effects observable many

months after exposure (Vijayalaxmi et al., 2004). Recently, oxidative stress is possibly involved in the pathology of some diseases and other inborn errors of lipid and protein metabolism (Onody et al., 2003). Oxidative stress with subsequent production of reactive oxygen species (ROS) has been postulated as one of the mechanisms of radiation toxicity (Finkel and Holbrook, 2000). It was also reported that ionizing radiation affected liver and kidney functions (El-Kashef et al., 1986; Roushdy et al., 1984).

Radiation damage, is to a large extent, caused by the overproduction of ROS, including superoxide anion ( $O_2^{\bullet-}$ ), hydroxyl radical ( $\bullet OH$ ), and hydrogen peroxide ( $H_2O_2$ ), that decrease the levels of antioxidants, resulting in oxidative stress and cellular damage. ROS cause damage by reacting with cellular macromolecules such as nucleotides in nucleic acids, polyunsaturated fatty acids found in cellular membranes, and sulfhydryl bonds in proteins. If this damage is irreparable, then injury, mutagenesis, carcinogenesis, accelerated senescence, and cell death can occur (Spitz et al., 2004).

Ionizing radiations interact with biological systems through free radicals generated by water radiolysis. This indirect action plays an important role in the induction of oxidative stress leading to cellular damage and organ dysfunction (Berroud et al., 1996).

It became apparent that radiation can penetrate the living cells and deposit within them in random fashion, leading to radiation damage. The blood chemistry measures the levels of chemicals, enzymes, and organic waste products that are normally found in the blood. It was of particular interest to investigate the toxicity associated with oxidative stress and the damage to rats induced by gamma-radiation exposure. In medicine, there is a limited knowledge on the levels of chemicals, enzymes, and organic waste products that are normally found in the blood after irradiating with different gamma radiation doses. Thus, it is of particular interest to investigate the hepatic and nephritic toxicity by determining serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), creatinine (CREA), uric acid (UAC) and urea induced by different gamma radiation doses *in vivo* in an attempt to cover and understand the toxicity and potential therapeutic tool of gamma radiation.

## MATERIALS AND METHODS

### Chemicals

All reagents were of the highest purity available. All chemicals for biochemical analysis were purchased from Sigma Chemical Co.

### Animals

Male Wistar rats weighing 150 to 200 g, were used in the present

experiments. Experimental animals were housed in cages with free access to drinking water and diet and were maintained in the animal care facility throughout the duration of the experiment. The animals were kept at 20 to 25°C with the 12 h light/dark cycle. Animal husbandry and experimentation were consistent with the Public Health Guide for the Care and Use of Laboratory Animals and in accordance with protocols approved by King Saud University Local Experimental Animal Ethics Care and Use Committee.

### Experimental design

A total of 60 healthy male Wistar-Kyoto rats weighing 250 g were used in this study. All rats were whole body gamma irradiated with Co 60 source. The animals were randomly divided into groups, 4 gamma-irradiation treated rat groups (The 1st group was irradiated with 25 Gy gamma radiation (n = 10); the 2nd group was irradiated with 50 Gy gamma radiation (n = 10); the 3rd group was irradiated with 75 Gy gamma radiation (n = 10); the 4th group was irradiated with 100 Gy gamma radiation (n = 10) and one control group (NG: n = 20)). The 4 gamma-irradiation treated rat groups were maintained on standard laboratory rodent diet pellets and were housed in humidity and temperature-controlled ventilated cages for a period of 24 h day/night cycle. The irradiation process was carried out at Research Center, King Saud University using Co 60 source with dose rate 0.9 ( $\mu G/s$ ) at the beginning of the experiment

### Serum biochemical analysis

Biochemical analysis was performed 24 h after the irradiation with different gamma radiation doses. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. After sacrificing the animals, 2 ml of blood samples were collected and placed in chilled non-heparinized tubes, centrifuged at 3000 rpm for 10 min at 4°C. The serum was frozen at -20°C for biochemical measurements. The serum levels of the activity of ALT, AST, ALP, GGT, urea, CREA and UAC were measured using automated biochemical analyzer (Type 7170, Hitachi).

### Statistical analysis

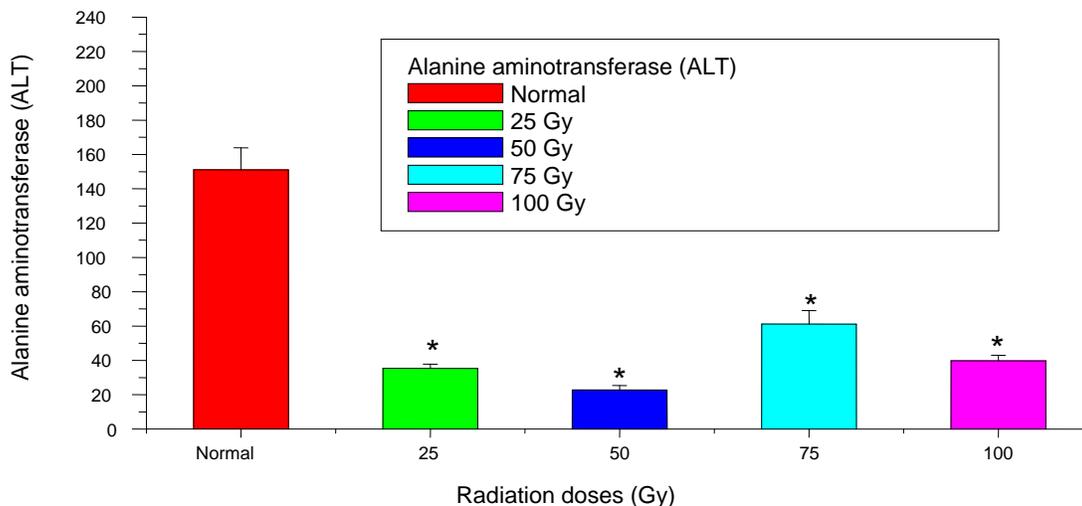
The results of this study were expressed as mean  $\pm$  standard error (Mean  $\pm$  SE). To assess the significance of the differences between the control group and the four gamma-irradiated rat groups (25, 50, 75 and 100 Gy), a statistical analysis was performed using one-way analysis of variance (ANOVA) for repeated measurements with the significance assessed at the 5% confidence level.

## RESULTS AND DISCUSSION

The ALT values significantly decreased with the different gamma radiation doses for rat blood serum when compared with the control (Figure 1). This study suggests that the liver might be damaged with irradiation with different gamma radiation doses. The liver enzyme ALT rearranges the building blocks of proteins.

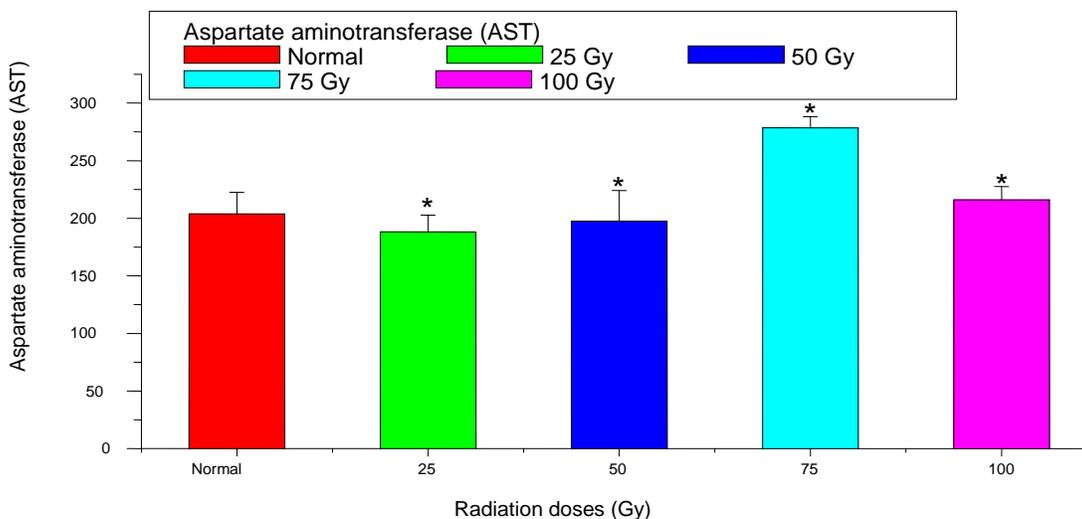
The AST values significantly decreased after irradiation with 25 and 50 Gy gamma radiation doses when compared with the control, while it significantly increased with 75 and 100 Gy gamma radiation doses (Figure 2).

The serum ALT and AST levels are common markers for hepatic toxicity; levels of these proteins were rapidly



**Figure 1.** The levels of alanine aminotransferase (ALT) versus the different gamma radiation doses for rat blood serum.

\*Means are significantly different ( $P < 0.05$ ).



**Figure 2.** The levels of aspartate transaminase (AST) versus the different gamma radiation doses for rat blood serum.

\*Means are significantly different ( $P < 0.05$ ).

increased when the liver is damaged by any cause, including hepatitis or hepatic cirrhosis. Transaminases play an important role in protein and amino acid metabolism. They are found in the cells of almost all body tissues and when diseases or injuries affected these tissues, they are released into blood stream (Kaplan, 1986).

Some investigators have reported significant elevation in the activity of liver enzymes (ALT and AST) and kidney function tests (CREA and UREA) after gamma-irradiation (Makhlouf and Makhlouf, 2012; El-Deeb et al., 2006; Bhatia and Manda, 2004), while other investigators have reported the opposite (Hanan et al., 2007). The increase

or decrease in the activity of liver enzymes and kidney function parameters might indicate occurrence of liver and kidney injury. Therefore, it is proposed that oxidative stress is linked to the organ damage following exposure to ionizing radiation. It is hypothesized that after the onset of oxidative stress in the tissue, antioxidant treatment should delay or prevent the progression of that damage (Halliwell and Whiteman, 2004).

A single irradiation dose of 6 Gy caused hepatic and renal damage manifested biochemically as an elevation in levels of ALT and AST as well as an increase in blood UREA. The rats' significantly countered radiation induced

biochemical disorder: liver enzymes and kidney function analysis, as well as, cholesterol level in the serum (Ibrahim, 2013). The increase in the serum aminotransferase activities could be due to liver damage induced by free radicals generated after radiation exposure (Jirtle et al., 1990).

The GGT values significantly decreased with the different gamma radiation doses for rat blood serum when compared with the control (Figure 3). A significant increase in serum GGT activity after exposure of rats to ionizing radiation has been observed (Makhlouf and Makhlouf, 2012). The GGT is a key enzyme in the catabolism of glutathione (GSH) (Djavaheri-Mergny et al., 2002; Lee et al., 2002). Recently, it has been reported that the extracellular cleavage of GSH by GGT induces production of ROS, suggesting that GGT plays a pro-oxidant role (Lee et al., 2004). The significant decrease observed in serum GGT activity might be due to the presence of oxidative stress and damage. The present results might indicate that GGT is considered as one of the enzymes related to oxidative stress after the exposure to gamma radiation dose. The GGT is inversely associated with antioxidants (Kilanczyk and Bryszewska, 2003).

ALP values significantly decreased with the irradiation with different gamma radiation doses for rat blood serum when compared with the control (Figure 4). ALP is processed in the liver and excreted into the digestive tract in the bile. A higher ALP levels than normal indicates liver problems.

It has been reported that a significant increase ( $P \leq 0.05$ ) in ALP activities, CREA and urea levels were recorded in the serum of irradiated rats which might result from radiation-induced cell membrane damage followed by the release of intracellular molecules to the blood stream (Kaplan, 1986).

In this study, the levels of urea values significantly decreased with the irradiation with 25 and 50 Gy gamma radiation doses when compared with the control while it significantly increased with the 75 and 100 Gy gamma radiation doses (Figure 5). Blood urea nitrogen is a part of urea; the waste product that is left over from the breakdown of protein. Urea circulates in the blood until it is filtered out by the kidneys and excreted in the urine. If the kidneys are not functioning properly, there will be excess urea levels in the bloodstream.

It has been reported that irradiation of male rats increased serum urea without significant changes in serum CREA. It is known that radiation causes an increase in glutamate dehydrogenase enzyme levels, which might increase carbamoyl phosphate synthetase activity leading to an increase in urea concentration (Roushdy et al., 1984). The increased serum CREA in the irradiated group indicates development of nephritis and renal dysfunction, a result in agreement with. This result may be attributed to impairment of glomerular selective properties caused by irradiation.

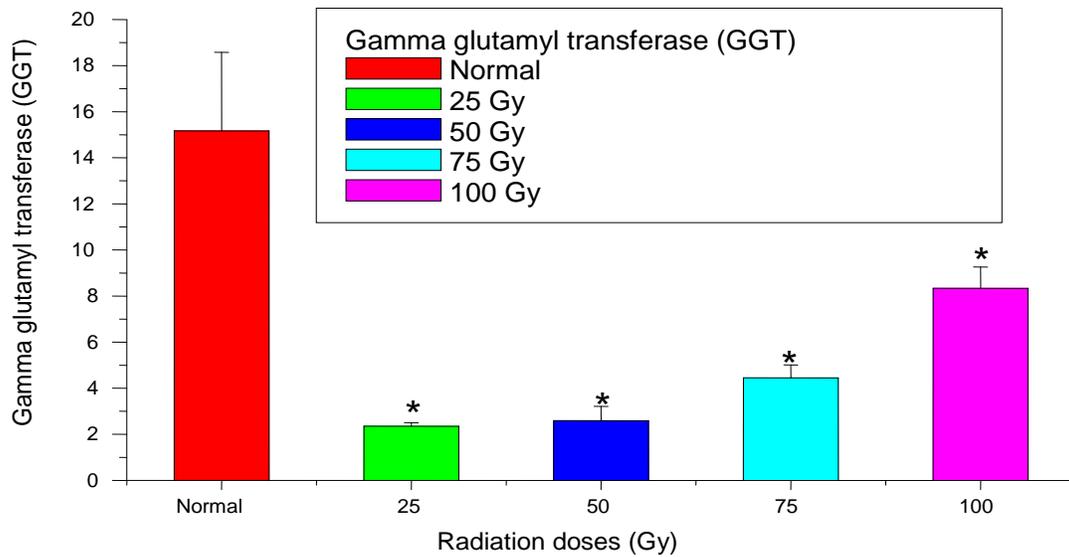
The major forms of cellular damage induced by radiation are DNA damage, lipid peroxidation, and protein oxidation. The increased concentration of thiobarbituric acid reactive substances (TBARS) and nucleic acid in the rat liver, indicating high level of oxidative stress, markedly enhanced with increasing radiation dose (Makhlouf and Makhlouf, 2012); similar observations were reported on radiation-induced oxidative damage in several organs (Bhatia and Manda, 2004; Sener et al., 2003) and mitochondrial. Ionizing radiation generates ROS as a result of water radiolysis. In actively metabolizing cells, there is considerable water apart from the target macromolecules. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes (Cadet et al., 2004). Lipid peroxidation such as malondialdehyde (MDA) and 4-hydroxynonenal (4HNE) have the ability to interact with and alter macromolecules, possibly resulting in diseases (Petersen and Doorn, 2004).

Oxidative damage to proteins, as assessed by formation of carbonyl groups is a highly damaging event, and may occur in the absence of lipid peroxidation (Dean et al., 1997). Thus, modification of lipids and proteins by ROS is implicated in the etiology of radiation-induced physiological disorders and diseases. This study suggests that the energy-radiation induced by cesium-137 source, produced a significant oxidative damage in the rats after whole body exposure. It has been reported that whole-body exposure of rats to high energy radiation from Co-60 causes tissue damage in several organs, as assessed by increased lipid peroxidation 2 and 12 h after irradiation (Sener et al., 2003).

In this study, the levels of CREA values decreased with no significant manner after irradiation with 25, 50, 75 and 100 Gy gamma radiation doses when compared with the control (Figure 6). CREA is a compound that is produced by the body and excreted in the urine. Compounds that leave the body in the urine are processed by the kidney; therefore CREA may be used to monitor the kidney function.

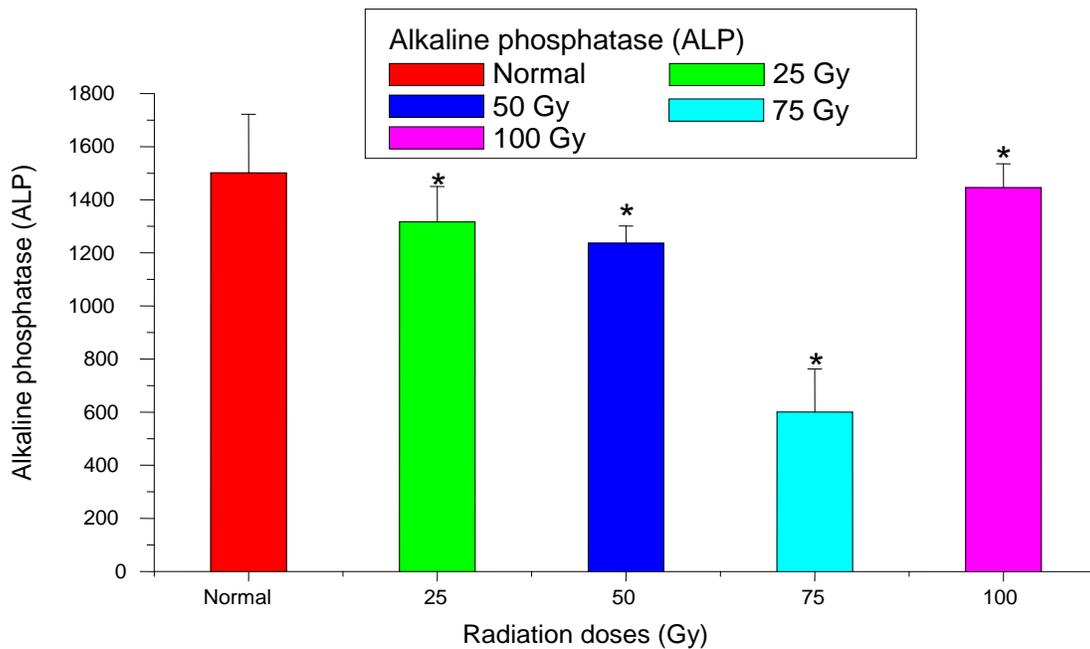
The levels of UAC values decreased with no significant manner with irradiation with 25 Gy gamma radiation dose when compared with the control, while it significantly increased with the 50, 75 and 100 Gy gamma radiation doses (Figure 7). UAC is the end product of the digestion of certain proteins and is normally eliminated through the urine. Excess UAC may be a side effect of some cancer treatments, and may lead to a condition called tumor lysis syndrome. When excess UAC is present, it is converted to crystal. These crystals may be deposited in the tiny tubes that are part of the kidney and cause acute kidney damage, which can ultimately lead to kidney failure.

Gamma rays act either directly or by secondary reactions to produce biochemical lesions that initiate series of physiological symptoms. Ionizing radiation is known to induce oxidative stress through the generation of ROS resulting in imbalance of the prooxidant and antioxidant



**Figure 3.** The levels of gamma glutamyltransferase (GGT) versus the different gamma radiation doses for rat blood serum.

\*Means are significantly different ( $P < 0.05$ ).

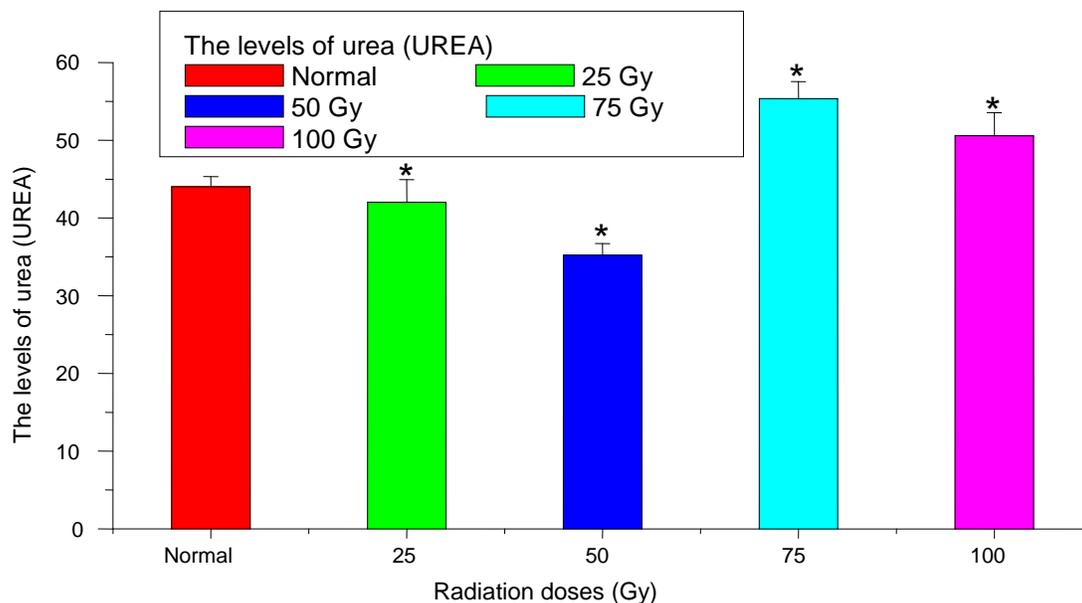


**Figure 4.** The levels of alkaline phosphatase (ALP) versus the different gamma radiation doses for rat blood serum.

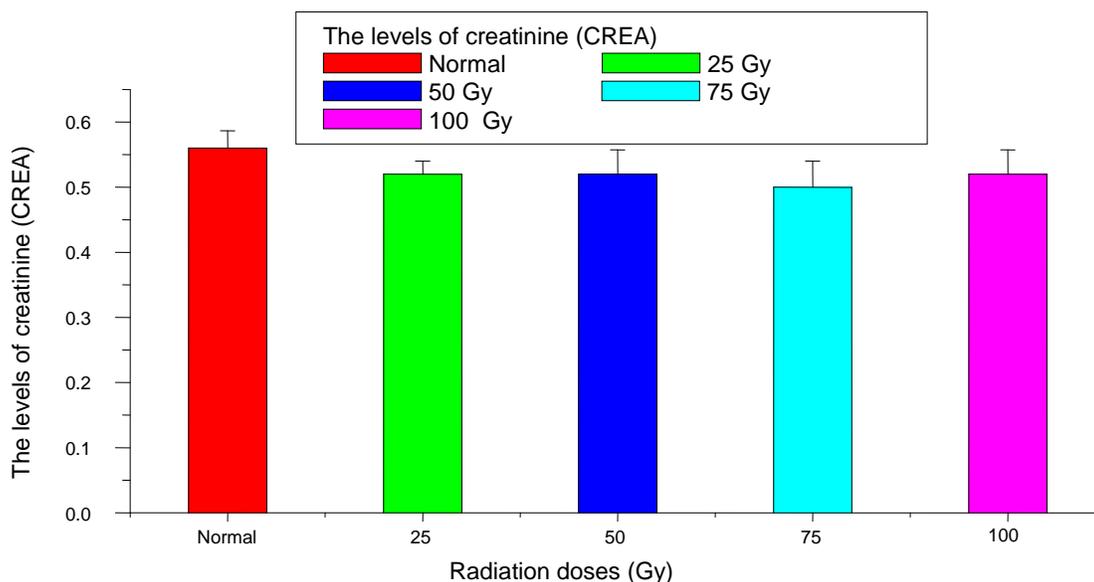
\*Means are significantly different ( $P < 0.05$ ).

activities, ultimately resulting in cell death (Srinivasan et al., 2006). Numerous attempts have been made to investigate different means for controlling and protection from radiation hazards using chemical, physical and biological means.

This study suggests that additional experiments will be performed taking into consideration the oxidative stress through the generation of ROS such as hydrogen peroxide and oxygen free radicals; resulting in imbalance of the prooxidant and antioxidant activities, ultimately leading



**Figure 5.** The levels of urea (UREA) versus the different gamma radiation doses for rat blood serum. \*Means are significantly different ( $P < 0.05$ ).

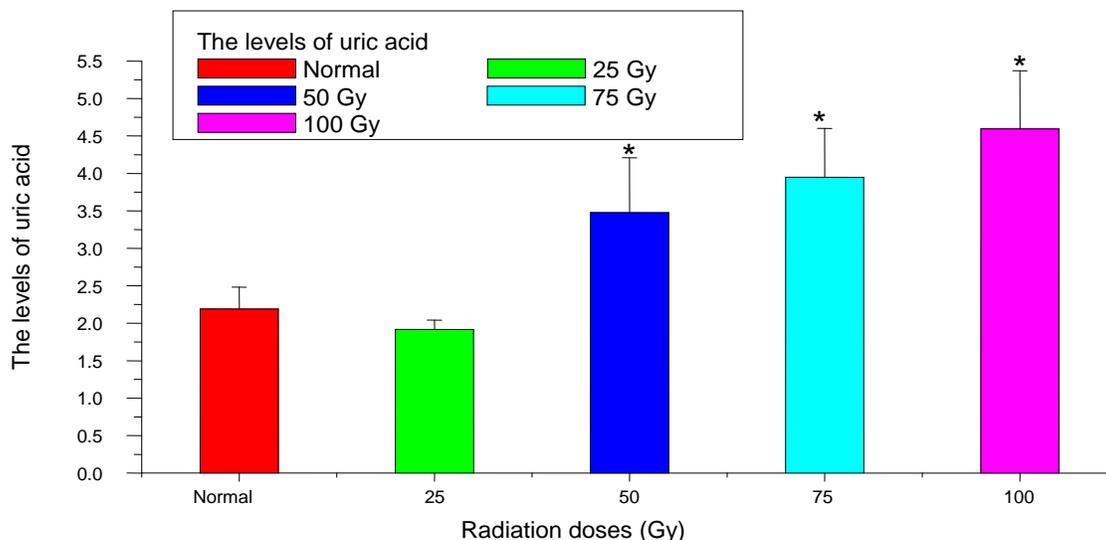


**Figure 6.** The levels of creatinine (CREA) versus the different gamma radiation doses for rat blood serum. \*Means are significantly different ( $P < 0.05$ ).

leading to cell death. Investigation of the different means of controlling and protection from radiation hazards is through the use of chemical, physical and biological means through the prior administration of radioprotection agent to the irradiation with different gamma radiation doses preventing the oxidative stress.

Significant increase in the levels of serum lipid profile and low density lipoprotein (LDL) are demonstrated post

radiation exposure of rats, possibly as a result of liver injury. These changes are in agreement with previous studies (Feurgard et al., 1999). This indicates that ionizing-radiation-induced oxidative stress which might alter hepatic lipid metabolism and serum lipoproteins. It seems that there is an association between radiation-induced oxidative stress and elevated levels of lipid fractions and LDL (Onody et al., 2003). This association is



**Figure 7.** The levels of uric acid (UAC) versus the different gamma radiation doses for rat blood serum. \*Means are significantly different ( $P < 0.05$ ).

similarly observed in other conditions characterized by increased oxidative stress (El-Missiry et al., 2004; Zwirska-Korczala et al., 2003). Therefore, this study suggests that additional experiments will be performed taking into consideration the levels of LDL, high density lipoprotein (HDL), lipid peroxidation through MDA, GSH content, catalase (CAT) activity and DNA content.

## Conclusions

Some investigators have reported significant elevation in the activity of liver enzymes after gamma-irradiation, while other investigators have reported the opposite. ALT, AST and GGT levels significantly decreased with the different gamma radiation doses in rat blood serum when compared with the control. Serum ALT and AST levels are common markers for hepatic toxicity: levels of these proteins are rapidly increased when the liver is damaged by any cause, including hepatitis or hepatic cirrhosis. The extracellular cleavage of GSH by GGT might induce ROS, suggesting that GGT plays a pro-oxidant role, and GGT might be considered as one of the enzymes related to oxidative stress after exposure to gamma radiation dose and it is inversely associated with antioxidants. The increase or decrease in the activity of liver enzymes and kidney function parameters might indicate liver and kidney damage. It is proposed that oxidative stress is linked to the organ damage following exposure to ionizing radiation, and it is hypothesized that after the onset of oxidative stress in the tissue, antioxidant treatment should be applied to delay or prevent the progression of that damage. Elevation and reduction in ALP, CREA and urea levels might result from radiation-induced cell membrane damage followed by the release

of intracellular molecules to the blood stream. The decrease in ALP might be attributed to a transitory reduction in the release of alkaline phosphatase to the enzymatic circulation by rapidly metabolizing cells and/or injury to the intestinal mucosa after irradiation. The changes in urea might indicate kidneys failure. The changes in glutamate dehydrogenase enzyme levels might be attributed to carbamoyl phosphate syntheses activity leading to changes in urea concentration. The changes in serum CREA in the irradiated rats group might indicate nephritis and renal dysfunction due to impairment of glomerular selective properties. The major forms of cellular damage induced by radiation are DNA damage, lipid peroxidation, and protein oxidation. The increased concentration of TBARS and nucleic acid in the rat liver, indicates high level of oxidative stress, which markedly enhanced with increasing radiation dose; similar observations were reported on radiation-induced oxidative damage in several organs and mitochondrial.

The modification of lipids and proteins by ROS might be implicated in the etiology of radiation-induced physiological disorders and diseases. Excess of UAC level might be converted to crystals, depositing in the tiny tubes of the kidney and causing acute kidney damage, leading to kidney failure. The ionizing radiation might induce oxidative stress through the generation of ROS, resulting in imbalance of the prooxidant and antioxidant activities, resulting in cell death, altering hepatic lipid metabolism and serum lipoproteins, and producing biochemical lesions that initiate series of physiological symptoms.

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## REFERENCES

- Berroud A, Le-Roy A, Voisin P (1996). Membrane oxidative damage induced by ionizing radiation detected by fluorescence polarization. *Radiat. Environ. Biophys.* 35:289-295.
- Bhatia AL, Manda K (2004). Study on pre-treatment of melatonin against radiation-induced oxidative stress in mice. *Environ. Toxicol. Pharmacol.* 18:13-20.
- Cadet J, Bellon S, Douki T, Frelon S, Gasparutto D, Muller E, Pouget JP, Ravanat JL, Romieu A, Sauvaigo S (2004). Radiation-induced DNA damage: formation, measurement, and biochemical features. *J. Environ. Pathol. Toxicol. Oncol.* 23(1):33-43.
- Dean RT, Fu S, Stocker R, Davies MJ (1997). Biochemistry and pathology of radical mediated protein oxidation. *Biochem. J.* 324:1-18.
- Djavaheri-Mergny M, Accaoui MJ, Rouillard D, Wietzerbin J (2002). Gamma-glutamyl transpeptidase activity mediates NF-kappa B activation through lipid peroxidation in human leukemia U937 cells. *Mol. Cell Biochem.* 232(1-2):103-111.
- EI-Deeb AE, Abd El-Aleem IM, Abd El-Rahman AA (2006). The curative effect of some antioxidants on  $\gamma$ -irradiated rats, *J. Egypt. Soc. Toxicol.* 35:79-89
- EI-Kashef HS, Saada HN, Awadallah SSH (1986). Radiation effects of some enzymatic activities in tissues of rats subjected to whole body gamma irradiation. *Egypt J. Radiat. Sci. App.* 3(2):105-14.
- EI-Missiry MA, Othman AI, Amer MA (2004). L-Arginine ameliorates oxidative stress in alloxan-induced experimental diabetes mellitus. *J. Appl. Toxicol.* 24:93-97
- Feurgard C, Boehler N, Ferezou J, Serougne C, Aigueperse J, Gourmelon P, Lutton C, Mathe D (1999). Ionizing radiation alters hepatic cholesterol metabolism and plasma lipoproteins in Syrian hamster. *Int. J. Radiat. Biol.* 75(6):757-766
- Finkel T, Holbrook NJ (2000). Oxidants, oxidative stress and biology of aging. *Nature* 408:239-47
- Halliwell B, Whiteman M (2004). Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? *J. Pharmacol.* 142:231-255.
- Jirtle RL, Anscher MS, Alati T (1990). Radiation sensitivity of liver. In: Altman KI, Lett JT (eds.), *Advances in radiation biology*, Vol. 14. Relative radiation sensitivities of human organ systems, Part, 11. Academic Press, San Diego. pp. 369-411.
- Kaplan MM (1986). Serum alkaline phosphatase – another piece is added to the puzzle. *Hepatology* 6(3):526
- Kilanczyk E, Bryszewska M (2003). The effect of melatonin on antioxidant enzymes in diabetic skin fibroblasts. *Cell Mol. Biol.* 8(2):333-336.
- Lee DH, Blomhoff R, Jacobs Jr D (2004). Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Rad. Res.* 38(6):535-539.
- Lee DH, Ha MH, Kim JR, Gross M, Jacobs DR (2002). Gamma-glutamyltransferase, alcohol, and blood pressure: a four year follow up study. *Annual Epidemiol.* 12:90-96.
- Ibrahim NK (2013). Possible Protective Effect of Kombucha Tea Ferment on Cadmium Chloride Induced Liver and Kidney Damage in Irradiated Rats. *Int. J. Biol. Life Sci.* 9:1.
- Ramadan LA, Shouman SA, Sayed-Ahmed MM, El-Habit OH (2001). Modulation of radiation-induced organs toxicity by cremophor-el in experimental animals. *Pharmacol. Res.* 43(2):185-191
- Makhlouf R, Makhlouf I (2012). Evaluation of the effect of Spirulina against Gamma irradiation induced oxidative stress and tissue injury in rats. *Int. J. Appl. Sci. Eng. Res.* 1(2):152-164.
- Onody A, Csonka C, Giricz Z, Ferdinandy P (2003). Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. *Cardiovasc. Res.* 58(3):663-670.
- Petersen DR, Doorn JA (2004). Reactions of 4-hydroxynonenal with proteins and cellular targets. *Free Rad. Biol. Med.* 37(7):937-945.
- Roushdy HM, El-Huseini M, Saleh F (1984). Effect of whole body gamma irradiation and/or dietary protein deficiency on the level of plasma non-protein nitrogen and amino acids, plasma and urinary ammonia and urea in desert rodent and albino rats. *Egypt J. Radiat. Sci. App.* 1(2):157-66.
- Rubin P, Constine LS, Nelson DF (1992). Late effects of cancer treatment: radiation and drug toxicity. In: Perez CA, Brady LW (eds.), *Principles and practice of radiation oncology*, 2nd edn. Lippincott, Philadelphia. pp. 124-61.
- Sener G, Jahovic N, Tosun O, Atasoy BM, Yegen B (2003). Melatonin meliorates ionizing radiation-induced oxidative organ damage in rats. *Life Sci.* 74:563-572.
- Srinivasan M, Sudheer AR, Pillail KR, Kumar PR, Sudhakaran PR, Menon VP (2006). Influence of ferulic acid on gamma-radiation induced DNA damage, lipid peroxidation and antioxidant status in primary culture of isolated rat hepatocytes. *Toxicology* 228(2-3):249-258.
- Spitz DR, Azzam EI, Li JJ, Gius D (2004). Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: A unifying concept in stress response biology. *Cancer Metast. Rev.* 23:311-322
- Vijayalaxmi RJ, Tan DX, Herman TS, Thomas CR (2004). Melatonin as a radioprotective agent: a review. *Int. J. Radiat. Oncol.* 59:639-653.
- Yousri RM, Roushdy H, Gawish MA (1991). Changes in some blood lipid fractions in whole body irradiated rats as influenced by some radioprotectors. *Isotopenpraxis* 27(3):117-123.
- Zwirska-Korczała K, Jochem J, Rybus-Kalinowska B, Polaniak R, Birkner E (2003). Assessment of blood superoxide dismutase, glutathione peroxidase activity and malondialdehyde concentration as oxidation status parameters in obese women. *Poly. Arch. Med.* Wewn 110(1):725-731.

Full Length Research Paper

## Assessment of a modified release verapamil hydrochloride (HCl) matrix compacts: Effect of formulation composition on the *in vitro* release kinetic

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Verapamil hydrochloride is a drug used to treat arrhythmias. In this study, a D-optimal mixture design with 16 runs was used to select the best combination of three polymers into a matrix that rendered an 8 h *in vitro* release profile of verapamil hydrochloride (HCl) which fulfill a once-a-day modified release in compliance with the United States Pharmacopeia (USP) specifications. The Korsmeyer-Peppas model was used to fit the dissolution data since it presented the best fitting characteristics. A cubic model predicted the best formulation of verapamil.HCl containing carnauba wax, hydroxypropyl methyl cellulose and Avicel<sub>PH101</sub> at the 40, 20 and 40% levels, respectively. Validation runs confirmed the accuracy of the cubic models which include these three components.

**Key words:** Verapamil, controlled-release preparations, non-linear models, experimental design.

### INTRODUCTION

The number of United States (US) adults with some form of heart disease is expected to increase to 110 million by 2030. This is translated in a 37.3 and 9.3% of adults with hypertension and coronary disease, respectively. Obese people with a sedentary lifestyle are the most vulnerable group to acquire these diseases (Mathers and Loncar, 2006). Conventionally, verapamil.HCl (V.HCl), administered in the form of an immediate release dosage form has been the main drug used for the treatment of heart disease, especially that related with supraventricular tachyarrhythmias. On the other hand, V.HCl suffers from a first pass effect, has a short biological half-life (4 to 6 h), a low bioavailability, and requires a high frequency of administration (3 times per day) to maintain effective plasma levels. As a result, the incidence of side effects such as constipation, dizziness and headache is boosted, leading to patient non-compliance and hence, therapeutic

ineffectiveness (Deshmane et al., 2009).

In order to solve these problems, modified release dosage forms have been attempted to provide a lower but controlled drug concentration. In one study, chitosan was employed to create a 6 h-release gastro-retentive beads, but the drug loading efficiency was only 42% (Yassin et al., 2006). A different approach employed melt granulation of synthetic waxy materials such as glyceryl monostearate and stearic acid to achieve an 8 h sustained release matrix. However, the resulting compacts were difficult to swallow (Bhagwat et al., 2008). Further, transdermal drug delivery systems have been attempted with hydroxypropyl methylcellulose (HPMC), but large drug loads and high pHs were needed to enhance drug release and absorption through the skin (Ramírez and Villafuerte, 2004). Likewise, a 6 h sustained released buccal patch with chitosan and PVP K-30 has been reported

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K-30 has been reported. Nonetheless, this dosage form could be uncomfortable in the mouth for an extended period of time. Further, a 12 h matrix compacts made of carbopol and Eudragit NE30D has been reported. However the oral administration of carbopol is questionable (Elkhesheh, 2001; Khamanga and Walker, 2011).

A modified release of a drug could be achieved through the development of a matrix composed of a polymer and other excipients homogeneously distributed in a three dimensional network. Soluble active ingredients such as V.HCl when released are first wet, dissolved and disseminated in the matrix, whereas insoluble excipients remain in place until the surrounding matrix is eroded or dissolved (Chouldhury et al., 2008). Other factors affecting the release kinetics are the polymer swelling coupled with chain relaxation, erosion, drug dissolution/diffusion characteristics, homogeneity of the drug within the polymer matrix and geometry of the system (Wu and Zhou, 1998). However, the occurrence of multicomponent transport processes, composition, device geometries, drug loading and solubility in the matrix make the release mechanism more complex.

Currently, mixture designs are widely used to analyze a set of formulation components to render the desirable response. Mixture factors stand for the amounts of ingredients in a mixture. They are part of a formulation and add up to a value of 100%. In some cases, a D-optimal mixture design is employed if the components are used in a short range, giving a small number of experimental runs. Replication is used to measure the experimental error and it is usually done on the center point run. These runs are also used for curvature checking and error variance estimation (Montgomery, 2012).

In this study, V.HCl was selected as a model drug for designing a modified release matrix. This formulation was composed of carnauba wax, HPMC and microcrystalline cellulose (Avicel\_PH101) prepared by direct compression. These components were crucial to achieve a combined hydrophilic and lipophilic mechanism. Preliminary studies with the pure components failed to render an 8 h modified release. The resulting dissolution profiles and release kinetics of the matrices were also evaluated.

## EXPERIMENTALS

Verapamil Hydrochloride (lot YR3110) was donated from ECAR laboratories. Avicel\_PH101 (particle size, 43.5 µm; lot 1430) was obtained from FMC BioPolymers. Hydroxymethylpropylcellulose (HPMC Type 2919 with methoxyl content of 28 to 30% and hydroxypropyl content of 7 to 12%, lot 506825) was obtained from Dow Wolff Cellulosics, and Carnauba Wax lot 07110 was purchased from Carnauba do Brazil. Monobasic potassium phosphate (lot 8N117059B) and concentrated hydrochloric acid (37%, lot 2612KLHV) were purchased from Carlo Erba and Mallinckrodt Specialty Chemicals Co., respectively.

## D-optimal mixture design

A D-optimal mixture design with 3 components (Carnauba wax, HPMC and Avicel\_PH101) and 16 runs was employed. The selected dependent variables were release rate (k) and release order (n). The non-linear fitting model was conducted using the software Statgraphics (StatPoint, Inc. Warrenton, VA). The coefficients of the model estimated the variation in the experimental parameters. The results were analyzed by performing an analysis of the coefficients of the various polymers, and by the analysis of variance (ANOVA). Preliminary fitting of the data to the Higuchi, Hixson-Crowell, Baker and Lonsdale, Jorgensen and Christensen and Korsmeyer-Peppas (KP) determined the latter as the most suitable release model based on the comparison of the relevant correlation coefficients and thus, only the results and discussion from this model were included in this study. Restrictions of the components were set up from preliminary studies in order to obtain an 8 h release of V.HCl. These constraints ranged from 0.3 to 0.6 for carnauba wax and Avicel\_PH101 and from 0.1 to 0.4 for HPMC, respectively.

## Preparation of matrix compacts

Dry powder mixtures of ~10 g each were prepared on a mortar and pestle (Table 1). Powder mixtures were then passed through a 250 µm sieve. Cylindrical compacts, each weighing ~1,240 mg were then made on a single punch tablet press (060804 Compac, Indemec, Itagüí, Columbia) using a 13 mm cylindrical punches and die set at a pressure of 150 MPa at a dwell time of 1 s. The porosity of the resulting compacts was ~20%. The upper punch was equipped with a load cell (LCGD-10K, Omega Engineering, Inc., Stamford, CT) and a strain gauge meter (DPiS8-EI, Omega Engineering, Inc., Stamford, CT).

## In vitro dissolution studies

Matrixes containing 240 mg of V.HCl were agitated at 50 rpm in a 900 ml dissolution type II apparatus (DT6-K, Erweka GmbH, Milford, CT) at 37°C. The first treatment employed a simulated gastric fluid (0.01 N) for 1 h, followed by treatment with a simulated intestinal fluid (pH of 6.8) for 7 h according to the USP specifications. Five milliliter aliquots were withdrawn periodically and immediately replaced by a fresh dissolution medium. Aliquots were then filtrated through a 0.45 µm filter and diluted using a dilution factor of 1/10. Samples were analyzed by ultra violet (UV) spectroscopy (HACH DR500, HACXH Company, Loveland, CO) at 280 nm. The V.HCl concentration was determined by interpolation from a calibration curve built using 10, 20, 30 40 and 80 µg/ml concentrations. The uniformity of content was determined on three individual compacts and analyzed for drug content as described.

## Dissolution profile models

The release models of Higuchi, Hixson and Crowell, Baker and Korsmeyer-Peppas were used to analyze the *in vitro* data to find the model that best represents the data. The Korsmeyer-Peppas model was employed due to the best fit to the experimental data:

$$F = k \cdot t^n \quad (1)$$

Where F is the fraction of drug released within the range of 0.1 to 0.60 at a time t, k is the release rate constant which incorporates structural and geometric factors. On the other hand, n is the exponent

**Table 1.** D-Optimal mixture design matrix.

Batch	CW	HPMC	AV	V.HCl	UC (%)
F1	0.45	0.1	0.45	0.24	101.2±1.2
F2	0.45	0.25	0.3	0.24	104.2±0.8
F3	0.6	0.1	0.3	0.24	100.8±2.1
F4	0.3	0.1	0.6	0.24	98.9±1.8
F5	0.4	0.3	0.3	0.24	100.7±2.4
F6	0.45	0.1	0.45	0.24	102.3±1.2
F7	0.3	0.1	0.6	0.24	101.6±0.9
F8	0.6	0.1	0.3	0.24	103.6±1.3
F9	0.4	0.2	0.4	0.24	104.2±0.9
F10	0.3	0.25	0.45	0.24	100.9±2.4
F11	0.3	0.1	0.6	0.24	99.1±3.3
F12	0.35	0.15	0.5	0.24	98.8±1.9
F13	0.5	0.2	0.3	0.24	102.1±1.1
F14	0.3	0.3	0.4	0.24	101.6±2.1
F15	0.3	0.4	0.3	0.24	101.3±1.8
F16	0.3	0.4	0.3	0.24	100.5±2.3

CW = carnauba wax; HPMC = hydroxypropylmethylcellulose, AV = avicel\_PH101, V.HCl = verapamil.HCl; UC = uniformity of content.

exponent that characterizes the process of drug transport mechanism. If "n" is equal to 0.45;  $0.45 < n < 0.89$ ; and  $>0.89$  the diffusion process is Fickian, non-fickian, and zero order, respectively for a cylindrical matrix (Ritger and Peppas, 1987). The release mechanism is given once the polymer chains contact the solvent and the chains are reoriented to achieve a new equilibrium condition (Siepmann and Peppas, 2001). The time required for this reorganization is called polymer relaxation time ( $t_r$ ). If  $t_r$  is much smaller than the diffusion time ( $t_d$ ) required for the release, the process is then Fickian. When  $t_r \cong t_d$ , solvent absorption is non-

Fickian or anomalous (Grassi et al., 1998).

### Validation of the model

A cubic model including interaction terms were generated for "k" and "n" using the multiple linear regression analysis obtained from the Design Expert software (Stat-Ease, Inc, Minneapolis, MN). The statistical validity of the model was established on the basis of the ANOVA test. Further, the grid search from the contour plots was used to find the optimal formulation composition. Two check points were also selected to validate the model. Subsequently, the experimental data of the check points were quantitatively compared with that of the predicted values.

## RESULTS

Table 1 lists the experimental matrix composed of three independent factors and the uniformity of content of each batch. Further, Table 2 shows the parameters and correlation coefficient of all the release models used. The Korsmeyer-Peppas (KP) model was selected for the release analysis since in most cases it rendered the best fit to the experimental data. Table 3 shows the analysis of variance for the two responses (release rate and release order) obtained from the KP model. The first column present the terms of the fitted cubic models. The p-values determined the cubic models as significant, indicating that the relative composition of all factors had a strong effect on the two responses. The goodness of fit statistic of the mixture design was examined by the coefficient of determination ( $r^2$ ), which indicates how much variation in the responses was explained by the cubic models. The higher the  $r^2$ , the better the model fits the data. The fitted cubic models for the release rate and release order are:

$$\begin{aligned} \ln(k) = & +1159.6 * CW - 670.3 * HPMC - 899.8 * AV - 1128.9 * CW * HPMC - 460.6 * CW * AV \\ & + 2255.3 * HPMC * AV + 1648.4 * CW * HPMC * AV - 341.6 * CW * HPMC * (CW - HPMC) - \\ & 5247.9 * CW * AV * (CW - AV) + 2468.0 * HPMC * AV * (HPMC - AV) \\ n = & -181.6 * CW + 120.9 * HPMC + 139.6 * AV + 139.3 * CW * HPMC + 72.4 * CW * AV - 373.6 * HPMC \\ & * AV - 221.1 * CW * HPMC * AV + 92.0 * CW * HPMC * (CW - HPMC) + 815.6 * CW * AV * (CW - AV) \\ & - 395.7 * HPMC * AV * (HPMC - AV) \end{aligned}$$

Figure 1a and b shows the contour plots for the release rate and release order, respectively. The lowest values are depicted with a blue color font and the highest values with a red color font, respectively. In general, HPMC and Avicel\_PH101 levels between 0.3 and 0.4 rendered high values of release rates. Likewise, high levels of Avicel\_PH101 ( $> 0.5$ ) rendered high release rates. Conversely, formulations that possessed a large level of

carnauba wax such as F1, F2, F3, F5, and F6 exhibited the slowest release rates. The optimal range of the desired 8 h drug release rate is depicted in the contour plot in green color and ranged between 0.0006 and 0.0054. This area is composed of HPMC, carnauba wax and Avicel\_PH101 at ranges of 0.3 to 0.4, 0.4 to 0.5 and 0.35 to 0.4, respectively.

Surprisingly, the regions of the contour plot that showed

**Table 2.** Parameters and correlation coefficients obtained from the different release models.

Batch	Higuchi model		Hixon-Crowell model		Baker model		Korshmeier-Peppas model		
	$k_H$	$r$	$k_{HC}$	$r$	$k_B$	$r$	$k_{kp}$	$n$	$r$
F1	0.024	0.9403	0.0004	0.8644	0.0001	0.9558	0.04	0.483	0.953
F2	0.0144	0.8403	0.0002	0.7179	$3 \times 10^{-5}$	0.7642	0.035	0.338	0.932
F3	0.0156	0.4237	0.0001	0.4971	0.00003	0.9441	0.076	0.216	0.883
F4	0.0271	0.9814	0.0005	0.9442	0.0002	0.9854	0.025	0.515	0.982
F5	0.0153	0.7256	0.0002	0.7145	$4 \times 10^{-5}$	0.7084	0.072	0.233	0.966
F6	0.0185	0.9786	0.0003	0.9452	$7 \times 10^{-5}$	0.9759	0.02	0.486	0.979
F7	0.032	0.9757	0.0007	0.9857	0.0003	0.965	0.017	0.614	0.991
F8	0.0403	0.9334	0.001	0.9231	0.0004	0.978	0.035	0.524	0.934
F9	0.0292	0.8628	0.0008	0.9030	0.0004	0.978	0.003	0.921	0.956
F10	0.0199	0.6579	0.0005	0.7662	0.0001	0.5829	$3 \times 10^{-6}$	1.996	0.865
F11	0.0528	0.7699	0.0014	0.7642	0.001	0.8234	0.065	0.461	0.804
F12	0.0528	0.8012	0.0015	0.7860	0.001	0.814	0.071	0.446	0.775
F13	0.0402	0.9192	0.001	0.9529	0.0005	0.9681	0.021	0.616	0.939
F14	0.0221	0.7894	0.0005	0.9098	0.0001	0.797	0.001	0.996	0.913
F15	0.013	0.8214	0.0001	0.8418	$3 \times 10^{-5}$	0.9113	0.04	0.299	0.982
F16	0.0122	0.9197	0.0001	0.8590	$3 \times 10^{-5}$	0.9441	0.029	0.348	0.987

$k_H, k_{HC}, k_B$  and  $k_{kp}$  corresponds to release constants of the different models and  $n$  is the release order of the Korshmeier-Peppas model

showed a high release rate also exhibited non-Fickian release orders ( $< 0.4$ ). On the contrary, the F11 and F12 experimental batches composed of  $\sim 0.3$ , 0.1 and 0.6 of carnauba wax, HPMC and Avicel PH<sub>101</sub>, respectively exhibited a Fickian-like release with an “ $n$ ” value of  $\sim 0.45$  (Figure 2). The optimal range of an 8 h modified release formulation is shown in Figure 3. F9 was the only formulation that falls under the specifications. It is composed of 0.4, 0.2 and 0.4 levels of carnauba wax, HPMC and Avicel PH<sub>101</sub>, respectively.

## DISCUSSION

Batches that had a high HPMC and low carnauba wax composition presented a high swelling. On the other hand, matrices with a low HPMC loads and high Avicel PH<sub>101</sub> levels showed more erosion. Swelling is caused by the overlap of the polymer chains and intermolecular entanglement leading to a three dimension (3D) network structure. As a result, the mobility of polymer chains is reduced, leading to an increase of viscosity of the polymeric matrix. On the other hand, batches containing a high level of carnauba wax did not show swelling, but partial erosion.

The multiple regression coefficients indicate that 98.3 and 97.6% of the experimental variance for “ $k$ ” and “ $n$ ,” respectively were explained by the fitted cubic models. The other variability (1.7 and 2.4%, respectively) was due to a random error as demonstrated by the lack of fit test.

The latter evaluates whether the variation due to the lack of fit of the model is small enough to be accepted as a negligible portion of the pure error. In this case, the null hypothesis established the lack of fit error as zero. These results show that the experimental variations observed to the release rate and release order could be attributed to randomized errors.

Formulations with a high composition of Avicel<sub>PH101</sub> ( $> 0.5$ ) rendered high release rates. This is explained by its high hydrophilicity. On the other hand, batches containing high levels of HPMC in the matrix system increased the swelling tendency and hence, increased the rapid V.HCl release. Further, a large amount of Avicel<sub>PH101</sub> and a low level of HPMC made the matrix more erodible and hence, increased the release rate. In most cases, high values of carnauba wax and low values of HPMC made hydrophobicity more prevalent than swelling, resulting in a decrease of drug release rate. The optimal range of the desired 8 h drug release rate is depicted in the contour plot in green color and ranged between 0.0006 and 0.0054. This area is composed of HPMC, carnauba wax and Avicel<sub>PH101</sub> at ranges of 0.3 to 0.4, 0.4 to 0.5 and 0.35 to 0.4, respectively. In this area, a combination of the swelling and erosion mechanisms was predominant. Most batches had a release order different from 0.5 for indicating that a non-Fickian release kinetics predominated and the drug release mechanism involved a combination of erosion and matrix swelling by water filled pores (polymer chain-relaxation). Thus, results showed that swelling and erosion phenomena

**Table 3.** ANOVA table of the release rate (k) and release order (n).

Source	Sum of Squares	DF	Mean square	F-value	p-value ( $\alpha=0.05$ )
Model	92.15	9	10.24	39.37	0.0001
Linear mixture	10.01	2	5.01	19.25	0.0025
AB	0.021	1	0.021	0.081	0.7859
AC	0.16	1	0.16	0.60	0.4683
BC	69.69	1	69.69	267.97	<0.0001
ABC	1.95	1	1.95	7.50	0.0338
AB(A-B)	0.88	1	0.88	3.40	0.1147
AC(A-C)	23.36	8	23.36	89.83	<0.0001
BC(B-C)	13.40	3	13.40	51.51	0.0004
Residual	1.56	6	0.26	-	-
Lack of Fit	$3.991 \times 10^{-3}$	1	$3.991 \times 10^{-3}$	0.013	0.9143
Pure error	1.56	5	0.31	-	-
SD=0.51	$r^2$	0.9833	-	-	-
<b>Release order (n)</b>					
Model	2.73	9	0.30	27.59	0.0003
Linear mixture	0.27	2	0.14	12.47	0.0073
AB	$3.429 \times 10^{-3}$	1	$3.429 \times 10^{-3}$	0.31	0.5967
AC	$1.574 \times 10^{-3}$	1	$1.574 \times 10^{-3}$	0.14	0.7181
BC	2.04	1	2.04	185.17	<0.0001
ABC	0.04	1	0.04	3.19	0.1242
AB(A-B)	0.064	1	0.064	5.84	0.0522
AC(A-C)	0.56	1	0.56	51.32	0.0004
BC(B-C)	0.34	1	0.34	31.32	0.0014
Residual	0.066	6	0.011	0.62	0.5750
Lack of Fit	$5.650 \times 10^{-3}$	1	$5.650 \times 10^{-3}$	0.47	0.5242
Pure error	0.060	5	0.012	-	-
SD=0.10	$r^2$	0.9764	-	-	-

A = carnauba wax; B = HPMC; C = Avicel\_PH101; DF = degrees of freedom.

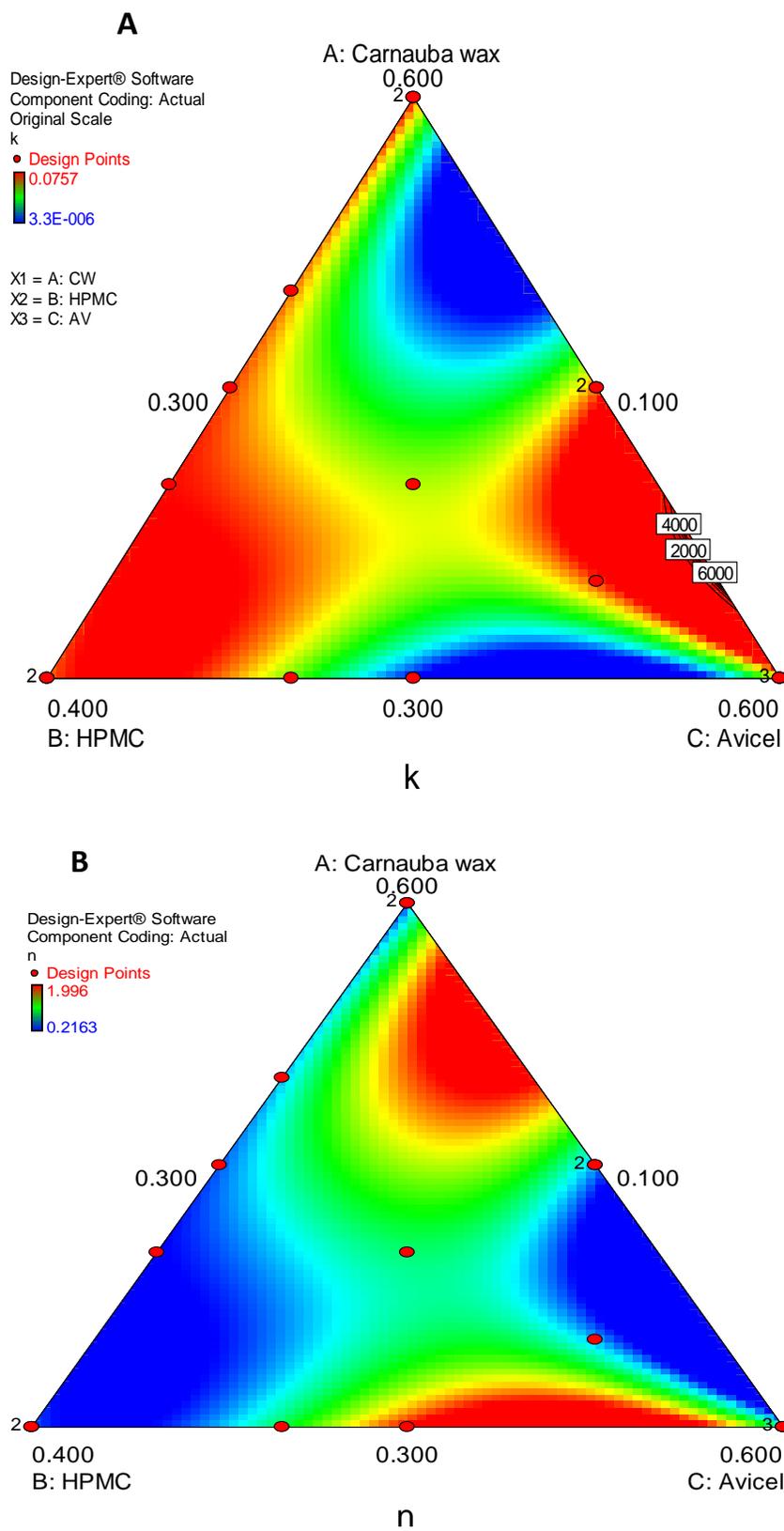
dictated the kinetics and mechanism of drug release from modified release formulations, but one process predominated over the other because of different excipient characteristics.

Formulations that showed a high release rate also exhibited low release orders (< 0.4), indicating a fast disruption of the eroding excipient matrix leading to a non-Fickian release profile. Batches F11 and F12 presented a rapid release in the intestinal medium due to the high water wicking power of Avicel\_PH101 (Figure 2). This is due to the large formation of hydrogen bonds of cellulose upon water contact which counteracted the hydrophobic and swelling effect of carnauba wax and HPMC, respectively. The grid search within the contour plots was employed to select the optimal region that presented an “n” value between 0.86 and 1.19 and is shown in green color. This area also matches the region of Figure 1a which had the optimal values of release rate. This region was composed of HPMC (0.3 to 0.4), carnauba

wax (0.4 to 0.5) and Avicel (0.35 to 0.4). Matrices produced in this study are considered porous systems and thus, drug release also depended on the drug dissolution/diffusion rate through the pores. Since drug loading was constant (240 mg), polymer swelling also affected the drug release kinetics.

The *in vitro* dissolution profiles shown in Figure 2 simulate the gastrointestinal transit of the drug at a pH of 1 for 1 h followed by treatment at pH of 6.8 for 7 h. It is evident that the drug release increased with time, but a larger dissolution was achieved mainly at a pH of 6.8 due to the larger residence time. The increase in dissolution rate with increasing pH could be attributed to the ions of the buffer which enhanced the hydration properties of the drug. The hydrodynamic particle consists of the solute and solvent molecules bound or adsorbed to the surface. The molecule transport takes place through interstices of polymer chains filled with the solvent.

Formulations F7 and F9 fall within the desirable limits



**Figure 1.** Contour plot for (A) the “k” release rate and (B) the “n” release order.

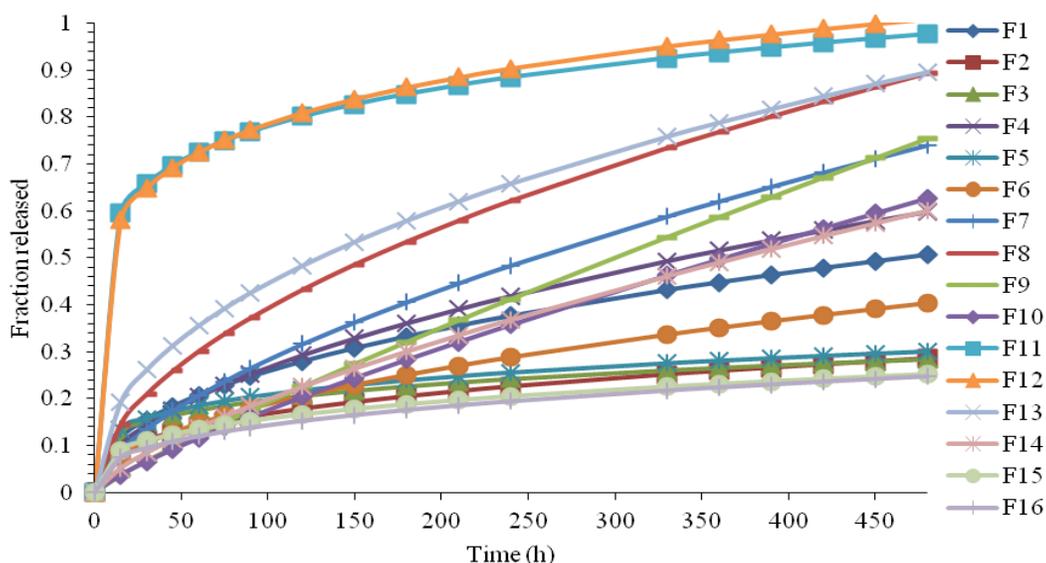


Figure 2. Release profiles obtained from the 16 formulations of the mixture design.

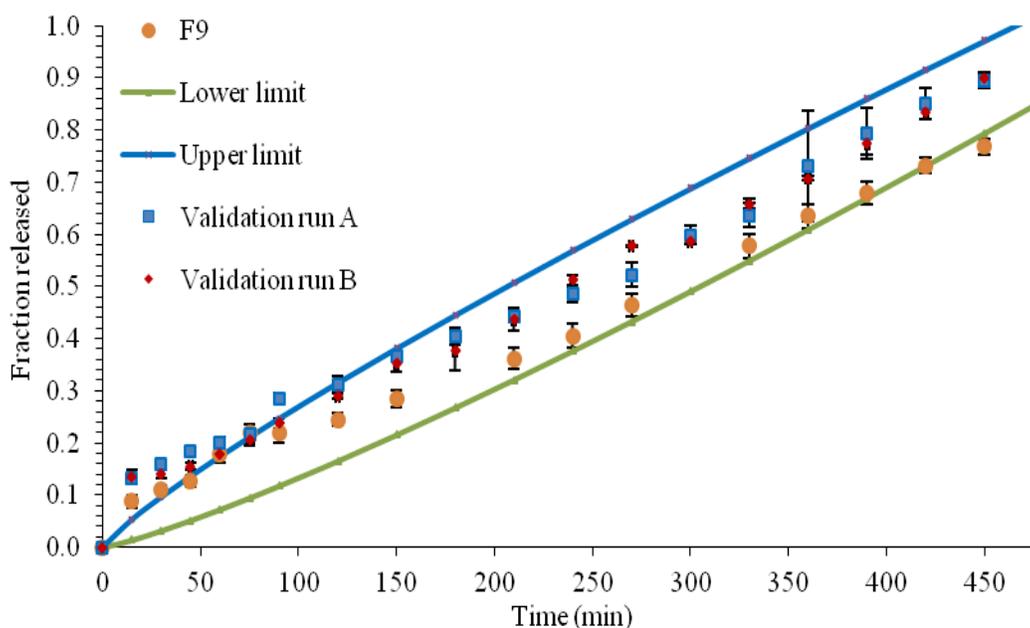


Figure 3. Validation runs of formulation 9 compared to the limits established by the USP.

for an optimal 8 h release profile. However, the cubic models presented F9 as the most optimal formulation composed of carnauba wax, HPMC and Avicel\_PH101 at the 0.4, 0.2 and 0.4 levels, respectively. Two more experimental runs of a formulation with this composition were conducted to assess the validity of the cubic models and results are depicted in Figure 3. This composition fall within the USP specifications and hence, was selected to

achieve the desired 8 h release profile of V.HCl.

**ACKNOWLEDGEMENTS**

Our sincere gratitude to The Drug and Food Research Institute of the University of Antioquia for providing the resources needed to carry out this project.

## REFERENCES

- Bhagwat DA, Kawtikwar PS, Sakarkar DM (2008). Sustained release matrices of verapamil HCl using glyceryl monostearate and stearic acid. *Res. J. Pharm. Tech.* 1:405-409.
- Chouldhury PK, Kar M, Chauhan CS (2008). Cellulose acetate microspheres as a floating depot system to increase gastric retention of antidiabetic drug: formulation, characterization and *in vitro- in vivo* evaluation. *Drug Dev. Ind. Pharm.* 34:349-354.
- Deshmane SV, Channawar MA, Chandewar AV, Josh UM (2009). Chitosan based sustained release mucoadhesive buccal patches containing verapamil HCl. *Int. J. Pharm. Pharm. Sci.* 1:216-239.
- Elkhesheh A (2001). Interaction of verapamil hydrochloride with carbopol 934P and its effect on the release rate of the drug and the water uptake of the polymer matrix. *Pharm. Dev. Ind. Pharm.* 27:925-934.
- Khamanga SD, Walker RB (2011). Drug transport mechanisms from carbopol/Eudragit verapamil sustained-release tablets. *Dissol. Technol.* 1:30-38.
- Mathers CD, Loncar D (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* 3:e442.
- Montgomery DC (2012). *Design and Analysis of Experiments*, 7th Edition. Wiley, NY. 680p
- Ramírez M, Villafuerte L (2004). Effect of formulation variables on verapamil hydrochloride release from hydrated HPMC matrices. *Rev. Soc. Quím. Méx.* 48:326-331.
- Ritger PL, Peppas NA (1987). A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *J. Control Release* 5:37-42.
- Siepmann J, Peppas NA (2001) Modeling of drug release from delivery systems based on hydroxypropylmethylcellulose (HPMC). *Adv. Drug Rev.* 48:139-157.
- Wu XY, Zhou Y (1998). Finite element analysis of diffusional drug release from complex matrix systems II. Factors influencing release kinetics. *J. Control Release* 51:57-71.
- Yassin AE, Alsarra IA, Al-Mohizea AM (2006). Chitosan beads as a new gastroretentive system of verapamil. *Sci. Pharm.* 74:175-188.

Full Length Research Paper

## Effect of dose on disposition kinetics of isometamidium chloride/hydrochloride in trypanosomiasis induced calves

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Effect of dose on disposition kinetics of isometamidium (ISMM) in Indian buffalo calves was studied in view of limited applications of the drug in India. Buffalo calves were pre grouped and trypanosomiasis was induced using *Trypanosoma evansi*. ISMM was administered intravenously at 0.25, 0.5 and 1 mg/kg (body weight) to the grouped animals. Blood samples were collected at 0.08, 0.16, 0.33, 0.66, 1, 2, 4, 6, 8, 12, 24 and 36 h post drug administration for disposition kinetics. ISMM chloride/hydrochloride in plasma was estimated by trial and error method using high performance liquid chromatography (HPLC). The time to reach the maximum plasma concentration ( $T_{max}$ ), was 0.08 h for all the doses. The maximum plasma concentration ( $C_{max}$ ) declined rapidly and the drug could not be detected in plasma samples collected beyond 8 h post dose of 0.25 mg/kg and 24 h post dose of the other two doses, that is, 0.5 and 1 mg/kg. The distribution rate constant ( $\alpha$ ) in groups I (0.25 mg/kg), II (0.5 mg/kg) and III (1 mg/kg) were  $4.77 \pm 1.54$ ,  $7.44 \pm 0.55$  and  $6.91 \pm 2.57 \text{ h}^{-1}$ , respectively, while  $t_{1/2\beta}$  values were  $2.01 \pm 0.16$ ,  $3.09 \pm 0.30$  and  $2.46 \pm 0.16 \text{ h}$ , respectively. The apparent volume of distribution like  $V_{d_{area}}$ ,  $V_{d_c}$  and  $V_{d_B}$  were  $0.50 \pm 0.01$ ,  $0.69 \pm 0.06$  and  $0.03 \pm 0.005 \text{ L/kg}$  in group I;  $0.35 \pm 0.02$ ,  $1.98 \pm 0.02$  and  $0.07 \pm 0.005 \text{ L/kg}$  in group II;  $0.26 \pm 0.01$ ,  $3.88 \pm 0.49$  and  $0.035 \pm 0.003 \text{ L/kg}$  in group III, respectively. The value of  $K_{12}$  and  $K_{21}$  were  $1.97 \pm 0.83$  and  $0.67 \pm 0.07 \text{ h}^{-1}$  in group I;  $4.92 \pm 0.45$  and  $1.60 \pm 0.07 \text{ h}^{-1}$  in group II;  $4.12 \pm 0.21$  and  $0.89 \pm 0.39 \text{ h}^{-1}$  in group III, respectively. It was concluded that ISMM follows dose independent kinetics after intravenous administration in buffalo calves.

**Key words:** Disposition kinetics, isometamidium chloride/hydrochloride, trypanosomiasis, intravenous, calves.

### INTRODUCTION

“Trypanosomiasis,” is one of the most widely distributed pathogenic, mechanically transmitted vector borne haemoprotozoan (*Trypanosoma evansi*) disease of domestic and wild animals in India (Juyal, 2011). It leads to severe anaemia, oedema, immunosuppression and various neurological disorders causing huge economic losses to the farmers in terms of morbidity, mortality, abortion, infertility, reduced milk yield, etc (Juyal, 2011). The economic losses due to disease are underestimated

in cattle and buffaloes mainly because of its sub-clinical nature. Chemotherapy and chemoprophylaxis has been the mainstay of control in domestic animals in India. Currently, drugs in practice, in India include diaminazene aceturate (berenil), quinapyramine sulphate and chloride (Triquin, Antrycide Prosalt) and quinapyramine sulphate (Triquin-S and Antrycide) for treatment and prophylactic use against trypanosomiasis in domestic animals (Juyal, 2011). However, this approach has also been associated

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with major problems such as high price of drugs, availability of few drugs and the development of drug resistance.

Isometamidium (ISMM) chloride/hydrochloride, synthesized by combining diazotized Para-Amino Benzamide moiety of diaminazene with homidium in the presence of sodium acetate (Kinabo et al., 1988) has been used for chemotherapy and chemoprophylaxis of disease in cattle, sheep, and goats under conditions of natural tsetse challenge for more than 35 years (Geerts and Holmes, 1998; Anene et al., 2001) in countries other than India. Mode of action of ISMM is not fully understood, but evidence is there that kinetoplastic topoisomerase type II of trypanosoma is selectively inhibited by the drug (Kaminsky et al., 1997; Boibessot et al., 2002; Mehlhorn, 2008). The claimed duration of protection afforded by ISMM is as long as 5 months (Whitelaw et al., 1991; Mehlhorn, 2008). Use of ISMM as remedial measure against trypanosomiasis has been cited (Magona et al., 2004; Delespaux and de Koning, 2010; Karaye, 2012) and disposition kinetics have been studied in cow (Dowler et al., 1989; Moloo and Kutuza, 1990), goats and sheep (Braide and Eghianruwa, 1980; Wesongah et al., 2000) and camel (Ali and Hassan, 1984). To determine the dosage regimen in buffalo and thereby its use against trypanosomiasis in buffalo, disposition kinetics of administered ISMM is essentially required. Keeping these points in view, the present study was undertaken to determine the disposition kinetics of intravenously administered ISMM in buffalo calves.

## MATERIALS AND METHODS

### Test drug and chemicals

ISMM chloride/hydrochloride, a trypanocidal drug (technical grade) was procured from M/S Alembic Ltd., Veterinary Division, Mumbai (India). The purity of the compound was >90%. All the other chemicals used in this experiment were obtained from E. Merck (India) and Sigma Chemicals Co. (USA).

### Experimental animals

Clinically healthy calves (6 months age) weighing between 70 and 90 kg were used in this experiment. They were kept in animal room of clinical complex at Mohanpur campus. The animals were stall fed and were also allowed to graze. Water was provided *ad-libitum*. The composition of feed was 3 parts paddy straw, 1 part mustard cake and 1 part wheat husk. Before starting the experiment, the animals were dewormed with Fluzan® (suspension of Oxytoclozanide 3% w/v and levamisole hydro-chloride, 1.5% w/v) at 10 mg/kg body weight. Animals were also dewormed against cestode with Cestophane® (Dichlorophan) at 0.5 g/2.5 kg body weight orally after 7 days of Fluzan® administration. After 21 days of deworming, the animals were acclimatized in experimental environment for 7 days.

### Experimental grouping of animals

Eighteen calves of both sexes were grouped into 3, each containing

6 animals. ISMM was administered as a single intravenous dose at 0.25, 0.5 and 1 mg/kg (as 1% solution in normal saline) to animals of Groups I, II and III respectively.

### Induction of infection (trypanosomiasis)

Strain of *Trypanosoma evansi* was brought from Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, U.P and the strain was serially passaged in mice in the Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Science. About 1 ml of heart blood was taken from mice and was mixed with equal volume of Alsevers solution. The mixed solution (2 ml) was administered in the calves subcutaneously.

### Confirmation of trypanosomiasis

Blood (0.2 ml) from each infected calf was injected in mice intraperitoneally. Wet blood film of mice was examined at every 12 h interval under microscope and *Trypanosoma* was visible in the film after 48 h post induction.

### Development of signs and symptoms

After 28 days of induction of infection, different signs and symptoms were observed in calves like posterior paresis, rise in temperature (103.6 to 104.8°F), anorexia, dullness and depression, urticarial patches with hemorrhage in some calves and staggering gait.

### Collection of blood samples

Blood samples were collected from jugular vein of calves in Groups I, II and III in test tubes containing ethylenediaminetetraacetic acid (EDTA; 99.5%) at 0.08, 0.16, 0.33, 0.66, 1, 2, 4, 6, 8, 12, 24 and 36 h. About 4 ml of blood was collected at the aforementioned time period. Plasma was then separated by centrifugation at 3000 rpm for 20 min. One milliliter of plasma was utilized for the analysis of ISMM concentration.

### Analysis of ISMM in blood

Analysis of ISMM in blood method was developed by trial and error. About 3 ml of blood was collected from the jugular vein of the calf and was centrifuged at 3000 rpm for 20 min. Plasma (1 ml) was separated and 3 ml of acetonitrile was added to it and was well mixed. Then, the test tubes were allowed to centrifuge at 3500 rpm for 20 min. The supernatant was collected to another test tube. Acetonitrile (3 ml) was added in the test tubes containing sediment and was shaken well. Then, the test tubes were allowed to centrifuge at 3500 rpm for 20 min. The supernatant was collected and was added in the first supernatant. The aforementioned process was repeated for 5 times for complete extraction of the drug. The test tube containing supernatants were completely dried using vacuum evaporator at 40°C and the dried residue was dissolved in small quantity of high performance liquid chromatography (HPLC) grade water and final volume was made to 1 ml for subsequent analysis by HPLC.

### Apparatus

SHIMADZU LC-20AT liquid chromatography coupled with diode-array detector (UV/V) attached with computer SPDMXA 10 software were used.

### Condition of HPLC

Mobile phase: Phosphate buffer: Acetonitrile (77:23) (This mixture was subjected to membrane filtration); column: 5  $\mu$  Luna C<sub>18</sub> (2), 125  $\times$  4 mm LiChrospher 60 (RP); flow rate: 1 ml/min; wavelength: 320 nm; injection: (25  $\mu$ l loop with Hamilton syringe). Standard and samples (20  $\mu$ l) were injected into the injector part of liquid chromatography with the first and last being the standard.

### Calibration

A stock solution of 100  $\mu$ g/ml of technical grade of ISMM was prepared in 70 ml HPLC grade water and 30 ml HPLC grade acetonitrile.

### Buffer

For buffer, 2.7 g of potassium dihydrogen phosphate was dissolved in 950 ml of distilled water, pH was adjusted to 2.5 with orthophosphoric acid and volume was made to 1000 ml with distilled water. Three peaks were observed with retention time (RT) of ISMM at 5.81, 7.09 and 8.22 min, respectively. The ISMM concentration in blood was calculated using following equation:

$$\text{Concentration of ISMM in blood } (\mu\text{g/ml}) = \frac{a_2 \times v_2 \times c}{a_1 \times v_1}$$

where  $a_1$  = area of standard chromatogram,  $a_2$  = area of sample chromatogram,  $v_2$  = final volume of sample (ml)  $v_1$  = volume of blood taken (ml), and  $c$  = concentration of standard (ppm).

### Recovery of ISMM from plasma

Recovery of ISMM from calf plasma was carried out *in vitro* to ascertain the reliability of analytical method after fortifying with 5, 10, 20, 50 and 100  $\mu$ g/ml of ISMM in plasma. The limit of detection for ISMM was below 0.1 ppm. The linearity for different concentrations of ISMM was plotted on graph paper and linearity was found to be maintained. The recovery was 85 to 92% and therefore, analytical method was considered ideal for estimation of ISMM in this experiment.

### Pharmacokinetic parameters

Pharmacokinetic parameters of ISMM were determined from computerized curve fitting programme 'PHARMKIT' supplied by the Department of Pharmacology, JIPMER, Pondicherry, India as the following.

1) A, B: Zero time blood ISMM concentrations intercepts of biphasic intravenous disposition curve. The co-efficient A is the point of intercept of regression line of distribution phase and coefficient B is based on the terminal elimination phase. These are expressed in  $\mu$ g/ml.

2)  $C^*p$ : The theoretical zero time plasma ISMM concentration:  $C^*p$  ( $\mu$ g/ml) = A + B

3)  $\alpha$  and  $\beta$ : The hybrid rate constants of disposition curve. Values of  $\alpha$  and  $\beta$  are related to the slope of distribution and elimination curve. These rate constants are obtained from the terminal slope of semi-logarithmic plot of blood ISMM concentrations versus time and are expressed as  $h^{-1}$   $t_{1/2}$   $\alpha$  and  $t_{1/2}$   $\beta$ , the half-lives of the ISMM in distribution and elimination phase, respectively. They are expressed in hour (Baggot, 1977).

$$t_{1/2} \alpha = \frac{0.693}{\alpha}$$

$$t_{1/2} \beta = \frac{0.693}{\beta}$$

4)  $K_{12}$ : The first order rate constant for transfer of ISMM from central to peripheral compartment.

5)  $K_{21}$ : The first order rate constant for transfer of ISMM from peripheral to central compartment. Rate constants are expressed as per hour ( $h^{-1}$ ).

6)  $K_{el}$ : First order rate constant for drug elimination from the central compartment.

$$K_{21} = \frac{A \beta + B \alpha}{A + B}$$

$$K_{12} = \alpha + \beta - K_{21} - K_{el}$$

$$K_{el} = \frac{\alpha \beta}{K_{21}}$$

7)  $V_{d_c}$ : The apparent volume of distribution of ISMM in central compartment is expressed as L/kg, where D is the dose (mg/kg).

$$V_{d_c} = \frac{D}{A+B}$$

8)  $V_{d_{area}}$ : The apparent volume of ISMM distribution based on total area under blood concentration versus time area (area method) is expressed as L/kg where D is the dose (mg/kg).

$$V_{d_{area}} = \frac{D}{\text{Area} \times \beta}$$

$$= \frac{D}{(A/\alpha + B/\beta) \times \beta}$$

(Baggot, 1977)

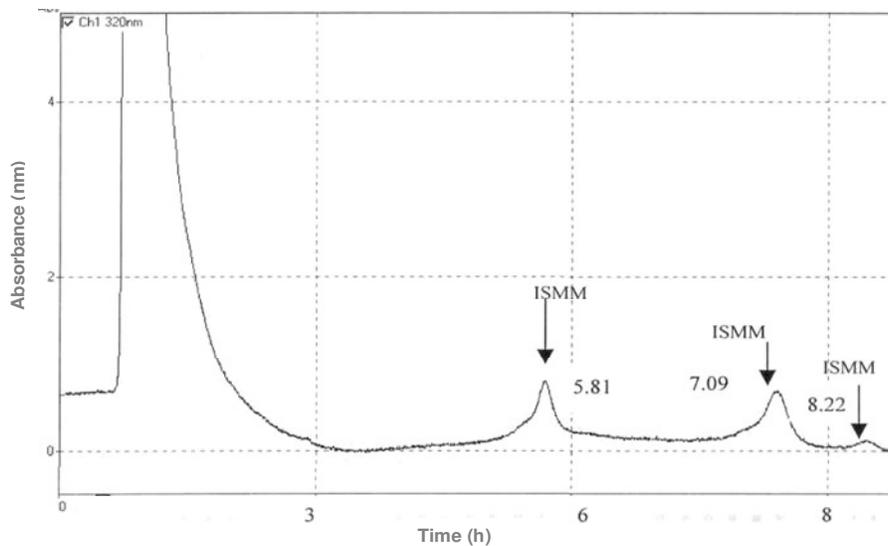
$$= \frac{D}{\text{AUC} \times \beta}$$

9)  $V_{d_B}$ : The apparent volume of drug distribution obtained by avoiding the distribution phase of drug distribution and is expressed as L/kg.

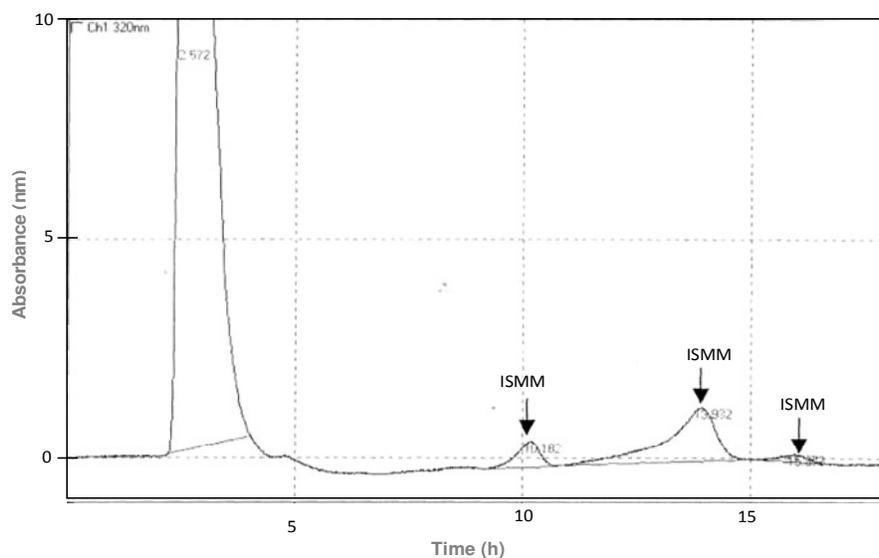
$$V_{d_B} = \frac{D}{B}$$

10)  $V_{d_{ss}}$ : Apparent volume of distribution at steady state is expressed as L/kg.

$$V_{d_{ss}} = (K_{12} + K_{21}) \times \frac{V_{d_c}}{K_{21}}$$



**Figure 1.** Chromatogram of ISMM chloride/hydrochloride, standard (100 ppm).



**Figure 2.** Chromatogram of Isometamidium chloride/hydrochloride recovered from plasma fortified with 100 ppm.

11) AUC: The total area under the blood ISMM concentration versus time curve from 't<sub>0</sub>' to 't' after administration. The unit of measurement is  $\mu\text{g}/\text{h}/\text{ml}$  (for two compartment).

$$\text{AUC} = \frac{A}{\alpha} + \frac{B}{\beta}$$

12) Cl<sub>B</sub>: The total body clearance of ISMM representing the sum of all clearance process in the body and is expressed as  $\text{Lkg}^{-1}\text{h}^{-1}$ .

$$\text{Cl}_B = Vd_{\text{area}} \times \beta$$

#### Statistical analysis of data

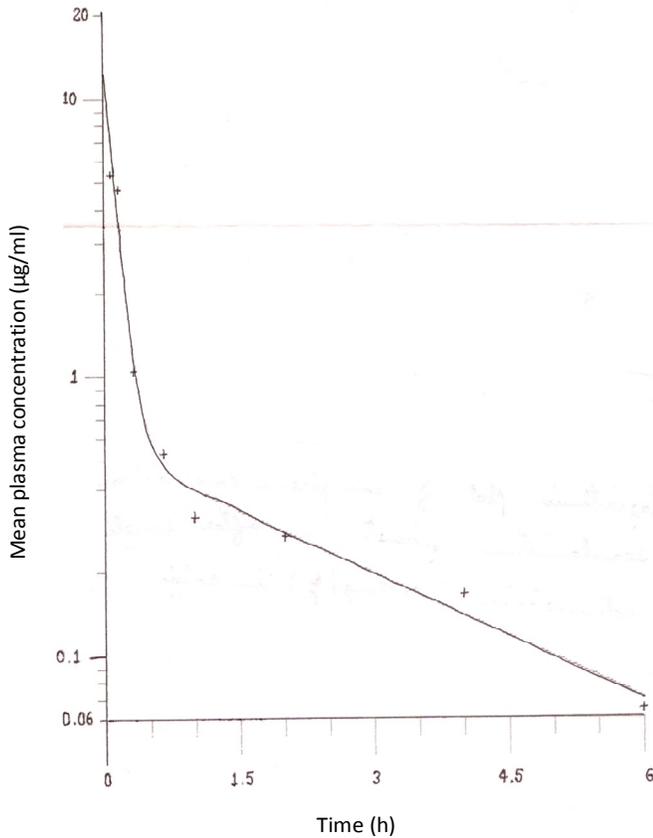
Analysis of variance (ANOVA) using SPSS (10) was used for the

analysis of data where applicable.

## RESULTS

### Recovery experiment

The chromatogram of HPLC, showed three peaks of ISMM and the retention time (RT) was found to be 5.81, 7.09 and 8.22 min under the operating conditions as described earlier (Figure 1). The recovery of ISMM from plasma varied from 85 to 92% (Figure 2). The sensitivity was found to be below 0.1 ppm.



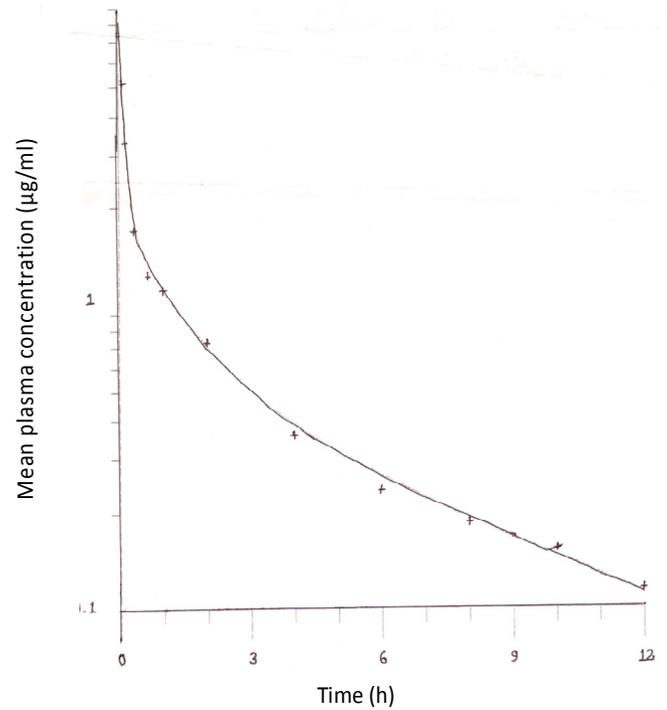
**Figure 3.** Semilogarithmic plot of mean plasma concentration of ISMM against time after single intravenous administration at the dose rate of 0.25 mg/kg body weight in calf.

**Plasma level of ISMM chloride/hydrochloride**

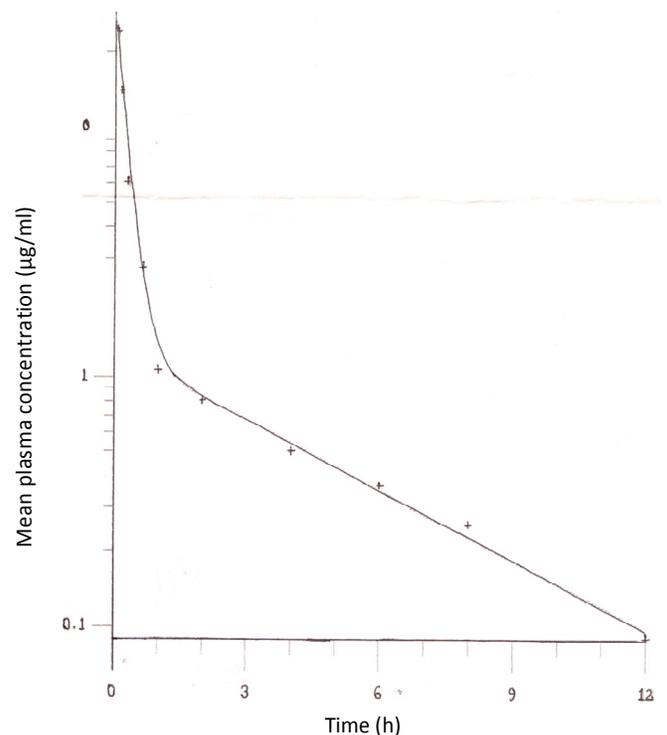
Mean values with standard error of plasma concentration of ISMM in calves at different time intervals after single dose intravenous administration at 0.25, 0.5 and 1 mg/kg are presented in Table 1 and Figures 3, 4 and 5. Maximum plasma concentration of ISMM at 0.25 mg/kg was  $5.28 \pm 0.14 \mu\text{g/ml}$  at 0.08 h and the minimum plasma concentration of  $0.07 \pm 0.008 \mu\text{g/ml}$  was recorded at 6 h post dosing (pd) (Figure 3). ISMM could not be detected in plasma collected beyond 8 h at the dose of 0.25 mg/kg. The highest plasma concentration of ISMM at 0.5 mg/kg dose level was  $9.85 \pm 0.15 \mu\text{g/ml}$  at 0.08 h and the lowest plasma concentration of  $0.10 \pm 0.01 \mu\text{g/ml}$  was found at 12 h pd (Figure 4). ISMM could not be detected in plasma collected at 24 h. Maximum plasma concentration of ISMM at 1 mg/kg was  $19.92 \pm 1.17 \mu\text{g/ml}$  at 0.08 h and minimum plasma concentration of  $0.09 \pm 0.01 \mu\text{g/ml}$  was at 12 h pd. No drug in plasma could be detected at 24 h (Figure 5).

**Kinetic profile**

Semilogarithmic plot of mean plasma level time profile of



**Figure 4.** Semilogarithmic plot of mean plasma concentration of ISMM against time after single intravenous administration at the dose rate of 0.5 mg/kg body weight calf.



**Figure 5.** Semilogarithmic plot of mean plasma concentration of ISMM against time after single intravenous administration at the dose rate of 1 mg/kg body weight in calf.

**Table 1.** Plasma concentration of isometamidium ( $\mu\text{g/ml}$ ) following single dose intravenous administration in calves at 3 dose levels (Mean of 6 replicates with SE).

Time (h)	Mean plasma concentration ( $\mu\text{g/ml}$ )		
	Group I	Group II	Group III
0.08	$5.28^a \pm 0.14$	$9.85^a \pm 0.15$	$19.92^b \pm 1.17$
0.16	$4.66^a \pm 0.16$	$5.36^a \pm 0.14$	$11.91^b \pm 0.95$
0.33	$2.21^a \pm 0.23$	$3.90^a \pm 0.09$	$6.41^b \pm 0.27$
0.66	$0.88^a \pm 0.23$	$1.30^a \pm 0.07$	$3.06^b \pm 0.14$
1	$0.40^a \pm 0.04$	$1.05^b \pm 0.06$	$1.94^b \pm 0.24$
2	$0.27^a \pm 0.01$	$0.82^b \pm 0.04$	$1.27^b \pm 0.17$
4	$0.18^a \pm 0.02$	$0.43^b \pm 0.02$	$0.72^b \pm 0.10$
6	$0.07^a \pm 0.008$	$0.27^b \pm 0.02$	$0.51^b \pm 0.09$
8	BDL	$0.18 \pm 0.01$	$0.26 \pm 0.02$
12	BDL	$0.10 \pm 0.01$	$0.09 \pm 0.01$
24	BDL	BDL	BDL

Mean value with dissimilar superscript in a row vary significantly ( $P < 0.05$ ) and denoted as superscript a,b. BDL: Below detection limit; SE: standard error.

**Table 2.** Pharmacokinetic parameters of Isometamidium following single dose intravenous administration in calves at 3 dose levels (Mean of 6 replicates with SE).

Kinetic parameter	Group I	Group II	Group III
$C_p$ ( $\mu\text{g/ml}$ )	$5.24^a \pm 0.83$	$7.27^a \pm 0.38$	$29.18^b \pm 2.97$
$\alpha$ ( $\text{h}^{-1}$ )	$4.77 \pm 1.54$	$7.44 \pm 0.55$	$6.91 \pm 2.57$
$\beta$ ( $\text{h}^{-1}$ )	$0.34 \pm 0.01$	$0.23 \pm 0.02$	$0.28 \pm 0.01$
$t_{1/2 \alpha}$ (h)	$0.35 \pm 0.15$	$0.11 \pm 0.01$	$0.11 \pm 0.02$
$t_{1/2 \beta}$ (h)	$2.01 \pm 0.16$	$3.09 \pm 0.30$	$2.46 \pm 0.16$
AUC ( $\mu\text{g/h/ml}$ )	$3.30^a \pm 0.16$	$6.16^b \pm 0.3$	$13.89^c \pm 0.84$
$Cl_B$ (L/kg/h)	$14 \times 10^{-4} \pm 1 \times 10^{-4}$	$15 \times 10^{-4} \pm 1 \times 10^{-4}$	$14 \times 10^{-4} \pm 1 \times 10^{-4}$
$V_d$ area (L/kg)	$0.5 \pm 0.01$	$0.46 \pm 0.02$	$0.35 \pm 0.01$
$V_{dB}$ (L/kg)	$0.69^a \pm 0.06$	$1.98^b \pm 0.02$	$3.88^c \pm 0.49$
$V_{dC}$ (L/kg)	$0.03 \pm 0.005$	$0.07 \pm 0.005$	$0.04 \pm 0.003$
$K_{el}$ ( $\text{h}^{-1}$ )	$2.52 \pm 0.69$	$1.17 \pm 0.03$	$2.17 \pm 0.19$
$K_{12}$ ( $\text{h}^{-1}$ )	$1.97^a \pm 0.83$	$4.92^b \pm 0.45$	$4.12^b \pm 2.11$
$K_{21}$ ( $\text{h}^{-1}$ )	$0.67^a \pm 0.07$	$1.60^b \pm 0.07$	$0.89^a \pm 0.39$
$C_{max}$ (cal) ( $\mu\text{g/ml}$ )	$6.97^a \pm 0.82$	$7.28^a \pm 0.54$	$29.18^b \pm 2.97$
$f_c$	$0.18 \pm 0.04$	$0.21 \pm 0.01$	$0.12 \pm 0.02$
$T-P$	$5.88^a \pm 0.24$	$3.64^b \pm 0.15$	$6.44^a \pm 0.69$

Mean value with dissimilar superscript in a row vary significantly ( $P < 0.05$ ) and denoted as superscript a,b, c. Group I – at dose rate of 0.25 mg/kg body weight, Group II – at dose rate of 0.5 mg/kg body weight, Group III – at dose rate of 1 mg/kg body weight.

ISMM obtained from computerized pharmacokinetic programme "PHARMKIT" and disposition kinetic parameters of ISMM in calves following single dose intravenous administration at 0.25, 0.5 and 1 mg/kg have been depicted in Table 2. It was observed that plasma concentration of ISMM was maximum at 0.08 h which rapidly declined till 1 h and then gradually decreased in concentration till 6 h (0.25 mg/kg) and 12 h (0.5 and 1 mg/kg).

Mean value with standard error of  $C_p$  (The theoretical zero time plasma ISMM concentration),  $t_{1/2 \alpha}$  (half life of

ISMM in distribution phase),  $t_{1/2 \beta}$  (half life of ISMM in elimination phase),  $\beta$  (hybrid rate constants of elimination phase),  $\alpha$  (hybrid rate constant of disposition phase), AUC (total area under the blood ISMM concentration versus time curve),  $Cl_B$  (total body clearance of plasma),  $V_{d\text{area}}$  (apparent volume of ISMM distribution based on total area under blood concentration versus time area),  $C_{max}$  cal,  $V_{dB}$  (apparent volume of drug distribution obtained by avoiding the distribution phase of drug distribution),  $V_{dC}$  (apparent volume of distribution of ISMM

in central compartment),  $K_{el}$  (first order rate constant for drug elimination from the central compartment),  $K_{12}$  (first order rate constant for transfer of ISMM from central to peripheral compartment),  $K_{21}$  (first order rate constant for transfer of ISMM from peripheral to central compartment),  $f_c$  (fraction of drug in the body that is contained in the central compartment), T~P (tissue to plasma ratio) at 0.25, 0.5 and 1 mg/kg dose level are presented in Table 2.

The data presented in Table 2 showed that the mean values of  $C_p$ ,  $C_{max}$ , AUC and  $V_{dB}$  were significantly ( $P < 0.05$ ) greater in Group III animals as compared to animals of Groups I and II. The values for  $V_{d_{area}}$  and  $V_{dC}$  at three dose levels were comparable among themselves, whereas  $V_{dB}$  values increased significantly with increase in dose level. There was insignificant difference in  $K_{el}$  values at three dose levels. The value for  $K_{12}$  and  $K_{21}$  were the highest at 0.5 mg/kg body weight, significantly higher than the values at 0.25 mg/kg body weight, but comparable to the values at 1 mg/kg body weight,  $C_{max}$  was significantly higher at 1 mg/kg body weight than the other two which were comparable between themselves. T~P value was lower at 0.5 mg/kg body weight than the other two which shares a comparable values in between them.

## DISCUSSION

There have been much studies on the distribution and pharmacokinetics of ISMM in several animal species, including rats, mice, dogs, monkeys, goats, pigs, camels and cattle (Hill and McFadzean, 1963; Philips et al., 1967; Braide and Eghianruwa, 1980; Kinabo and Bogan, 1988a, b; Kinabo et al., 1991; Eisler, 1996; Murilla et al., 1996; Wesongaha et al., 2004). In our recovery experiment study, the retention time showed a linear trend with dose and recover value ranged between 85 and 925 much higher than usual standard of 80% indicative of standard operating conditions and satisfactory recovery level. Mean plasma concentration of ISMM in animals of Group III at 0.08, 0.16, 0.33 and 0.66 h were significantly ( $P < 0.05$ ) higher in comparison to Groups I and II, whereas at 1, 2, 4 and 6 h, mean values of ISMM in Groups II and III were higher significantly ( $P < 0.05$ ) as compared to Group I. Plasma concentration of ISMM was increased in all the three groups with enhanced doses at different times except at 0.08, 0.16 and 0.33 h in animals of Group II suggesting dose dependent increase in drug concentration in calves. Similar dose dependent increase in concentration was also reported by Ardelli and Woo (2001) and Eze et al. (2012) in aquatic animals. The plasma concentration of ISMM falls to below detection level within 24 h at all the dose level, indicates rapid distribution of drug in the tissues. Similar findings for rapid distribution were reported by Boibessot et al. (2006).

Dose dependent significant increase in the value of

AUC was obvious and in consistence with total systemic clearance of the drug.  $V_{d_{area}}$  value at three dose levels indicates moderate distribution of ISMM in the body. But  $V_{dC}$  value was very low ( $0.03 \pm 0.005$  to  $0.07 \pm 0.005$  L/kg) suggesting persistence of the drug in the peripheral compartment/tissue compartment. This suggests that disposition kinetics of ISMM in buffalo calves were best fitted to a two-compartment open model at all three dose levels. Higher  $K_{12}$  values and lower  $K_{21}$  values along with higher T~P ratio of ISMM in all three groups might have cause longer persistence of the drug in tissues leading to lower  $Cl_B$  value. Murilla et al. (1995) reported that 80% of ISMM was excreted within 21 days out of which only 18% was through urine and remaining through faeces when administered at 1 mg/kg intravenously in cattle. Kinabo et al. (1990) found that the elimination of half life of the drug was 3.2 h, and the mean residence time was 2.4 h following intravenous (I/V) administration at 0.5 mg/kg in lactating goats. The apparent volume of distribution averaged 1.52 L/kg and the mean total body clearance was 0.31 L/kg/h. Uptake of ISMM chloride demonstrated Michaelis-Menten-type kinetics. The difference in parameters with the present findings might be due to species variation. An exceptionally higher value for  $C_{max}$  at 1 mg/kg body weight than the other two dosages suggested for its accumulation as depots in tissues at higher dose level. The reports of prophylactic use of ISMM against trypanosomiasis also substantiate the same (Awa and Ndamkou, 2006). The values of  $t_{1/2\beta}$ ,  $Cl_B$ ,  $V_{d_{area}}$  and  $V_{dC}$  showed mild alteration at three dose level which may suggest that ISMM does not show dose-dependent kinetics.

It is concluded that selection of the optimal dose of ISMM in buffalo intravenously, should be based on the information regarding its disposition kinetics, efficacy and safety. The results of the present study have established that ISMM does not show dose-dependent kinetics while administered intravenously, and the same would be helpful in designing appropriate drug regimens in the strategic use of ISMM chloride in buffalo.

## REFERENCES

- Ali H, Hassan AP (1984). Estimation of ISMM in induced trypanosomiasis in camel. *Indian Vet. J.* 71:191-192.
- Anene BM, Onah DN, Nawa Y (2001). Drug resistance in pathogenic African trypanosomes: What hopes for the future? *Vet. Parasitol.* 96:83-100.
- Awa DN, Ndamkou CN (2006). Response of *Trypanosoma vivax* and *Trypanosoma congolense* in zebu cattle in North Cameroon to prophylactic treatment with two formulations of isometamidium. *Prev. Vet. Med.* 15:90-96
- Baggot JD (1977). Principles of Drug Disposition in Domesticated animals. The Basis of Veterinary clinical Pharmacology W.B. Saunders company, Philadelphia
- Boibessot I, Tettey JN, Skellern GG, Watson DG, Grant MH (2006). Metabolism of isometamidium in hepatocytes isolated from control and inducer-treated rats. *J. Vet. Pharmacol. Ther.* 29(6):547-53
- Boibessot I, Turner CM, Watson DG, Goldie E, Connel G, McIntosh A, Grant MH, Skellern GG (2002). Metabolism and distribution of

- phenanthridine trypanocides in *Trypanosoma brucei*. *Acta Trop.* 84:219-228.
- Braide VB, Eghianruwa KI (1980). Isometamidium residues in goat tissues after parenteral administration. *Res. Vet. Sci.* 29:111-113.
- Delepoux V, de Koning HP (2007). Drugs and drug resistance in African trypanosomiasis. *Drug Resist. Update* 10:30-50.
- Dowler ME, Schillinger D, Connor RJ (1989). Routine intravenous use of Isometamidium in the control of bovine trypanosomiasis on the Kenya coast. *Trop. Anim. Health Prod.* Feb. 21(1):4-10.
- Eisler MC (1996). Pharmacokinetics of the chemoprophylactic and chemotherapeutic trypanocidal drug isometamidium chloride (Samorin) in cattle. *Drug Metab. Dispos.* 24:1355-1361.
- Eze JI, Agwubilo CI, Anene BM (2012). Efficacy Of Increasing Doses Of Diminazene Diacetate Or Isometamidium Chloride In Treatment Of Mice Infected With Isometamidium Chloride-Resistant *Trypanosoma Congolense*. *Folia Vet.* 56:1-5.
- Hill J, McFadzean JA (1963). Studies on isometamidium: depots of isometamidium in mice and rats and their importance for prophylaxis against *Trypanosoma congolense*. *Transactions of the Royal Society: Trop. Med. Hyg. Trans.* 57:476-484.
- Kaminsky R, Schmid C, Lun ZR (1997). Susceptibility of dyskinetoplastic *Trypanosoma evansi* and *T. equiperdum* to isometamidium chloride. *Parasitol. Res.* 83:816-818.
- Karaye GP (2012). The Efficacy Of Isometamidium Chloride In The Treatment Of Trypanosomiasis In Red Sokoto Bucks Experimentally Infected With *Trypanosoma Congolense* And *Trypanosoma Brucei* Single And Mixed Infection Of The Two. M.Sc. (Veterinary sciences) thesis submitted to the School Of Postgraduate Studies, Ahmadu Bello University, Zaria, Nigeria.
- Kinabo LD, McKellar QA, Bogan JA (1990). Solid phase extraction and ion pair reversed phase HPLC of isometamidium in lactating goat serum and tissues. Dept. of veterinary Pharmacology, University of Glasgow, Great Britain. *Acta Trop.* 45(2):165-170.
- kinabo LDB, Bogan LA (1988). The pharmacology of isometamidium. *J. Vet. Pharmacol. Ther.* 11:233-245.
- Kinabo LDB, McKellar QA (1990). Isometamidium in goats. Disposition kinetics, mammary excretion and tissue residues. *Br. Vet. J.* 146:405-412.
- Kinabo LDB, Bogan JA (1988a). Solid-phase extraction and ion-pair reversed phase HPLC of isometamidium in bovine serum and tissues. *Acta Trop.* 45:165-170.
- Kinabo LDB, McKellar QA, Eckersall PD (1991). Isometamidium in pigs: disposition kinetics, tissue residues and adverse reactions. *Res. Vet. Sci.* 50:6-13.
- Magona JW, Mayende JSP, Okiria R, Okuna NM (2004). Protective efficacy of isometamidium chloride and diminazene aceturate against natural *Trypanosoma brucei*, *Trypanosoma congolense* and *Trypanosoma vivax* infections in cattle under a suppressed tsetse population in Uganda. *Onderstepoort J. Vet. Res.* 71(3):231-237. doi: 10.4102/ojvr.v71i3.265.
- Mehlhorn H (2008). *Encyclopedia of parasitology*, 1-2:381 Volume 1 (3rd edn). Springer. Verlag, Heidelberg, Germany.
- Moloo SK, Kutuza SB (1990). Expression of resistance of isometamidium and diminazene in *Trypanosoma Congolense* in Boran Cattle infected by *Glossina Morsitans Centralis*. *Acta Trop.* Feb. 47(2):79-89.
- Murilla GA, Mdachi RE, Karanja WM (1995). Pharmacokinetics bioavailability and tissue residue of [<sup>14</sup>C] isometamidium in non-infected and *T. congolense* infected Boran cattle. The Radioisotope Laboratory, Kenya Trypanosomiasis Research Institute.
- Murilla GA, Mdachi RE, Karanja WM (1996). Pharmacokinetics, bioavailability and tissue residues of <sup>14</sup>C-isometamidium in non infected and *Trypanosoma congolense*-infected Boran cattle. *Acta Trop.* 61:277-292.
- Juyal PD (2011). "Newer Perspectives in the Diagnosis and Control of Trypanosomiasis (Surra) in Domestic Livestock in India". *TROPMED - Internationale Wissenschaftliche Publikationen*. pp. 1-13.
- Philips FS, Sternberg SS, Cronin AP, Sodergren JE, Vidal P (1967). Physiologic disposition and intracellular localisation of isometamidium. *Cancer Res.* 27:333-349.
- Geerts S, Holmes PH (1998). Drug management and parasite resistance in bovine *trypanosomiasis* in Africa. The programme against African *Trypanosomiasis*, Technical and Scientific Series 1. FAO, Rome. pp. 5-31.
- Wesongah JO, Murilla GA, Kibugu JK, Jones TW (2000). Determination of serum isometamidium levels in sheep and goats under field conditions using isometamidium-ELISA. *J. Protozool. Res.* 10:191-201.
- Wesongaha JO, Jones TW, Kibugua JK, Murilla GA (2004). A comparative study of the pharmacokinetics of isometamidium chloride in sheep and goats. *Small Rumin. Res.* 53:9-14.
- Whitelaw DD, Gault EA, Holmes PH, Sutherland IA, Rowel, FJ, Pihps A, Urquhart GM (1991). Development of an enzyme-linked immunosorbent assay for the detection and measurement of the trypanocidal drug isometamidium chloride in cattle. *Res. Vet. Sci.* 50:185-189.

Full Length Research Paper

## Formulation of pyridoxine hydrochloride sustained release capsules: Effect of propylene glycol co-solvent on the *in vitro* release

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The objectives of the present study were to formulate sustained release pyridoxine hydrochloride capsules and to study the effect of propylene glycol co-solvents on the *in vitro* properties of the capsules. All batches of formulations were made with fixed concentrations of binder-disintegrants, diluents and equal load of active pharmaceutical ingredient. The granules were prepared by wet granulation using propylene glycol water co-solvent as the wetting agent, sodium carboxymethylcellulose (SCMC) and maize starch were used as binder-disintegrant and kaolin was used as the diluents. The micromeritic properties of the granules were analysed by direct and indirect methods. The granules were encapsulated in hard gelatin capsule No. 1. The capsule weight uniformity, disintegration time and drug content were determined. *In vitro* dissolution test was performed in 0.1 N HCl, simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2). The results showed that the particles size of granules ranged from 245 to 259  $\mu\text{m}$  and had good flowability. The capsules complied with British Pharmacopoeia (BP) requirement for capsule weight uniformity. The drug content was within 90 to 110% of the average values. The results of *in vitro* drug release in SGF (pH, 1.2) showed that the release of pyridoxine hydrochloride was very slow and was significantly ( $P < 0.05$ ) lower than the release in 0.1 N HCl and in SIF (pH, 7.2), respectively. Therefore, pyridoxine hydrochloride sustained release capsules could be formulated with kaolin as the diluent and propylene glycol co-solvent as the moistening agent in order to reduce the frequency of administration of this drug and improve patient compliance.

**Key words:** Pyridoxine hydrochloride, sustained release, capsules, particle size analysis.

### INTRODUCTION

Over the years, much work has been carried out to improve patient compliance of conventional oral and parenteral single dosage forms meant for repeated administration (Ravi-Kumar, 2000; Wissing et al., 2004; Mathew and Devi, 2007; Rhee et al., 2007; Jia et al., 2008; Kim et al., 2010). This has led to the development of effective sustained release dosage forms. Among all the factors that make for an ideal therapy, the most difficult to achieve is maintaining serum concentrations of drugs at therapeutic levels for a long period of time. This

is usually done by repeated administration and poses serious problem of patient compliance. Sustained release dosage forms are preparations with controlled rate of absorption of drug into the body, which is achieved mainly by controlling the dissolution rate of the formulations (Umeyor et al., 2012). Sustained release dosage forms provide an initial release of drug sufficient to provide a therapeutic dose soon after administration, and then a gradual release over an extended period. The rationale for the controlled delivery of drugs is to promote therapeutic

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benefits, while at the same time minimizing toxic effects (Sinko, 2006). Capsules and tablets are the most common oral dosage forms. These formulations differ from each other in that material in capsules is less impacted than in compressed tablets. Once a capsule dissolves, the contents generally disperse quickly (Welling, 2002). In the formulation of capsules, particle, flowability is of paramount importance (Welling, 2002). The flow of powder during manufacturing dictates the quality of the product in terms of weight and content uniformity of the capsules (Aulton, 2007). The measurement of the flow properties of powders is essential before capsule filling, because variation in particle flow will automatically cause variation in capsule weight and active ingredient variation. The flow property of bulk material results from the cohesive forces acting on individual particles such as van der Waals, electrostatic, surface tension, interlocking, and friction (Aulton, 2007).

Pyridoxine is a water-soluble vitamin involved mainly in amino acid metabolism, and is also involved in carbohydrate and fat metabolism. It is required for the formation of haemoglobin and is usually given as the hydrochloride, although other salts such as the citrate, oxoglurate, phosphate, and phosphoserinate, have also been used. Pyridoxine is used in the treatment and prevention of pyridoxine deficiency states (Sean, 2011). It is usually given orally, the preferred route, but may also be given by the subcutaneous, intramuscular, or intravenous routes (Sean, 2009). It is used to treat certain metabolic disorders such as homocystinuria or primary hyperoxaluria, seizures due to hereditary syndromes of pyridoxine deficiency and premenstrual syndrome. The aim of this work was to formulate pyridoxine sustained release capsules and to study the effect of propylene glycol co-solvent on the *in vitro* release.

## MATERIALS AND METHODS

The following materials were used: pyridoxine hydrochloride (BDH Chemicals Ltd., England), sorbic acid, sodium carboxymethylcellulose (SCMC), maize starch, ferric chloride, hydrochloric acid, sodium hydroxide, sodium chloride and mono-basic potassium phosphate, sodium benzoate (BDH Chemicals Ltd., England), kaolin, propylene glycol (Merck, Darmstadt, Germany). All other reagents and solvents were of analytical grade and were used as supplied.

### Preparation of granules

The granules were prepared by wet granulation using propylene glycol water co-solvent as the wetting agent; details of formulation are shown in Table 1. SCMC and maize starch were used as binder-disintegrant, sorbic acid and sodium benzoate were used as the preservative and kaolin was used as the diluents. The powders were mixed for 10 min in a tumbler mixer (Rotor mixer S42P43, Forster Equipment Co. Ltd., England) together with pyridoxine hydrochloride. The powder mixtures were moistened with the appropriate amount of the wetting agent and were triturated in a mortar to a homogeneous mix. The homogeneous wet mass was then

screened through a 1.7 mm sieve and the wet granules were dried in a hot air oven at 55°C for 1 h (Memmer, U25, Western Germany). Thereafter, the dried granules were screened through a 1.0 mm sieve.

## Evaluation of granules

### Particle size distribution

The particle size of the granules was determined using nest of sieves (numbers 16, 52, 100 and 200) arranged in descending order of aperture size with a pan collector underneath. Eighteen grams quantity of each batch of granulations was accurately weighed using an electronic weighing balance (Ohaus Adventurer, SNR – 1121 R53860, China), and transferred to the top most of a series of sieves. The sieve arrangement was transferred to an Endecott mechanical sieve shaker (Endecott 1 MK 11, 6315, London, England) and was shaken for 5 min. At the end of 5 min, the fraction of powder retained by each sieve was weighed. Three determinations were carried out and the mean particle diameter ( $d_{av}$ ) was determined using the relation (Ansel et al., 2007; Okoye et al., 2012):

$$d_{av} = \frac{\sum(\text{Percentage powder retained} \times \text{Mean aperture size})}{100} \quad (1)$$

### Bulk and tapped densities

A 25 g quantity of each granule sample was placed in a 100 ml measuring cylinder and the volume occupied by the sample was noted as the bulk volume. The bulk density ( $\ell_B$ ) was calculated using the equation:

$$\text{Bulk density } (\ell_B) = \frac{\text{Mass of powder (M)}}{\text{Bulk volume of powder (V}_B)} \quad (2)$$

The tapped volume was determined by tapping the cylinder on a wooden flat surface from a height of 1 inch at 2 s interval until there was no significant change in volume reduction (Aulton, 2007; Ngwuluka et al., 2010; Chime et al., 2012). The volume occupied by the sample was then recorded as the tapped volume. The tapped density ( $\ell_T$ ) was calculated using the formula:

$$\text{Tapped density } (\ell_T) = \frac{\text{Mass of powder (M)}}{\text{Tapped volume of powder (V}_T)} \quad (3)$$

### Flow rate and angle of repose

A 25 g quantity of pyridoxine hydrochloride granules was weighed out and gradually placed into the funnel clamped onto a retort stand; the funnel orifice was closed with a shutter. The time taken for all the granules to flow through the orifice was noted. The flow rate was calculated using Equation 4:

$$\text{Flow rate (w)} = \frac{\text{Mass of powder (g)}}{\text{Time of flow (s)}} \quad (4)$$

The angle of repose was determined by measuring the height of heap of powder formed using a cathetometer; the radius was gotten by dividing the diameter by two. Angle of repose ( $\theta$ ) for each granule

**Table 1.** Composition of pyridoxine hydrochloride sustained release capsules.

Ingredient	Weight/capsule (mg)				
	F1	F2	F3	F4	F5
Pyridoxine hydrochloride (mg)	50.00	50.00	50.00	50.00	50.00
SCMC (mg)	50.00	50.00	50.00	50.00	50.00
Maize starch (mg)	25.00	25.00	25.00	25.00	25.00
Sorbic acid (mg)	1.25	1.25	1.25	1.25	1.25
Sodium benzoate (mg)	1.25	1.25	1.25	1.25	1.25
Propylene glycol (ml)	1.00	2.00	3.00	4.00	5.00
Magnesium stearate (1%)	2.50	2.50	2.50	2.50	2.50
Kaolin q.s (mg)	250.00	250.00	250.00	250.00	250.00

SCMC: Sodium carboxymethylcellulose.

granule sample was calculated using the formula:

$$\theta = \tan^{-1} \frac{\text{height of powder heap}}{\text{radius of powder}} \quad (5)$$

#### Compressibility index and Hausner's quotient

Carr's compressibility index (%) of the granules was obtained using the formula:

$$\text{Carr's index (\%)} = \frac{f_T - f_B}{f_T} \times 100 \quad (6)$$

While Hausner's ratio was obtained using Equation 5:

$$\text{Hausner's ratio} = \frac{f_T}{f_B} \quad (7)$$

where  $f_T$  and  $f_B$  are tapped and bulk densities, respectively.

#### Preparation of capsules

The granules were treated with magnesium stearate as shown in Table 1, and then filled manually using capsule shell No 1.

#### Evaluation of capsules

##### Weight uniformity

Twenty capsules were selected from each batch, and weighed individually, the contents of the capsule were emptied and weighed and also the empty capsule shell was also weighed. The mean standard deviation and percentage coefficient of variation of the mean weight was calculated.

##### Disintegration time test

Disintegration time test was conducted using an Erweka ZT 120 basket and rack assembly. Distilled water maintained at  $37.0 \pm 1.0^\circ\text{C}$  was used as the disintegration medium. Ten capsules from each batch were used for the test and the procedure being as stipulated in the British Pharmacopoeia (BP, 2009) for disintegration

time of capsules.

#### Content of active ingredient

Beer's calibration curve for pyridoxine hydrochloride was obtained at a concentration range of 2.0 to 10.0 mg% in 0.1 N HCl at a pre-determined wavelength of 450 nm. Twenty capsules were randomly selected from each batch of the tablets. The capsules were emptied and the content weighed together. An amount equivalent to the average weight of the capsule was weighed out in an analytical balance. The weighed amount was dispersed in the medium and was filtered. Two drops of ferric chloride was added to an aliquot of the filtrate and was assayed using spectrophotometer (Pye Unicam SP6 450 UV/VIS spectrophotometer, England) at 450 nm. The concentration of the drug in each capsule was calculated using the absorbance readings.

#### In vitro release studies

Beer's plot was obtained for pyridoxine hydrochloride in 0.1 N HCl, simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2), respectively at a concentration range of 2 to 10 mg%. USP paddle method was adopted in the study. The dissolution medium consisted of 900 ml of freshly prepared medium maintained at  $37 \pm 1^\circ\text{C}$ . A capsule from each batch was placed inside a tightly secured basket and the basket was placed in the bottom of the beaker. The paddle was rotated at 100 rpm. About 5 ml was withdrawn from the dissolution medium at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 h, and was filtered with a non adsorbent filter paper (Whatman No. 1). Two drops of ferric chloride was added to an aliquot of the filtrate and assayed using spectrophotometer (Pye Unicam SP6 450 UV/VIS spectrophotometer, England) at predetermined wavelengths of 450 nm in 0.1 N HCl and SGF and 296 nm in SIF. An equal volume of the withdrawn sample was replaced with a fresh medium to maintain sink condition in each case. The amount of drug released at each time interval was determined with reference to the standard Beer's plot for each drug. The experiment was repeated two times for each sample and the mean was calculated.

#### In vitro release kinetics

The dissolution data for the capsules were analysed to determine the *in vitro* release kinetic mechanism using three kinetic models including the first order equation, Higuchi square root equation and Ritger-Peppas empirical model. Drug release is said to be of first-

order if it obeys the following equation:

$$\ln Q_t = \ln Q_0 - K_1 t \quad (8)$$

where  $Q_t$  is the amount of drug released or dissolved at time  $t$ ,  $Q_0$  is amount of drug released or dissolved at time  $t = 0$ ,  $K_1$  is first-order release rate constant (Singh et al., 2011).

According to Higuchi relationship, the amount of drug released per unit surface area is proportional to the square root of time. This equation explains diffusion release rate as indicated below:

$$Q_t = K_H t^{1/2} \quad (9)$$

where  $k_H$  is Higuchi rate constant,  $Q_t$  has same meaning as defined earlier (Singh et al., 2011). The integral form of Higuchi equation is employed in seeking to establish whether mixed order release kinetics exists. Diffusion controlled process is dominant where the log-log plot of the integral form of Higuchi equation approaches 0.5 (Ofoefule and Chukwu, 2002).

Ritger and Peppas (1987a,b) developed an empirical equation to analyze both Fickian and non-Fickian release of drug from swelling as well as non-swelling polymeric delivery systems. The equation is represented as:

$$M_t/M_\infty = Kt^n \quad (10)$$

where  $M_t/M_\infty$  is the fraction of drug released at time  $t$ ,  $n$  is diffusion exponent indicative of the mechanism of transport of drug through the polymer,  $K$  is the kinetic constant (having units of  $t^{-n}$ ) incorporating structural and geometric characteristics of the delivery system. The release exponent  $n \leq 0.5$  for Fickian diffusion release from slab (swellable matrix),  $0.5 < n < 1.0$  for non-Fickian release (anomalous), this means that drug release followed both diffusion and erosion controlled mechanisms and  $n = 1$  for zero order release, that is, drug release is independent of time (Ritger and Peppas, 1987a, b).

### Statistical and data analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) Version 16.0 (SPSS Inc. Chicago, IL, USA). All values were expressed as mean  $\pm$  standard deviation (SD). Data were analysed by one-way analysis of variance (ANOVA). Differences between means were assessed using student's  $t$ -test.  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

### Granule properties

#### Particle size of granules

The results of particle size of pyridoxine granules are shown in Table 2. From the results, the particle size ranged from 245 to 259  $\mu\text{m}$ . As the dimensions of particles increase and the particles change in nature, the forces acting on them change. Fine powder particles less than 100  $\mu\text{m}$  in diameter are acted upon primarily by surface forces, while particles above 1000  $\mu\text{m}$  in diameter are governed by gravitational forces (Fukumori and Ichikawa, 2002). Therefore, the balance of interactive forces determines powder behavior. With relatively small particles, the flow through an orifice may be restricted,

because the cohesive forces between the particles are of the same magnitude as gravitational forces. Large particles however with respect to the orifice through which it has to flow can cause arching that can block flow from hopper to die cavity (Fukumori and Ichikawa, 2002). The flowability of powders decreases as the shapes of particles become more irregular. Efforts to relate various shape factors to powder and their surfaces such as size, shape, surface morphology, packing conditions, and interparticle forces must therefore be considered. To make the situation more complex, the interparticle forces can be of a number of types: mechanical forces, surface tension, electrostatic forces, van der Waals forces, solid-bridge forces, or plastic welding forces; none of these can be readily quantified (Fukumori and Ichikawa, 2002). The results therefore showed that the granules were within limits for good flow of powders as shown in Table 2.

### Flow properties

The results of the flow properties of pyridoxine hydrochloride granules are shown in Table 2. The results of loosed densities (bulk and tapped densities) showed that the granules had reduced densities and hence had good flowability. The flow of powder during manufacturing dictates the quality of the product in terms of weight and content uniformity of the capsules (Lachman et al., 1990). The measurement of the flow properties of powders is essential before capsule filling because variation in particle flow will automatically cause variation in capsule weight and active ingredient variation. The flow property of bulk material results from the cohesive forces acting on individual particles such as van der Waals, electrostatic, surface tension, interlocking, and friction (Lachman et al., 1990). Bulk and tapped densities are important, because they are used as an indirect method of assessing powder flowability. Hausner's ratio, determines the degree of interparticulate friction and values  $\leq 1.25$  indicates good flow, while Hausner's ratio  $> 1.25$  indicates poor flow. The results indicated that Hausner's ratio ranged from 1.10 to 1.22; therefore, they were within the limits for good powder fluidity. Carr's compressibility index also reveals the degree of interparticulate friction and values between 5 and 17 indicates good flow (Aulton, 2007; Yüksel et al., 2007). The results showed that Carr's index ranged from 9.1 to 17.3% and hence exhibited good flowability. Values for angles of repose  $\leq 30^\circ$  generally indicate a free flowing material and  $\geq 40^\circ$  suggest a poorly flowing material. Angle of repose was also used as an indirect method of assessing flowability of granules and the results also showed that the granules had low interparticulate friction and hence had good flowability. The results of flow rate (that is, flow under gravity) also showed that the granules had good flowability.

### Capsule properties

The results of the weight uniformity of the capsules are

**Table 2.** Micromeritic properties of pyridoxine hydrochloride granules.

Batch	Particle size ( $\mu\text{m} \pm \text{SD}$ )	$\ell_B$ (g/ml $\pm$ SD)*	$\ell_T$ (g/ml $\pm$ SD)*	AR ( $^\circ \pm \text{SD}$ )*	HR	CI (%)	FR (g/sec $\pm$ SD)
F1	259.0 $\pm$ 0.1	0.68 $\pm$ 0.27	0.75 $\pm$ 0.07	27.82 $\pm$ 0.03	1.10	9.10	8.62 $\pm$ 0.05
F2	256.0 $\pm$ 0.2	0.61 $\pm$ 0.23	0.74 $\pm$ 0.06	29.94 $\pm$ 0.01	1.21	16.70	6.65 $\pm$ 0.07
F3	245.0 $\pm$ 0.1	0.53 $\pm$ 0.17	0.63 $\pm$ 0.12	29.93 $\pm$ 0.09	1.19	15.80	5.12 $\pm$ 0.03
F4	252.0 $\pm$ 0.3	0.51 $\pm$ 0.11	0.61 $\pm$ 0.19	21.80 $\pm$ 0.11	1.20	16.00	8.32 $\pm$ 0.05
F5	249.0 $\pm$ 0.2	0.50 $\pm$ 0.17	0.61 $\pm$ 0.21	24.86 $\pm$ 0.05	1.22	17.30	8.61 $\pm$ 0.07

Values shown are mean  $\pm$  SD (\*n = 3);  $\ell_B$  and  $\ell_T$  = Bulk and tapped densities, AR = Angle of repose, HR = Hausner's ratio, CI = Carr's compressibility index, FR = Flow rate; batches F1, F2, F3, F4 and F5 contain propylene glycol 1, 2, 3, 4 and 5 ml respectively.

**Table 3.** Properties of pyridoxine capsules.

Batch	Capsule weight (mg $\pm$ CV)*	Disintegration time (min $\pm$ SD) <sup>a</sup>	Drug content (mg $\pm$ SD) <sup>a</sup>
F1	253.00 $\pm$ 0.94	3.54 $\pm$ 0.37	52.50 $\pm$ 0.13
F2	260.00 $\pm$ 1.01	3.31 $\pm$ 0.17	49.00 $\pm$ 0.27
F3	255.00 $\pm$ 0.92	3.30 $\pm$ 0.29	49.00 $\pm$ 0.15
F4	263.00 $\pm$ 2.64	3.40 $\pm$ 0.11	50.00 $\pm$ 0.31
F5	257.00 $\pm$ 0.81	3.56 $\pm$ 0.32	53.50 $\pm$ 0.11

\*Mean for 20 capsules, <sup>a</sup>Mean for 10 capsules, CV: coefficient of variation, SD: standard deviation, batches F1, F2, F3, F4 and F5 contain propylene glycol 1, 2, 3, 4 and 5 ml, respectively, P < 0.05 was considered significant.

**Table 4.** Release kinetics of sustained release pyridoxine hydrochloride in SIF.

Batch	Higuchi			First order		Ritger-Peppas		
	(r <sup>2</sup> )	(n)	K (h <sup>-1</sup> )	(r <sup>2</sup> )	K (h <sup>-1</sup> )	(r <sup>2</sup> )	(n)	K (h <sup>-n</sup> )
F1	0.936	0.097	69.2	0.867	1.559	0.953	0.099	0.81
F2	0.937	0.169	76.7	0.975	1.687	0.953	0.173	1.26
F3	0.943	0.230	65.3	0.878	1.655	0.998	0.183	1.34
F4	0.958	0.036	93.3	0.953	1.002	0.954	0.040	1.07
F5	0.961	0.048	64.9	0.750	1.578	0.866	0.022	1.08

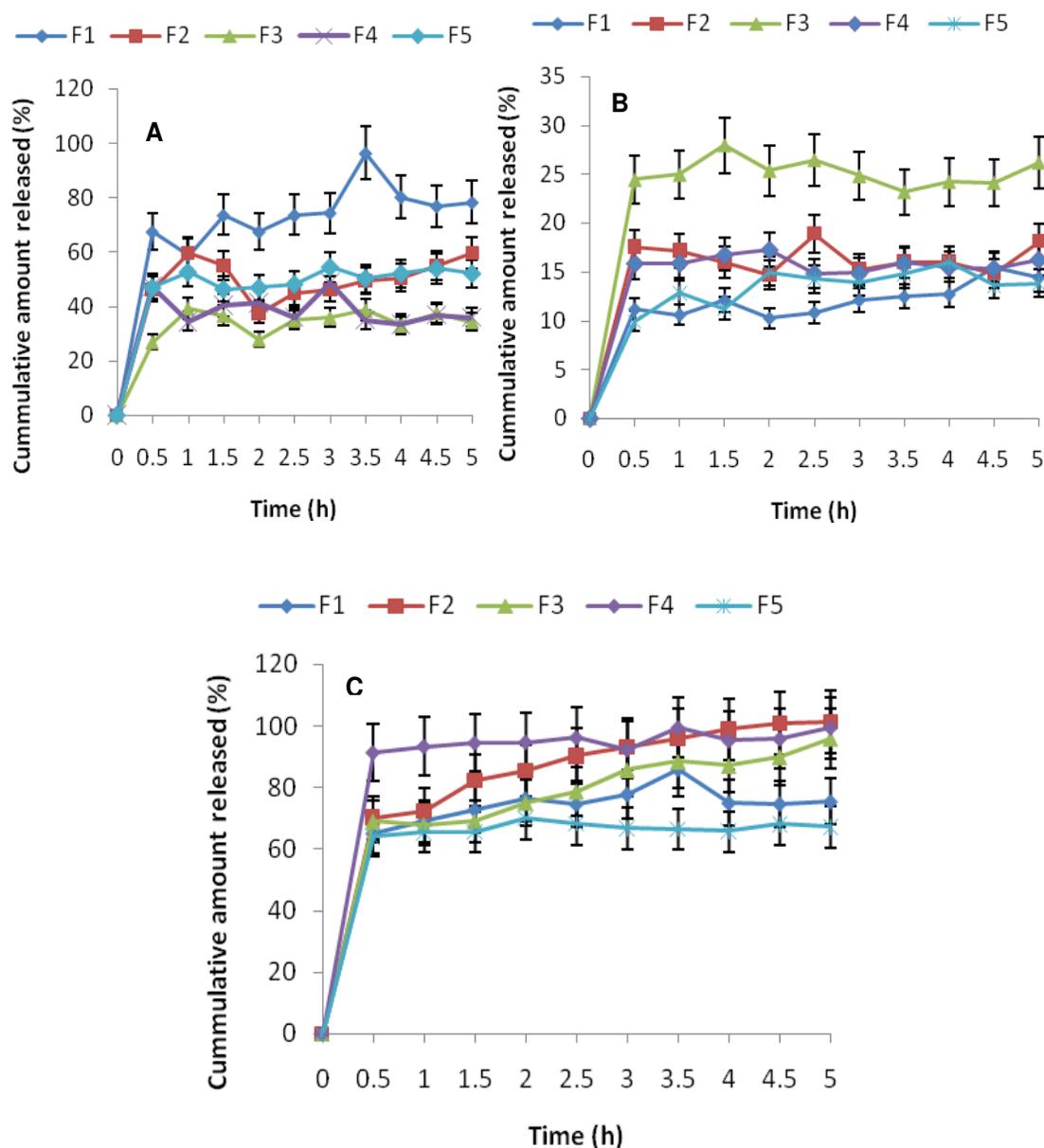
Batches F1, F2, F3, F4 and F5 contain propylene glycol 1, 2, 3, 4 and 5 ml, respectively.

shown in Table 3. The results showed that all the capsules complied with requirement for capsule weight uniformity (BP, 2009) and the percentage deviations obtained from the capsule weight uniformity test were significantly below 5%. The results of the disintegration time of the capsules showed that they disintegrated within 3.31 to 3.56 min, and hence did not vary significantly within the batches (P < 0.05). This may be due to the kind of capsule shell used. The product being a modified release product, one would ordinarily expect prolonged disintegration time, but the granules were not encapsulated with gastro-resistant capsules. Therefore, the capsule shell was not the cause of the sustained release and was not designed as such. The results of the drug content of the capsules also showed that pyridoxine

hydrochloride capsules had high percentage drug content and complied with BP standards for drug content. The results showed that the drug content were within 90 to 110% of the average values specified in the official book (BP, 2009) as shown in Table 3.

### *In vitro* drug release

The results of the *in vitro* drug release are shown in Figure 1a to c. From the results of the *in vitro* release of pyridoxine hydrochloride capsules in 0.1 N HCl (Figure 1a), the results showed an initial high release of drug between 0.5 and 2 h before maintaining the sustained drug release over time between 2 and 3 h. Batch F1 containing



**Figure 1.** Release profile of pyridoxine hydrochloride in (A) 0.1 N HCl, (B) SGF (pH 1.2), and (C) SIF (pH 7.2). Batches F1, F2, F3, F4 and F5 contain propylene glycol 1, 2, 3, 4 and 5 ml, respectively.

1 ml of propylene glycol had 96% drug release at 3.5 h, however,  $T_{100\%}$  could not be attained in other formulations. Increase in the amount of propylene glycol significantly ( $P < 0.05$ ) delayed the release of pyridoxine hydrochloride as shown in Figure 1 a to c. The results of *in vitro* drug release in SGF (pH, 1.2) showed that the release of pyridoxine hydrochloride was significantly ( $P < 0.05$ ) lower than the release in 0.1 N HCl and in SIF (pH, 7.2), respectively. Generally, batch F5 formulated with 5 ml of propylene glycol had 46.87 and 54.42% drug release at  $T_{120}$  and  $T_{180}$  (120 and 180 min), respectively in 0.1 N HCl, also F5 had 15.04 and 13.96% drug release at  $T_{120}$  and  $T_{180}$ , respectively in SGF (pH, 1.2) and 69.87 and

66.6% drug release at  $T_{120}$  and  $T_{180}$ , respectively in SIF (pH, 7.2). Therefore, pyridoxine hydrochloride sustained release capsule formulated exhibited higher drug release in SIF (pH, 7.2).

#### ***In vitro* release kinetics**

From the results of drug release kinetics shown in Table 4, the regression coefficients had  $r^2$  of  $\approx 0.9$ . Also, the release exponent in the Ritger-Peppas model ( $n$ ) for all the batches suggested that the mechanism that led to the release of pyridoxine hydrochloride from the capsule was

by diffusion with release rate adequate for a sustained release dosage form. Higuchi's kinetics seconds the Ritger–Peppas in the linearity of their plot with  $r^2$  of  $\approx 0.9$ . The linearity of Higuchi's kinetic explains why the drug diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred to as square root kinetics (or Higuchi's kinetics) (Rawat et al., 2011; Singh et al., 2011). The linearity of the plot indicates that the release of pyridoxine hydrochloride from the samples followed diffusion mechanism. The results of first order kinetics showed that drug release was not dissolution controlled ( $r^2 \neq 0.9$ ) in most of the formulations, however, batches F2 and F4 showed some level of linearity ( $r^2 = 0.9$ ). This suggested that the release of drug from these batches followed mixed mechanism of drug release (Rawat et al., 2011; Singh et al., 2011).

## Conclusion

Pyridoxine hydrochloride sustained release capsules with fixed concentrations of binder-disintegrants, diluents and equal load of active pharmaceutical ingredient were successfully formulated using propylene glycol co-solvent as the moistening agent. The results showed that free flowing spherical particles were produced. The capsules complied with BP (2009) specifications for capsule weight uniformity and drug content. The *in vitro* release properties of the pyridoxine capsules showed that increase in the amount of propylene glycol increased the time of drug release. Therefore, pyridoxine hydrochloride sustained release capsules could be formulated with kaolin as the diluent and propylene glycol co-solvent as the moistening agent in order to reduce the frequency of administration of this drug and improve patient compliance.

## REFERENCES

- Ansel HC, Allen Jr LV, Poporich NG (2007). Powders and granules. Ansel's Pharmaceutical Dosage forms and Drug Delivery Systems. 8<sup>th</sup> Edn., Lippincott Williams and Wilkins, New Delhi, pp: 189.
- Aulton ME (2007). Pharmaceutics; The Science of Dosage Form Design, 3<sup>rd</sup> Edn. Churchill Living Stone, Edinburgh. pp. 483-499.
- British Pharmacopoeia (2009). The Commission Office London. Vol. III: 6578-6585.
- Chime SA, Attama AA, Agubata CO, Ogbonna JD, Onunkwo GC (2012). Micromeritic and antinociceptive properties of lyophilized indomethacin-loaded SLMs based on solidified reverse micellar solutions. J. Pharm. Res. 5(6):3410-3416.
- Fukumori Y, Ichikawa H (2002). Fluid Bed Processes for Forming Functional Particles. In: James Swarbrick, James C, Boylan (Eds), Encyclopedia of Pharmaceutical Technology, 2<sup>nd</sup> ed., Vol 3. Marcel Dekker Inc., New York. pp. 1392-1400.
- Jia Y, Joly H, Omri H (2008). Liposomes as a carrier for gentamicin delivery: Development and evaluation of the physicochemical properties. Int. J. Pharm. 359:254–263.
- Kim JK, Park JS, Kim CK (2010). Development of a binary lipid nanoparticles formulation of itraconazole for parenteral administration and controlled release. Int. J. Pharm. 383:209–215.
- Lachman L, Herbert A, Liberman J (1990). *In: The theory and practice of Industrial Pharmacy*. Varghese publishing House, Hind Rajasthan Building Dadar Mumbai 400001, 3rd Edn. P 318.
- Mathew ST, Devi SG (2007). Formulation and evaluation of ketorolac tromethamine-loaded albumin microspheres for potential intramuscular administration. AAPS Pharm. Sci. Tech. 8(1):34–43.
- Ngwuluka NC, Idiakhwa BA, Nep EI, Ogaji I, Okafor SI (2010). Formulation and evaluation of paracetamol tablets manufactured using the dried fruit of *Phoenix dactylifera* Linn as an excipient. Res. Pharm. Biotech. 2(3):25-32.
- Ofoefule SI, Chukwu A (2002). Sustained release dosage forms: design and evaluation of oral products. In: Ofoefule S.I (ed.), Text Book of Pharmaceutical Technology and Industrial Pharmacy. Samakin (Nig.) Enterprises, Lagos. 94-120.
- Okoye EI, Onyekweli AO, Fatoki OO (2012). Evaluation of LD50 of Cashew Gum and the Comparative Study of its Functionality in Cotrimoxazole Granule and Tablet Formulations. Br. J. Pharm. Toxicol. 3(4):156-164.
- Ravi-Kumar MNV (2000). Nano and microparticles as controlled drug delivery devices. J. Pharm. Pharm. Sci. 3:234–58.
- Rawat SM, Singh D, Saraf S (2011). Formulation optimization of controlled delivery systems for antihypertensive peptides using response surface methodology. Am. J. Drug Dis. Dev. 1(3):174-187.
- Rhee YS, Park CW, Nam TY, Shin YS, Chic SC, Park ES (2007). Formulation of parenteral emulsion containing itraconazole. Arch. Pharm. Res. 30:114–23.
- Ritger PL, Peppas NA (1987a). A simple equation for description of solute release I. Fickian and non-Fickian release from non swellable device in the form of slabs, spheres, cylinders and discs. J. Cont. Rel. 5:23-36.
- Ritger PL, Peppas NA (1987b). A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J. Cont. Rel. 5: 37-42
- Sean CS ed (2011). Martindale: The Complete Drug Reference, (36<sup>th</sup> Edn). London: Pharmaceutical Press. ISBN 978 0 85369 840 1:289-291.
- Singh J, Gupta S, Kaur H (2011). Prediction of *in vitro* drug release mechanisms from extended release matrix tablet using SSR/SR<sup>2</sup> techniques. Trends App. Sci. Res. 6(4):400–409.
- Sinko PJ (2006). Martins Physical Pharmacy and Pharmaceutical Sciences; Physical, Chemical and Biopharmaceutical Principles in Pharmaceutical Sciences, (6<sup>th</sup> Edn.). Lippincott Williams and Wilkins, USA. 665-669.
- Umeyor EC, Kenechukwu FC, Ogbonna JD, Chime SA, Attama AA (2012). Preparation of novel solid lipid microparticles loaded with gentamicin and its evaluation *in vitro* and *in vivo*. J. Microencapsul. 1-12. DOI: 10.3109/02652048.2011.651495.
- Welling PG (2002). Absorption of drugs. In: James Swarbrick, James C, Boylan (Eds), Encyclopedia of Pharmaceutical Technology, 2<sup>nd</sup> ed., Vol 3; Marcel Dekker Inc., New York. 3606:1-21.
- Wissing SA, Kayser O, Müller RH (2004). Solid lipid nanoparticles for parenteral drug delivery. Adv. Drug Deliv. Rev. 56(9):1257–72.
- Yüksel N, Türkmen B, Kurdoğlu AH, Başaran B, Erkin J, Baykara T (2007). Lubricant efficiency of magnesium stearate in direct compressible powder mixtures comprising cellactose® 80 and pyridoxine hydrochloride. FABAD J. Pharm. Sci. 32:173-183.

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