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EFFECT OF TAMOXIFEN ON THE HISTOMORPHOLOGICAL AND BIOCHEMICAL COMPONENTS OF THE LIVER OF GUINEA PIGS

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ABSTRACT

Aim: Tamoxifen, once praised for its importance in preventing breast cancer recurrence, is now implicated in dangerous side-effects. The authors are not aware of studies on the effect of tamoxifen on the histomorphological and biochemical components of the liver of guinea pig, hence the present one.

Methods: Thirty guinea pigs weighing between 650g and 700g were assigned randomly into 6 groups of five and were separately given 0.70 and 7.0mg/kg body weight (b.w) tamoxifen for 2 and 4 weeks. Liver function tests and conventional tissue processing haematoxylin and eosin staining method were used in the study.

Results: The results showed a significant loss in body weight ($p<0.05$) of the guinea pigs administered with low and high doses of the tamoxifen for 4 weeks when compared with the controls. The mean serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin and conjugated bilirubin increased significantly than the controls in high dose groups exposed for 2 weeks and 4 weeks respectively ($p<0.05$). Similarly, histomorphological changes such as enlargement and sclerotic condition of central vein, enlargement and non-hexagonal radiating arrangement of sinusoids were observed in the liver of guinea pigs in the high dose tamoxifen

Key words: Tamoxifen, Liver, Breast cancer

INTRODUCTION

Tamoxifen (brand name Nolvadex) was developed in the late 1960's by the UK-based Imperial Chemical Industries (ICI), one of the world's largest multinational chemical corporations. Zeneca, an ICI subsidiary, is responsible for manufacturing and marketing the hormone (Sherrill, 1998). The use of tamoxifen for the treatment of cancers by oncologists began in 1970 and it is now the most widely prescribed cancer medication in the world. In the United States alone, about one million American breast cancer patients are currently being treated with the drug and about 20% of them have been on the drug for more than five years. According to Sherrill (1998), doctors now recommend tamoxifen for all pre-menopausal women with hormone-positive cancers and for most postmenopausal women with breast cancer and/or a growing number of women with hormone-negative cancers. Sherrill (1998) reported abnormal changes in the cells of the women taking tamoxifen. Activities of tamoxifen appear to be conflicting in the sense that whereas it acts as anti-eostrogen in the breast, it is also observed to act as eostrogen to the uterus and, to a lesser extent, the heart, blood vessels and the bone. Thus, tamoxifen which may show tendency to counter reoccurrence of breast cancer may end up actually promoting the aggressive types of liver and uterine cancers (Sherrill, 1998). Reports of acute hepatitis in patients treated with tamoxifen have been reported. A six-fold increase in liver cancer was reported among women taking tamoxifen for more than two years in the most human studies (Sherrill, 1998). Even Zeneca, the pharmaceutical company which produces tamoxifen was reported to have admitted that tamoxifen is a liver carcinogen, although it still pursues rigorous promotion of its use (Sherrill, 1998). Toxicity of tamoxifen to the liver has also been observed in every animal treated with tamoxifen. Gary Williams, medical director of the American Heart Foundation had demonstrated that tamoxifen is a "rip-roaring" liver carcinogen in his animal studies. He was able to induce serious aggressive cancers in

about 12% of rats (Clorfene-Casten, 1996). Murav'eva et al., (1982) studied the activity of tamoxifen on the sex organs of guinea pigs for long-term administration of tamoxifen (3-5 months) in a dose of 0.4 mg/kg to guinea-pigs with estrogen-induced benign uterine tumors and found out that the drug had no toxic effects on the animals. According to their report, tamoxifen caused no definite differences in the ovarian mass: however, 40% of the guinea-pigs showed ovulation inhibition. Evaluation of vaginal smears showed that most animals were in the stage of oestrus. Long-term intake of tamoxifen singly did not lead to the growth of uterine polyps or tumors. Tamoxifen independently or together with diethylstiboestrol stimulated a noticeable reduction in the level of free cytoplasmic estrogen and glucocorticoid receptors in the uterine of the guinea-pigs. In the course of long-term intake of tamoxifen alone, a tendency towards progesterone receptor decrease in the uterus was demonstrated. The study of the effect of the drug on liver function and the liver histomorphology are pertinent due to the principal function of the liver in metabolism and drug biotransformation hence the present study.

MATERIALS AND METHODS

Animals

Twelve adult female and eighteen adult male guinea pigs of about three months old, obtained from Egbu road Owerri, Imo State, Nigeria were used for the experiment. They were allowed to acclimatize for 2 weeks. They weighed between 650g and 700g. The animals were housed in cages under standard conditions of temperature (25 to 29^0C) and 12 hours light/dark cycle. They were maintained ad-libitium on water and commercial feeds (manufactured by Guinea feeds Ltd, Ewu, Edo State, Nigeria).The cages were cleaned and water and feed changed. The weights were taken after two weeks of acclimatization.

Drug Administration

The drug, 20mg tamoxifen tablet was purchased from Ovi's pharmaceuticals, Amakohia Uratta Owerri and manufactured by GENERICS (UK) LIMITED, It was dissolved in water to obtain the concentrations required and administered to the animals once daily by oral intubation for a period of 2 and 4 weeks.

Experimental Design

A total of 30 adult (12 female and18 male) guinea pigs were purchased for the research. They were randomly assigned into six equal groups (1 to 6), each group consisting of 5 (2 females and 3 males) guinea pigs. Group 1 received 7.0mg per kilogram body weight (7.0mg/kg. b.w.) tamoxifen for four weeks (4 weeks high dose). Group 2 guinea pigs were given 0.7mg/kg. b.w. tamoxifen for 4 weeks (4 weeks low dose). Group 3 received 7.0mg./kg. b.w. tamoxifen for two weeks (2 weeks high dose). Group 4 received 0.7mg/kg. b.w. tamoxifen for two weeks (2 weeks low dose). Group 5 animals were given 3.5ml normal saline for four weeks (4 weeks control). Group 6 received 3.5ml normal saline for two weeks (2 weeks control).The drug and normal saline were administered by oral intubation. The weights of the animals were taken before and after treatment.

Sample Collection and Processing

The animals were fasted for 24 hours after the last administration and sacrificed under chloroform anaesthesia. The blood samples were collected by cardiac puncture and put into centrifuge tubes. The blood was allowed to clot for about 30 minutes after which it was centrifuged at 2500rpm for 10 minutes using Wisperfuge centrifuge (model 1384 Sampson, Holland) and the sera subsequently retrieved for biochemical analysis. Livers of the Guinea pigs were excised, washed in normal saline to remove excess blood and immediately fixed in 10% buffered formalin to enable further histological investigations. The tissues were fixed in normal buffered formalin, thin slices cut from them and processed by the paraffin wax method according to the method of Ochei and Kolhalter (2004) and Baker et al., (1998). The tissues were sectioned at 5µ using a rotary microtome (KD-202 Wheel Microtome) and stained with H&E according to the method of Harris, 1990 cited by Baker et al., 1998. Blood samples were analyzed biochemically for Aspartate Aminotransferase (Reitman, 1957), Alanine Aminotransferase (Reitman and Frankel, 1957), alkaline phosphatase (Remnik, 2006) and bilirubin (Jendrassik and Grof method cited by Ramnik 2006).

Statistical Analysis

The results were expressed as mean standard deviations. Statistical analysis was carried out by one way Analysis of variance (One Way ANOVA). $P< 0.05$ was considered statistically significant.

RESULTS

Normal liver features were observed in guinea pigs which received low doses of tamoxifen (0.7mg/kg.b.w.) for a short period of time (2 weeks). The liver of the control guinea pigs (Groups 5 and 6) as well as those of Group 4 showed normal histology of the hepatocytes, sinusoids and central veins (figures 8-10). In contrast, the liver of those which received higher tamoxifen dose (7.0mg/kg. b.w.) for short period (2 weeks), the following abnormal features were observed: non-prominent sinusoids (Figure 1), hypertrophy and sclerosis of the central vein (Figure 2), disarray of hepatocytes (Figure 3). Similar observations were made on the histology of liver of Guinea pigs which received higher dose (7.0mg/kg. b.w.) for 4 weeks (Group 1). There was hypertrophy of sinusoids (Figure 4) and disarray of hepatocytes (Figure 5). There were clues to liver toxicity in Guinea pigs administered with high tamoxifen dose (7.0mg/kg.b.w.) and low tamoxifen dose (0.7mg/kg. b.w.) for 4 weeks. Table 1 shows the changes in the mean values of the initial and final body weights of the guinea pigs treated for two weeks and four weeks respectively. The tamoxifen did not have any significant effect ($P > 0.05$) on the mean values in body weights of the guinea pigs that received both the low and high doses for two weeks. However, there was a significant weight loss ($P < 0.05$) in the rats administered with low and high doses of the tamoxifen for 4 weeks when compared with the control. Table 2 and 3 show the changes in the mean values of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and conjugated bilirubin (CB) in low and high dose group administered with the tamoxifen for 2 and 4 weeks respectively. The study revealed a non-significant increase ($P>0.05$) in the mean values for AST, ALT, ALP, TB and CB in group 4 administered with low doses of tamoxifen (9.30 $\pm$ 1.71 IU/L, 8.00 $\pm$ 1.22 IU/L, 49.40 $\pm$ 8.77 IU/L, 0.46 $\pm$ 0.05mg/dl, and 0.20 + 0.10) When compared with those of control group 6 (8.20 $\pm$ 1.30 IU/L, 7.40 $\pm$ 0.89 IU/L, 46.60 $\pm$ 5.32 IU/L, 0.48 $\pm$ 0.04mg/dl and 0.22 $\pm$ 0.05mg/dl) respectively for 2 weeks (table 2). Similarly, the levels of AST, ALT, ALP, TB and CB in both high dose group 3 (table 2) and group 1 (table 3) which received 7.0mg/kg of the drug showed significant elevation ($P<0.05$) with mean values of (11.20 $\pm$ 1.29 IU/L, 9.80 $\pm$ 1.30 IU/L, 53.60 $\pm$ 8.17 IU/L, 0.64 $\pm$ 0.15mg/dl and 0.32 $\pm$ 0.08mg/dl for 2 weeks) and (12.60 $\pm$ 1.67IU/L, 10.60 $\pm$ 1.34 IU/L, 64.60 $\pm$ 11.15 IU/L, 0.72$\pm$ 0.08mg/dl and 0.42 $\pm$ 0.08mg/dl for 4 weeks) respectively when compared with the control group 6 and control group 5 with the mean values of (8.20 $\pm$ 1.30 IU/L, 7.40 $\pm$ 0.89 IU/L, 46.60 $\pm$ 5.32 IU/L, 0.48 $\pm$ 0.04mg/dl and 0.22 $\pm$ 0.05mg/dl for 2 weeks) and (8.80 $\pm$ 0.45 IU/L, 6.80 $\pm$ 0.84 IU/L, 51.80 $\pm$ 5.36 IU/L, 0.54 $\pm$ 0.09mg/dl and 0.30 $\pm$ 0.07mg/dl for 4 weeks) respectively.

Also, the present study recorded a significant increase ($p < 0.05$) in the mean values for AST, ALT, ALP, TB and CB (11.60 $\pm$ 1.51 IU/L, 9.60 $\pm$ 1.34 IU/L, 64.00 $\pm$ 10.51 IU/L, 0.70 $\pm$ 0.10mg/dl, and 0.40 $\pm$ 0.10mg/dl) in low dose group 2 which received 0.70mg/kg of the drug higher than those in control group 5 (8.80 $\pm$ 0.45 IU/L, 6.80 $\pm$ 0.84 IU/L, 51.80 $\pm$ 5.36 IU/L, 0.54 $\pm$ 0.09mg/dl and 0.30 $\pm$ 0.07mg/dl) respectively for 4 weeks (Table 3).

Table 1: Changes in body weight (g) of Guinea pigs administered tamoxifen for 2 weeks and 4 weeks.

Duration	Groups	Initial body weight	Final body weight
TWO	Group 6 (control)	682±4.1	684±3.6
WEEKS	Group 4 (0.7mg/kg)	680±3.5	683+3.7
	Group 3 (7.0mg/kg)	676+2.7	675±2.2
FOUR	Group 5 (control)	678±2.3	683±5.3
WEEKS	Group 2 (0.7mg/kg)	* 694±1.8	*689±2.1
	Group 1 (7.0mg/kg)	* 688±3.1	*676±2.8

Key: *=Significantly different from control.

Table 2: Biochemical parameters for short period of administration (2 weeks)

	Group 3 High Dose (7.00mg/kg)	Group 4 Low Dose (0.70mg/kg)	Group 6 Control	p-values
AST (IU/L)	*11.20 ±1.29	9.30±1.71	8.20±1.30	P<0.05
ALT (IU/L)	*9.80±1.30	8.00±1.22	7.40±0.89	P<0.05
ALP(IU/L)	*53.60±8.17	49.40±8.77	46.60±5.32	P<0.05
TB mg/dl	*0.64±0.15	0.46±0.05	0.48± 0.04	P<0.05
CB mg/dl	*0.32±0.08	0.20±0.10	0.22±0.05	P<0.05

Key: Aspartate Aminotransferase (AST); Alanine Aminotransferase (ALT); Alkaline Phosphatase (ALP); Total Bilirubin (TB); Conjugated Bilirubin (CB), *=Significantly different from control.

Table 3: Biochemical parameters for long period of administration (4 weeks)

	Group 1 High Dose (7.00mg/kg)	Group 2 Low Dose (0.70mg/kg)	Group 5 Control	p-values
AST (IU/L)	*12.6 ±1.67	*11.60±1.51	8.80±0.45	P<0.05
ALT (IU/L)	*10.60±1.34	*9.60±1.34	6.80±0.84	P<0.05
ALP(IU/L)	*64.6±11.15	*64.0±10.51	51.80±5.36	P<0.05
TB mg/dl	*0.72±0.08	*0.70±0.10	0.54±0.09	P<0.05
CB mg/dl	*0.42±0.08	*0.40±0.10	0.30±0.07	P<0.05

Key: Aspartate Aminotransferase (AST); Alanine Aminotransferase (ALT); Alkaline Phosphatase (ALP); Total Bilirubin (TB); Conjugated Bilirubin (CB), *=Significantly different from control.

Fig.1: Abnormal histology of the liver (High dose for 2 weeks). H&E (X100)

Fig. 2: Abnormal histology of the liver (High dose for 2 weeks) H&E (X100)

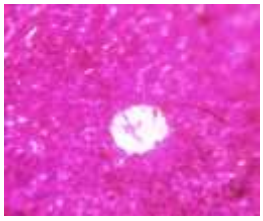

Fig. 3: Abnormal histology of the liver (High dose for 2 weeks) H&E (X400)

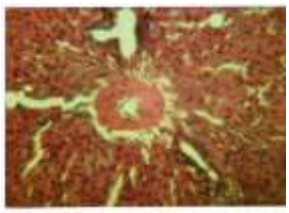

Fig.4: Abnormal histology of the liver (High dose for 4 weeks) H&E (X100)

Fig.5: Abnormal histology of the liver (High dose for 4 weeks) H&E (X100)

Fig. 6. Normal histology of the liver, (Low dose for 2 weeks) H&E (X100)

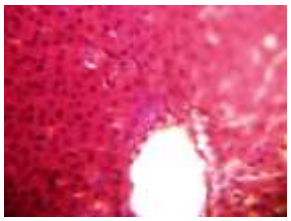

Fig. 7: Normal histology of the liver (Low dose for 2 weeks) H&E (X400)

Fig. 8: Normal histology of the liver (Low dose for 2 weeks). H&E (X100)

DISCUSSION

Tamoxifen is a competitive antagonist of the estrogen receptor that has been widely and globally used for treating breast cancer in premenopausal women and gynaecomastia in men receiving hormonal therapy for prostatic carcinoma (Rivera-Guevara and Camacho, 2011; Kennel et al., 2003 and Mati and Chen, 2003). Interestingly, the use of this anti-estrogenic drug has also proved to be effective against several protozoan parasites, including Leishmania major, L. braziliensis, L. chagasi, L. amazonensis, Trypanosoma cruzi (Eissa et al., 2011; Miguel et al., 2008; Miguel et al., 2009; Miguel et al., 2010) and Taenia crassiceps (Larralde et al., 1989). With the exception of its action on uterus of guinea pigs (Pasqualini et al., 1986), not much has been documented on tamoxifen's action on organs of guinea pigs. However, in humans and rats, it is known to be predominantly anti-estrogenic with residual estrogenic activities (Furr and Jordan, 1984). Histologically, evidence of inflammatory and immunological changes was observed in the guinea pig liver. These included hypertrophy and sclerotic condition of central vein and disarray of hepatocytes. Fatima et al., (2010) noted that the microscopical appearance of liver in rats receiving 20mg/kg b.w. of Nolvadex by an oral route for two weeks was characterized by vacuolar degeneration and hydropic degeneration. In contrast to this observation and apparent controversy, Kasahara et al., (2002) reported that no pathological changes could be noticed in the liver of rats treated with Nolvadex at dose level of 20 mg/kg b.w. for two weeks. According to Badawy et al., (2002) the treatment of rabbit with Nolvadex at dose level of 14 mg/kg b.w. daily for 60 days induced histopathological changes in the testis in the form of vacuolar degeneration of spermatogenic cells, atrophied and collapsed seminiferous tubules with azoospermia. The present study revealed a significant loss in body weight ($P < 0.05$) of the guinea pigs administered with low and high dose of the tamoxifen for 4 weeks when compared with the control (Table 1). Oze et al, (2010) reported the diuretic activity of a similar pharmacological agent in their toxicological study on experimental rats. The diuretic activity may result in loss of water and electrolytes especially sodium and consequently loss of body weight. The present findings also showed that the mean values of AST, ALT, ALP, Total Bilirubin and Conjugated Bilirubin of Guinea pigs in Group 2 (low dose for 4 weeks,) and those in Group 1 (High dose for 4 weeks) were significantly different ($P<0.05$) from those of Controls for the same period (Group 5, Control for 4 weeks, table 3). Similar results were obtained for AST, ALT, ALP, TB and CB levels in the high dose group 3 exposed for 2 weeks ($p<0.05$) according to Table 2. Tilkian et al., (1979) had enumerated the implications of elevation of liver function parameters. According to them, minor elevations of AST and ALT and ALP can be seen in cirrhosis, metastatic liver disease, hepatic disease or hepatic disease combined with other conditions. They noted that elevation of AST, ALT alongside with ALP as observed in the study indicates toxicity to the liver. Significant elevation of TB, CB and ALP were also associated with obstructive phase of hepatitis and lower biliary tree obstruction by either calculus or carcinoma. Thus high and low dose administration of tamoxifen to the Guinea pigs might have caused liver damage probably due to liver carcinoma. Earlier, Hirsimaki et al., (1993) reported that tamoxifen induces hepatocellular carcinoma in rat liver. The rate of the development of liver tumours in rats is strongly determined by the ability of tamoxifen to promote proliferation of hepatocytes, situation that has been associated with chronic cell death (Carthew et al., 1996). The present study has provided the evidence that administration of tamoxifen could be potentially hepatotoxic especially when prolonged, and more severe when the dose is high in guinea pigs. It is therefore recommended that caution should be applied with strict adherence to the recommended dose of tamoxifen for the treatment of breast cancer and other related health problems.

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