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Full Length Research Paper

Acute toxicity study of Carica papaya leaf extract in Sprague Dawley rats

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***Carica papaya* (CP) leaves are popularly used as food and have many traditional claims for herbal medicine. The leaf extract have been proven scientifically for their efficacy as wound healing and has protective effects against gastric damage in rats. However, toxicity study of the CP leaf extract is still lacking. The present study is to investigate the acute toxicity of CP leaf extract on Sprague Dawley rats at a dose of 2000 mg/kg body weight (BW). Sighting study was conducted in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg BW. No signs of toxicity and no deaths were observed. Based on the sighting study, we selected a dose of 2000 mg/kg BW and were observed for 14 days. The study includes control and treatment group, each consisting of 5 female rats. The single oral dose of the CP leaf extract did not produce mortality or significant changes in the body weight, food and water consumption. The relative weights of the internal organs were normal. However, hemoglobin (HGB), hematocrit (HCT), red blood cell (RBC) and total protein were significantly increase indicating dehydration. Apart from triglyceride, other biochemistry parameters demonstrated no significant changes as compared to the control.**

Key words: *Carica papaya*, leaf extract, acute toxicity.

INTRODUCTION

The *Carica papaya* (CP) is a member of the small family Caricaceae commonly grown in West Indies, Philippines, Sri Lanka, India, Bangladesh, Malaysia and other countries in tropical America. There are a lot of commercial products prepared from different parts of CP plant such as CP fruit juice, seed oil and supplement for health. The different parts of the CP plant (the fruits, leaves, latex and seeds) can be eaten and also have been used for medicinal purposes as claimed traditionally for treatment of different ailments (Wiart, 2002) and wound healing (Nor Suhada et al., 2008). Some of the traditional claims have been investigated scientifically using animal model and the efficacy have been proven (Indran et al., 2008; Imaga et al., 2009). Recent studies showed that CP leaf extract has been found to have potential anti sickling (inhibition of sickle cell formation) (Imaga et al., 2009) and has protective effect against gastric ulcer in rats (Indran et al., 2008). CP flowers are known to have antibacterial activities (Zakaria et al., 2006). CP seed extract oral administration could induce reversible male infertility and could be used for pharmaceutical development of a male contraceptive (Udoh et al., 2005). Acute and chronic toxicology of unripe fruit of the CP has been documented (Odoula et al., 2007). However, toxicology study of the CP leaf extract has not been carried out. Therefore, the present study is to investigate the acute toxicity of the CP leaf

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extract on Sprague Dawley rats.

MATERIALS AND METHODS

Test article

Test article is freeze dried CP leaf aqueous extract, supplied by Phytochemistry Unit, Herbal Medicinal Research Center. Young CP leaves from variety *Sekaki* were collected from a papaya farm in Salak Tinggi and the MARDI Experimental Plot in Selangor, Malaysia. The test article was stored in a refrigerator at 4°C before use. The dose were calculated based on the body weight of the rats and prepared in water before administering directly to the rats in the treatment group using intubation needle.

Test system and husbandry

Adult female Sprague Dawley (SD) rats, of age 6 to 7 weeks old, with body weight between 90 to 100 g (within ± 20% of the mean weight of individual rats) were used in the study. The animals were obtained from the Laboratory Animal Resources Unit, Medical Resource Research Center, Institute for Medical Research, Kuala Lumpur. The care and handling of the experimental animals are according to the Guidelines of Handling of Laboratory Animals by the Ministry of Health Malaysia (MOH, 2000). The study design and the use of the experimental animals were reviewed and approved by the Animal Care and Used Committee (ACUC) of the Institution (ACUC no.ACUC/KKM/02 (1/2009).

The experimental animals were housed individually in cages for the duration of the study. All the experimental animals were kept in one room and maintained under temperature and humidity of 27 ± 2°C and 65.85 ± 6.76% respectively. Room temperature and the humidity were monitored daily using a temperature and humidity data logger (TempRH Data logger BG-DL-01/01B). The SD rats were exposed to 12 h of artificial and natural light (alternating) with dark cycle. They were fed with certified rodent food (Zeigler Rodent NIH-31 Irradiated Auto Wafer Feeds from Zeigler Bros., USA) and drinking water was available *ad libitum* throughout the study. The experimental animals were acclimatized for 5 to 7 days before the commencement of the study and they were labeled appropriately.

Study design and selection of doses

Acute oral toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 420 (OECD, 2001). The study was initiated with a sighting study aimed to determine the dose for the acute toxicity study. The sighting study comprised of female SD rats dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. Starting with 5 mg/kg BW, the test article was administered orally to one rat. The rat was then observed for toxic effect for the first 30 min followed by hourly for 8 h for the first 24 h. If they are no signs of toxic effect or mortality observed on the rat within the 24 hours, we then dosed another rat with the next dose (50 mg/kg BW) and a similar procedure was carried out. A stepwise procedure was carried out until the highest dose, 2000 mg/kg BW is reached. If all the rats survived, they were monitored and observed once daily for the next 13 days. The sighting study showed that the rats dosed with 5, 50 300 and 2000 mg/kg BW with the test article survived. Based on this observation we decided to use the highest dose, 2000 mg/kg BW for the main test, the acute toxicity study. The acute toxicity study comprised of two groups, one control and one treatment group that consisted of 5 female rats in each group. Female rats were chosen because it is the most sensitive gender to see the effect of treatment (OECD, 2001; Lipnick et al., 1995). The treatment group received CP leaf extract that was diluted in water at a dose 2000 mg/kg BW given orally once, in a 2 ml volume. The control group received water delivered in the same volume and same procedure as the treatment group. The experimental animals were observed for 30 min after treatment, followed by observation hourly for 8 h and once daily for the next 13 days.

Physical observation and mortality

Clinical observation were made once a day for mortality, moribund, ill health or reaction to treatment, such as changes in skin and fur, eyes and mucus membranes, behavior pattern, tremors, salivation, diarrhea, sleep and coma.

Body weight, food and water consumption

The body weights (BW) of each rat were recorded once weekly. The differences of the BW were recorded. The amounts of test article to be given were calculated again weekly based on the new BW of the experimental rats to ensure a constant dose volume per kg BW of the test article given to the rats. The amounts of food place in the food tray were about 200 g and this amount was enough for a week. However, the amount of food left in the tray at the end of the week were calculated to obtain the amount of food consumed. The amounts of water placed in the bottle were 200 ml. The level of the water was measured weekly. The amounts of water consumed were calculated from this information.

Hematological and biochemical analysis

On the necropsy day, blood was withdrawn through cardiac puncture (or whenever possible) from the posterior vena cava of all rats under ether anesthesia. The blood was placed into EDTA bottles for hematological assay and in plain bottle for clinical biochemistry determination. The blood for hematological assay was immediately analyzed using a hematological analyzer (KX-21N Sysmex Cooperation, Japan). The parameters measured were white blood cell (WBC), hemoglobin (HGB), red blood cell (RBC), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte %, lymph no (lymphocytes number) and platelets (PLT). The blood in the plain bottle was allowed to stand for a minimum of 3 h for complete clotting. The serums were collected and transferred into another tubes to be centrifuged at 4 000 rpm at 4°C for 10 min. The serums were then kept at -20°C until analysis for clinical biochemistry measurements using the Vitalab Selecta, E-series, Netherlands. Clinical Biochemistry values determination were for liver profile (total protein, albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Renal profile parameters measured were urea and uric acid. Creatinine kinase (CK), lactate dehydrogenase (LDH) and α-hydroxybutyrate dehydrogenase (HBDH) were the cardiac profile parameters and other biochemistries were glucose and HDL-Cholesterol, cholesterol and triglycerides for lipid profile.

Relative organ weight (ROW)

All control and treatment rats were sacrificed using an overdose of ether on Day 15. A complete necropsy was performed. A comprehensive gross observation were carried out on the internal organs namely lung, heart, liver, stomach, spleen, gastro-intestinal tract (GIT), kidneys, ovaries, adrenals and urinary bladder. They

Table 1. Body weight (g), food consumption (g) and water intake (ml) by control and rats treated with CP leaf extract recorded during acute toxicity study.

	Body weight (g)		Food consumption (g)		Water intake (ml)	
	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
Control	190.00 ± 16.96	195.00 ± 31.02	84.00 ± 10.84	76.00 ± 30.08	227.00 ± 21.10	195.00 ± 7.07
2000 mg/kg	188.00 ± 16.43	208.00 ± 7.58	84.00 ± 8.94	95.00 ± 3.54	239.00 ± 2.24	198.80 ± 2.68

Values are expressed as mean ± standard deviation, n = 5.*p value less than 0.05, ($p < 0.05$) significant value.

were observed for any signs of abnormality and presence of lesions. The organs were then carefully dissected out, cleaned of any fats and weighed (absolute weight). The relative organ weight (ROW) of each organ was then calculated according to the following equation:

ROW =Absolute organ weight (g) / Body weight of rat on sacrifice day (g) x 100

Each organ was then preserved in 10% buffered formalin for subsequent histopathological examination.

Statistical analysis

All findings such as body weight changes, food and water consumption, hematology and blood chemistry were tabulated and analyzed. Statistical analysis involved use of the Statistical Package Sciences (SPSS) (14.0 Version). Data are expressed as the mean ± standard deviation. The mean value (x) and standard deviation (SD) were calculated for each variable measured and were analyzed statistically by analysis of variance (ANOVA) to determine significant differences between groups at $p<0.05$. The analysis and comparison were evaluated for significant at 5% ($\alpha = 0.05$). (Hinton, 2004)

RESULTS

Sighting study

The sighting study did not result with any signs of toxic effect at all, the dose level tested, 5, 50, 300 and 2000 mg/kg BW. All the rats survived.

Based on this observation we then selected a dose level of 2000 mg/kg BW for the main test (Acute Toxicity study).

Physical observation and mortality

The acute toxicity study did not result in any mortality of treatment rats and no toxic effect were observed throughout the 14 days study period. Physical observation of the test article-treated rats throughout the study indicated that none of the them showed signs of toxic effect such as changes on skin and fur, eyes and mucus membrane, behavior pattern, tremors, salivation, diarrhea, sleep and coma. No mortality was observed in any of the rats.

Body weight, food and water consumption

The body weight of the treatment and control rats were as shown in Table 1. There were gradual increases in body weight of treatment and control rats. The body weight of the treatment rats were not significant different as compared to the control rats.

The percentage increase in body weight of treatment rats measured weekly on Day 7 and 14 were found 11.24 and 11.00%, respectively. The food and water consumption of the treatment rats were also not significantly different as compared to the control rats measured throughout the study (Table 1).

Hematological and clinical biochemistry

Hematology and clinical biochemistry data are presented in Tables 2 and 3 respectively. Hematological values measured showed a significant elevation of HGB level and RBC level with $p=0.034$ and $p=0.015$ respectively in treatment group.

The value of HCT was significant increased as compared with the control group with $p=0.002$. Other hematology values, WBC, MCV, MCH, MCHC, Lymphocyte %, Lymphocyte no and PLT were not significantly different as compared to the control rats and they remained within normal limits (control values).

The clinical biochemistry values of total protein and triglycerides were elevated in the treatment rats as compared to the control rats, with significant value at $p=0.051$ and $p=0.032$ respectively (Table 3). Other clinical biochemistry parameters measured, albumin, ALP, AST, ALT, urea, uric acid, CK, LDH, HDL-cholesterol, cholesterol and glucose of the treatment rats were not significantly different as compared to the control.

Gross necropsy

Gross necropsy findings did not reveal changes in any of the organs examined. The relative organ weight per 100 g body weight recorded at the end of the study did not show any significant difference as compared with control (Table 4).

Table 2. Hematological values of control and rats treated with CP leaf extract measured during the acute toxicity study. The hematological data was measured by Hematology Analyzer, SYSMEX

Hematological values measured by Hematology analyzer, SYSMEX		
Hematology parameters measured	**Control**	**2000 mg/kg BW**
WBC (10^3/µL)	4.00 ± 2.40	3.94 ± 2.55
HGB(g/dL) *	13.28 ± 0.37	14.36 ± 0.87
RBC(10^6xµL)*	6.32 ± 0.43	7.08 ± 0.35
HCT (%) *	38.60 ± 1.43	42.36 ± 1.30
MCV(fL)	61.14 ± 2.21	59.86 ± 1.73
MCH(pg)	21.08 ± 1.07	20.28 ± 1.27
MCHC(g/dL)	34.42 ± 0.84	33.90 ± 1.43
Lymphocyte %	80.26 ± 7.65	88.12 ± 3.60
Lymphocyte no	3.24 ± 2.02	3.52 ± 2.35
PLT (10^3/µL)	975.40 ± 257.48	1028.40 ± 261.18

Values are expressed as mean ± standard deviation, *n* = 5. Group given 2000 mg/kg BW of CP leaf extract single dose orally and observed for 14 days. *p value less than 0.05, ($p < 0.05$): significant value, WBC; white blood cell, HGB; hemoglobin, RBC; red blood cells, HCT; hematocrit, MCV; mean corpuscular volume, MCH; mean cell hemoglobin, MCHC; mean corpuscular hemoglobin concentration, PLT; platelet, Lymphocyte no; Lymphocyte number.

Table 3. Clinical biochemistry values of control and rats treated with CP leaf extract measured during the acute toxicity study. The Clinical biochemistry data was measured by Vitalab Selecta, E-series, Netherlands.

Blood/serum biochemistry measured by Vitalab Selectra E-series		
Blood/serum biochemistry measured	**Control**	**2000 mg/kg BW**
Liver profile		
Total protein (g/L)*	150.00 ± 10.56	163.40 ± 7.64
Albumin (g/L)	39.02 ± 3.54	43.04 ± 2.07
ALP (U/L)	265.20 ± 60.30	270.40 ± 45.02
AST (U/L)	166.00 ± 25.33	171.60 ± 33.17
ALT (U/L)	47.40 ± 7.60	52.60 ± 3.85
Renal profile		
Urea (mmol/L)	5.89 ± 1.11	6.61 ± 1.11
Uric Acid (µmol/L)	160.26 ± 138.98	201.84 ± 113.80
Cardiac Profile		
CK (U/L)	1082.60 ± 78.33	1228.80 ± 457.34
LDH (U/L)	2467.60 ± 78.33	2239.0 ± 343.70
HBDH (U/L)	563.40 ± 70.00	577.40 ± 147.75
Lipid profile		
HDL-Cholesterol (mmol/L)	0.72 ± 0.09	0.85 ± 0.15
Cholestrol (mmol/L)	1.48 ± 0.19	1.70 ± 0.18
Triglycerides (mmol/L)*	0.80 ± 0.07	0.96 ± 0.12
Glucose (mmol/l)	9.55 ± 7.11	10.30 ± 2.36

Values are expressed as mean ± standard deviation, n = 5. ALP; alkaline phosphatase, AST; aspartate transminase, ALT; alanine aminotransferase, CK; creatinine kinase, LDH; lactate dehydrogenase, HBDH; α-hydroxybutyrate dehydrogenase, *p value less than 0.05, ($p < 0.05$) significant value.

DISCUSSION

The use of herbal preparations as a treatment of diseases is very common. In Malaysia, rural communities used herbs as food and traditional medicine. CP leaf extract has been used traditionally by some population as

Table 4. Values of control and rats treated with CP leaf extract measured during the acute toxicity study. The relative organ weight per 100 g body weight recorded at the end of the study.

Relative organ weight in gram per 100 g BW of control and rats treated with CP leaf extract.		
Organs	**Control**	**2000 mg/kg BW**
Lung	0.61 ± 0.20	0.60 ± 0.02
Heart	0.44 ± 0.03	0.39 ± 0.04
Liver	3.96 ± 1.13	4.06 ± 0.50
Stomach	0.75 ± 0.11	0.70 ± 0.08
Spleen	0.25 ± 0.05	0.24 ± 0.03
GIT	0.76 ± 0.16	0.64 ± 0.08
Kidney Left	0.38 ± 0.06	0.40 ± 0.05
Kidney Right	0.38 ± 0.06	0.41 ± 0.04
Ovary Left	0.03 ± 0.01	0.04 ± 0.01
Ovary Right	0.03 ± 0.01	0.03 ± 0.01
Adrenal Left	0.01 ± 0.00	0.02 ± 0.01
Adrenal Right	0.01 ± 0.01	0.01 ± 0.01
Urinary bladder	0.04 ± 0.02	0.03 ± 0.01

Values are expressed as mean ± standard deviation, $n = 5$.*p value less than 0.05, ($p < 0.05$) significant value.

digestive agent, food and skin healing. Some of these usages have been studied in *in vitro* and in animal model. Scientific evidence for their efficacy is widely studied but systemic safety studies are lacking. Therefore it is essential to evaluate the toxicity of the CP leaf extract in animals to ensure of its safety. The CP leaf extract did not affect the body weight of the treatment rats when compared to the control rats. Food and water intake of the treatment and control group were similar. The increase in body weight of both group weekly were about 11 g, which considered normal and gradually as observed in SD rats of similar age group in our previous studies and also other published reference (Taconic Technical Library, 2003). The increases in body weight were in line with the increase in food and water consumed by the rats. Gross pathological examination of treatment did not reveal any abnormalities, presence of lesions or changes in the color of the internal organs and the relative organ weight were not significant different to the control. For hematological parameter, HGB, RBC and HCT were found to be significantly increased as compared to the control rats.

The HGB level was increased in treated rats may result from increased in red blood cell production and increased in production of growth factors (Nancy, 2004). An increase in HCT could indicate an intracellular accumulation of water and it could sometimes appear in fasting rat (Hazelwood and Wilson, 1962) and may indicate spleen hyper function. However an increase in the following parameters (HGB, HCT and RBC) could be an indication of dehydration (Nancy, 2004). This is also supported by the findings of an increase of total protein in the treatment group which is another finding that indicate dehydration state (Edward et al., 1941). The other hematology parameters as well as biochemical parameters (except triglycerides and total protein) were normal. All this abnormal findings will be confirmed during the sub acute toxicity study. WBC, MCV, MCH, MCHC, lymphocyte %, lymphocyte no and PLT were not significantly different as compared to the control rats and fall within the normal range of the control rats. Liver profiles were not significantly different as compared to the control except for total protein value. The three most important and common liver enzyme in liver profile were AST, ALT and ALP (Pieme et al., 2006; Shashi, 2007) which were not affected by the administration of the CP leaf extract. Triglycerides level in the treatment rats was significant different as compared to the control rats. However, this value will be monitored in the sub acute toxicity study. Renal profile such as urea, uric acid, CK, LDH and HBDH were all normal as control group and indicated that there were no renal damage caused by CP leaf extract to the rats.

Conclusion

It was concluded that the acute toxicity study of CP leaf extract at 2000 mg/kg BW administered orally to Sprague Dawley rats did not caused any death or acute adverse effect on the clinical observation and mortality to the treatment rats. However, from the blood investigation, it showed that CP leaf extract consumption may cause dehydration as demonstrated by increased in HGB, HCT and RBC as well as total protein level. Other parameters except triglyceride were normal. This finding will be monitored in the sub acute toxicity study.

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