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Evaluation of Toxicological Effects Ethanol Extracts of *Mimosa pudica* in Adult Male Albino Rats

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Abstract

This study evaluated the *in vivo* toxicity effect of ethanolic extracts of *Mimosa pudica* in adult male albino rats. Acute toxicity test was carried out with Lorke′s method. Fifteen male rats weighing 130-160 g were used for the sub-chronic study. The rats were divided into three groups 1-3 (n=5), Group 3 served as control, Groups 1 and 2 received 200 mg/kg and 400 mg/kg body weight doses of the extracts respectively. Acute toxicity test carried out showed that the leaf extracts of *M. pudica* at a single dose of 5000 mg/kg produced treatment- related signs of toxicity and mortality in the test animals. The LD_{50} of this plant extract was 3808 kg/mg which is less than 5000 kg/kg. Sub-chronic oral toxicity study, the administration of 200 mg/kg and 400 mg/kg of *M. pudica* extracts per body weight revealed significant increase ($p < 0.05$) in the mean body weight, no significant difference ($p > 0.05$) for liver and renal function, oxidative stress and cardiac function, heamatological parameters, relative organ weight of the rats compared to the control groups. Biochemical parameters significantly increased ($p < 0.05$) in a dose dependent manner while haematological parameters significantly decreased ($p > 0.05$). Mild infiltration of the inflammatory cells and necrosis of the organs were observed. The use of leaf extracts of *M. pudica* is safe but intake of high doses and prolonged use may cause organ toxicity.

Keywords: *Mimosa pudica*, Toxicity, Mortality, Effects, Complication, Usage.

1. Introduction

Mimosa pudica commonly known as “touch and die” is a creeping annual or perennial herb often grown for its curiosity value, as the compound leaves fold inward when touched and reopen within minutes. This plant has other names such as Shame plant, Humble plant, Touch- me- not (Germplasm Resource information network, 2008), Sleeping grass (Tropical Biological Association., 2015) and Prayer plant. *M. pudica* is native to South and Central America. It is regarded as an invasive species in Tanzania, South Asia, South East Asia and many pacific Islands (Tropical Biology Association., 2015).

***Mimosa pudica* plant**

M. pudica has also been introduced to Nigeria, Seychelles, Mauritius and East Asia but not regarded as invasive. It is also found in tropics and sub-tropics, in Nigeria as common weeds widely distributed in open moist waste places and open grass land. *Mimosa* is usually a short prickly plant with its branches growing close to ground. The stem is erect in young plant, but becomes creeping or trailing with age, slender and well branched. It grows up to a height of about 0.5 m and spreads up to 0.3 m wide. The leaves are bipinnate (Saraswat and Pokharkar, 2012), fern-like and pale green in colour with tendency of closing when disturbed. *Mimosa* flowers are axillary in position and lilac pink in colour, usually occurring in globose heads. The calyxes are companulate and the petals are crenate towards the base. The fruits of *Mimosa* are pods, 1.5 – 2.5 cm long, falcate and closely prickly on sutures (Saraswat and Pokharkar, 2012). The fruit consists of clusters of 2-8 pods, breaks into 2-5 segments and contains pale brown seeds 2.5 mm long (US Forest Service, 2008).

Mimosa plant has a history of use for the treatment of various ailments. In folk medicine, it is used in arresting bleeding and in skin diseases. It has been reported to contain mimosine (an alkaloid), free amino acids, sitosterol, linoleic acid and oleic acid.

Several research works have showed the phytochemical components of this plant (Ahmad and Beg, 2001) and also about the antimicrobial activity of the plant (Ojalla *et al*., 1999). The leaves and stem of the plant have been reported to contain the alkaloid, mimosine. The leaves also contain mucilage and the roots contain tannins (Ghani, 1998). The leaves and roots are used in treating piles and fistula. Paste of the leaves is applied to hydrocele and cotton impregnated with juice of leaves is used for dressing Sinus. In Nigeria this plant has also been used in diseases arising from corrupted blood and bile, billious fever, piles, jaundice, leprosy, ulcers and small pox.

Mimosa is used for its hepato-protective (Gaurikarwani, 2011), hypolipidemic (Rekha, 2010), anti-convulsant (Ngo Bum, 2004), anti-depressant (Molina *et al*., 1999), antifertility (Maysumi Ganguly, 2007), anti- hepatotoxic (Nazeema and Brindha, 2009), wound healing (Dnyaneshwar *et al*., 2009) properties. Lozoya and Lozoya (1989) reported major chemical substances of interest from this plant as alkaloids and steroidal sapogenins. Methanolic extract of leaves of *M .pudica* showed the presence of bioactive components like terpeniods, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins and coumarin (Gandhiraja *et al*., 2009),. *M. pudica* leaves and roots revealed that tannin and protein (Ranjeet *et al*., 2013). The seed of the plant was also said have diuretic property (Krishnaraju *et al*., 2006). Also the roots of *Mimosa* contain tannin, ash, calcium oxalate crystals and alkaloid mimosine (Oudhia, *et al.*, 2006), antimicrobial activity (Gandhiraja *et al*., 2009) and wound healing activity of root of *M.pudica* (Kokane *et al.*, 2009, Muthusamy *et al*., 2008). The roots have anti-convulsant activity (Ngobum *et al.*, 2000; Burn *et al*., 2004). The present study was carried out to investigate the toxicological effects of *M. pudica* extracts on the morphology, biochemistry and histopathology of albino rats.

2. Materials and Methods

2.1 preparation of leaf extracts of *Mimosa pudica*

Fresh samples of *M. pudica* were collected from Imo state. The identification and verification of the plant species was conducted at Green Fingers Garden Okigwe Road Owerri, Imo State by a Taxonomist, Mr. Moore. The powdered *M. pudica* leaves were extracted by the solvent ethanol for a period of 72 hours. The extracts were concentrated *in-vacuo* at 40^{o} C, evaporated to dryness and the residues obtained were stored in a freezer at -80^{o} C until needed for further study. The given quantities were diluted in 1 % DMSO and distilled water and administered by oral gavage.

2.2 Acute oral toxicity testing

The acute toxicity profile was carried out and median lethal dose (LD_{50}) was calculated using method of Lorke (1983). Eighteen male rats aged 5- 9 weeks (130 g- 160 g) were used in this study. Nine rats (9) were divided into 3 groups of 3 rats each. *M. pudica* ethanol extracts were dissolved in 1% DMSO and administered to the rats orally in the first phase at a single dose of 10 mg/kg, 100 mg/kg and 500 mg/kg body weight. The rats were observed for behavioural changes and mortality. In the second phase, nine rats were divided into 3 groups of 3 rats each and administered orally at a single dose of 1000 mg/kg, 2900 mg/kg and 5000 mg/kg body weight and observed for behavioural changes and mortality.

2.3 Sub-chronic Oral toxicity testing

Fifteen male rats weighing 130 g -160 g were used in this study. The albino rats were divided into three groups labelled 1, 2 and 3 with each group consisting of five rats. Groups 1 and 2 were the experimental groups and Group 3 was the control group. Groups 1 & 2 were orally administered 200 mg /kg and 400 mg/kg body weight dose of ethanol extract of *M. pudica* dissolved in 1% DMSO daily for 28 days with the aid of an orogastric tube. The extracts were administered daily for twenty-eight days during which food and water were also given daily. The body weight of all the rats in the groups was recorded weekly.

2.4 Termination of the experiment

On the 29^{th} day, the rats were sacrificed by cervical decapitation and blood was collected by ocular puncture (media cantus) using ordinary capillary tube and cardiac puncture using syringe and needle. Blood samples were collected into non- heparinized and EDTA- containing tubes for biochemical parameters and haematological analyses, respectively. Each animal was laid on a dissecting board and a pair of scissors used to open the animal by cutting through vertical mid-line from neck to peritoneum (Osaro *et al*., 2016).

2.5 Organ weight and histopathology

The kidney, liver and heart were excised and weighed. Relative Organ Weight (ROW) was calculated by expressing absolute organ weight as percentage of the total body weight. The kidney, liver and heart were collected for histological studies. The tissues were washed in normal saline and fixed immediately in medium of 10 % solution of buffered formalin for 48 hours, dehydrated with alcohol, embedded in paraffin, cut into 4-5 µm thick sections, and stained with haematoxylin –eosin dye for photomicroscopic observation.

2.6 Statistical Analysis

All values were expressed as means ± SEM. Comparison between groups were performed using one way analysis of variance (ANOVA) and differences between treated groups and control accepted at $p<0.05$

3. Results

3.1 Acute oral toxicity

The acute oral toxicity result obtained showed that the ethanolic extract of *M.pudica* at a dose of 5000 mg/kg had adverse effect on the albino rats (Table 1). The LD_{50} of this plant was therefore estimated to be less than 5000 mg/kg**.** Result from the acute toxicity study may help in dose determination in animal studies, help to determine LD_{50} values that provide many indices of potential types of drug activity, help arrive at a dose of a new compound (Ukwuani *et al*., 2012). The acute oral toxicity study of ethanol extracts of *M. pudica* demonstrated mortality when the animals received orally up to 5000 mg/kg body weight of the extracts.

Table 1: Acute oral toxicity of ethanol extracts *M pudica.*

	M. pudica dosage (mg/kg) body weight	Mortality		*M. pudica* dosage (mg/kg) body weight	Mortality
Phase I			Phase II		
Group1	10	0/3	Group 4	1000	0/3
Group2	100	0/3	Group 5	2900	0/3
Group3	500	0/3	Group6	5000	2/3

3.2 Sub-chronic Oral Toxicity

Daily oral administration of *M. pudica* extracts for 28 days did not induce any obvious symptom of toxicity in rats, including the highest dose tested at 400 mg/kg body weight. No deaths or obvious clinical signs were found in any groups throughout the experimental period. Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioural changes, diarrhoea, tremors, salivation, sleep and coma. Weekly weight gains were observed during the study period compared to the control group.

3.3 The effect of ethanol extracts of *M. pudica* on body weights of albino rats.

The effect of ethanol extracts of *M. pudica* on the body weights of albino rats administered 200 mg/kg and 400 mg/kg, the results showed that there were significant differences in the mean body weight compared to control group (Fig.1)

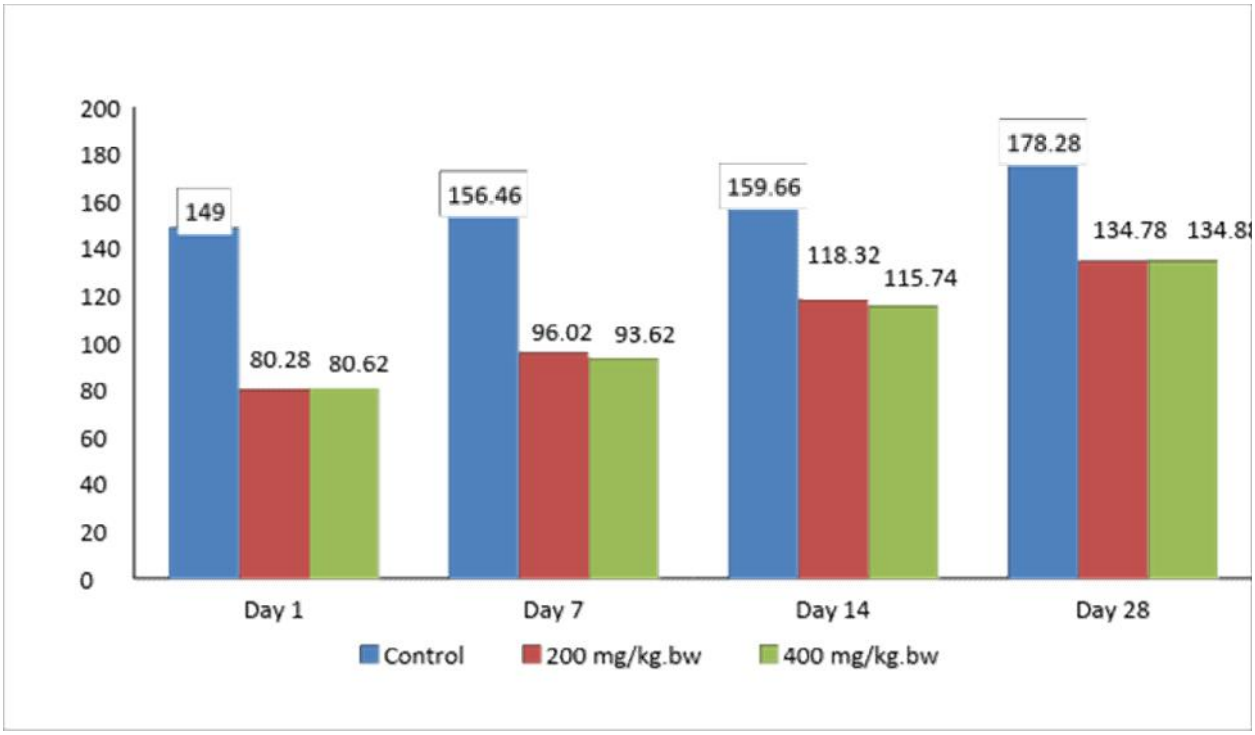

Figure 1: The effect of ethanol extracts of *M. pudica* on body weights of albino rats

3. 4 The effect of ethanol leaf extracts of *M. pudica* on liver and renal parameter in albino rats.

Result analysis of the effect of ethanol extracts of *M. pudica* on liver and renal parameter in albino rats showed that there was no significant difference in the liver and renal parameter compared to control. The result also showed that Total protein (TP), Albumin (ALB), Aspartate aminotransferase (AST) and urea activities significantly increased (P<0.05) in a dose-dependent manner when treated rats received doses 200 mg/kg and 400 mg/kg of the ethanol extract of *M. pudica* (Fig.2)**.** The Albino rats that received 200 mg/kg showed a higher Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities than those that received 400 mg/kg dose of the same extract compared to control group respectively. Creatinine activity was higher in treated groups with 200 mg/kg and 400 mg/kg doses than in control group. The result also showed that Total protein (TP), Albumin (ALB), Aspartate aminotransferase (AST) and urea activities significantly increased (P<0.05) in a dose-dependent manner when treated rats received doses 200 mg/kg and 400 mg/kg of the ethanol extracts of *M. pudica*. The Albino rats that received 200 mg/kg showed a higher Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities than those that received 400 mg/kg dose of the same extract compared to control group respectively. Creatinine activity was higher in treated groups with 200 mg/kg and 400 mg/kg doses than in control group

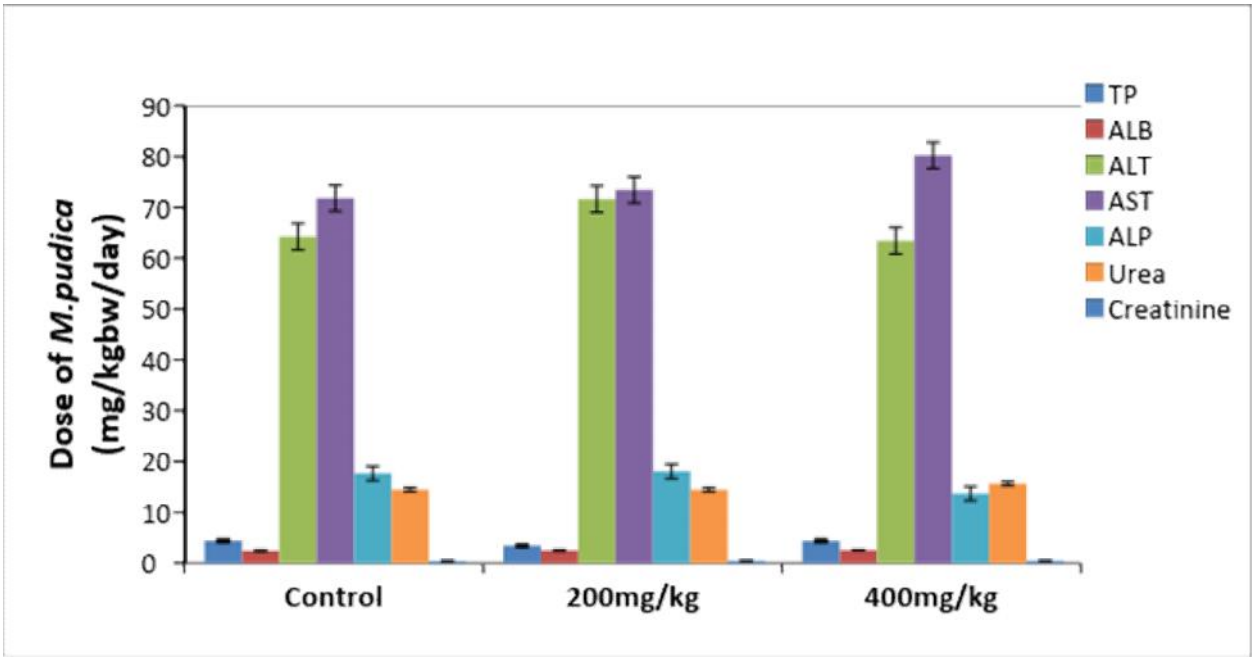

Figure 2: The effect of ethanol leaf extracts of *M. pudica* on liver and renal parameter in albino rats.

3.5 The effect of ethanol leaf extracts of *M. pudica* on oxidative stress and cardiac parameter in albino rats.

The result of the ethanol leaf extracts of *M. pudica* on oxidative stress and cardiac parameter in albino rats showed that there was no significant difference in the oxidative stress and cardiac parameter of the rats. The catalase (CAT), glutathione peroxidase (GPX), Reduced glutathione (GSH), Lactate dehydrogenase (LDH) activities significantly increased (P<0.05) in dose dependent manner compared to control group.(Fig .3). The rats that received 200 mg/kg dose of *M. pudica* had higher superoxide dismutase and C. Reactive protein (CRP) than those that received 400 mg/kg and the control. The lactate dehydrogenase (LDH) and glutathione peroxidase activities in rats that received 200 mg/kg and 400 mg/kg doses of *M. pudica* were higher in the treated groups and also in the control.

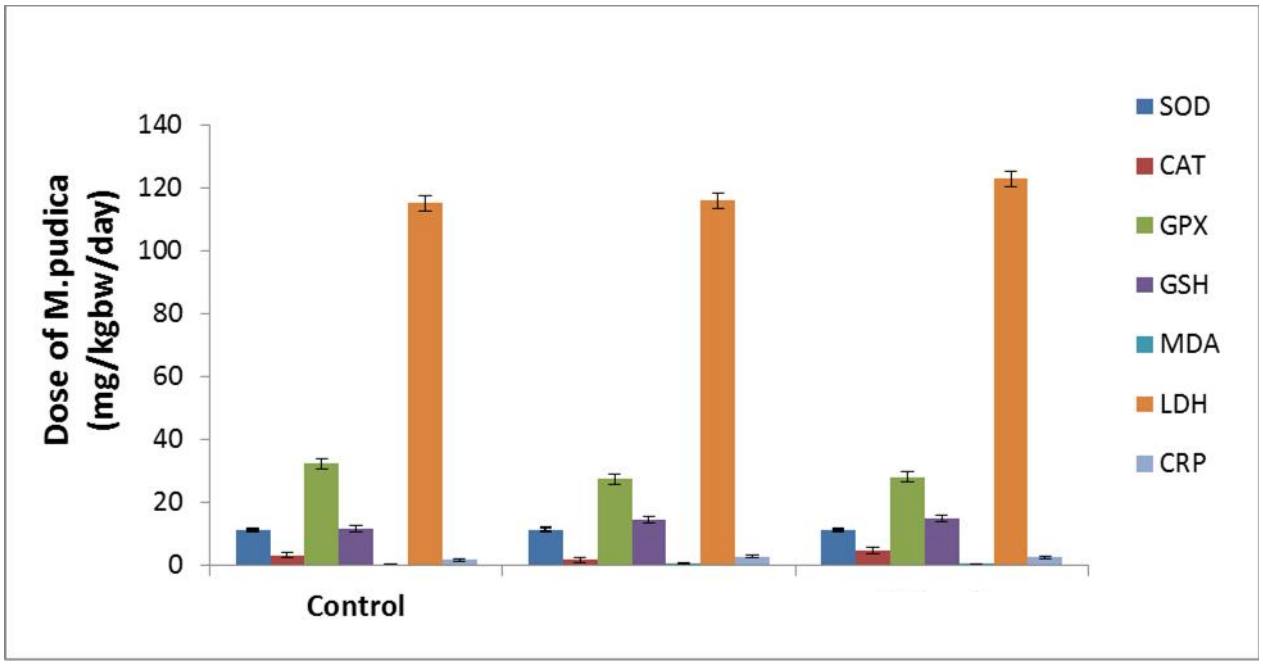

Figure 3: The effect of ethanol leaf extracts of *M. pudica* on oxidative stress and cardiac parameter in albino rats.

3.6 The effect of ethanol leaf extracts of *M. pudica* on haematological profile in Albino rats.

The result of the ethanol leaf extract of *M. pudica* on hematology profile in albino rats showed that there was no significant difference on hematological profile in albino rats. The concentration of the hemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), platelets and packed cell volume (PCV) significantly decreased (P>0.05) with increase in dosage (Fig.4). The concentration of the measured parameters was high in control group except hemoglobin (HB). The white blood cell concentration was higher in 200 mg/kg dose than in 400 mg/kg dose in treated group with *M. pudica* but highest in control group (Fig.4).

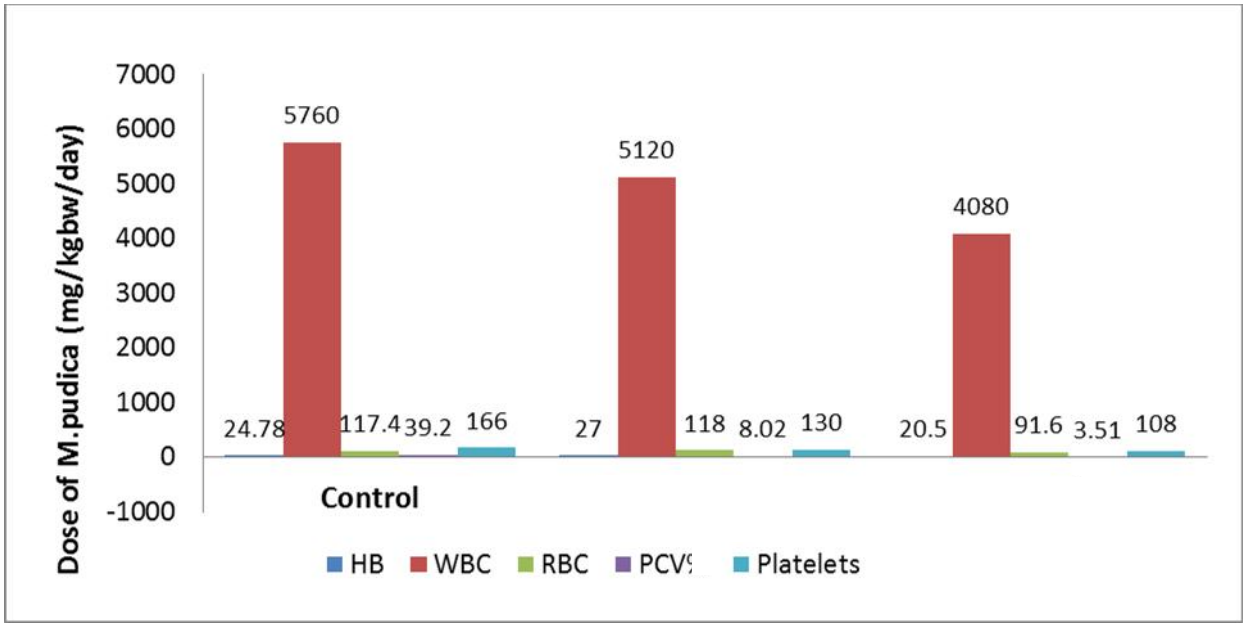

Figure 4: The effect of ethanol extracts of *M. pudica* on haematological profile in Albino rats

3.7 Relative organ weights of rats treated with ethanol leaf extracts in sub-chronic toxicity study.

The analysis showed that there was no significant difference ($p>0.05$) in the relative organ weights of each organ recorded in the treatment group compared to the control. The relative organ weight of the rats that received various doses of the ethanol extract of *M. pudica* was not significantly different ($p>0.05$) from that of the control group. The groups treated with 200 mg/kg and 400 mg/kg body weight of extracts showed no significant gain in weight of liver, kidney or heart.

Table 2: Relative Organ weights of Albino rats treated with *M. pudica* ethanol extracts in sub-chronic toxicity study.

Organ	Control	200 mg/kg	400 mg/kg	P- value
Heart	**0.435 ± 0.00707**	**0.3850 ± 0.00707**	**0.4000 ± 0.1414**	**0.005**
Liver	**2.9650 ± 0.00707**	**3.0050 ± 0.00707**	**3.0000 ± 0.02828**	
Kidney	**0.7250 ± 0.00707**	**0.7050 ± 0.00707**	**0.7350 ± 0.00707**	

Data are shown as Mean ± SEM (n= 5 for each group)

3.8 The effect of *M. pudica* leaf extracts (200 and 400 mg/kg) on liver histomorphology of albino rats in sub-chronic oral toxicity study.

Fig.5 (a & h) sections of the liver from the experimental animals showed normal hepatic histo-architecture. The liver collected from the experimental animals showed a mild infiltration of inflammatory cells into the periportal areas.

3.9 The effect of *M. pudica* extracts (200 and 400 mg/kg) on kidney histomorphologies of albino rats in sub-chronic oral toxicity study.

Fig. 5(c & d) the sections of the kidney showed mild inflammation and necrosis. Multifocal areas of tubular degeneration and necrosis with infiltration of the renal interstitium by inflammatory mononuclear phagocytes (Fig 5: c & d) involving mostly the outer medullar were observed. Sections of the kidney of group showed the normal renal histo-architecture for laboratory rodents.

3.10 Effects of *M. pudica* leaf extract (200 mg/kg and 400 mg/kg) on heart histomorphology of albino rats in sub-chronic oral toxicity study.

Fig.5 (e & f) sections of the heart showed mild infiltration of inflammatory cells into the highly vascularised myocardial connective tissue matrix. The sections of the heart showed a mild infiltration of inflammatory cells into the highly vascularised myocardial connective tissue matrix.

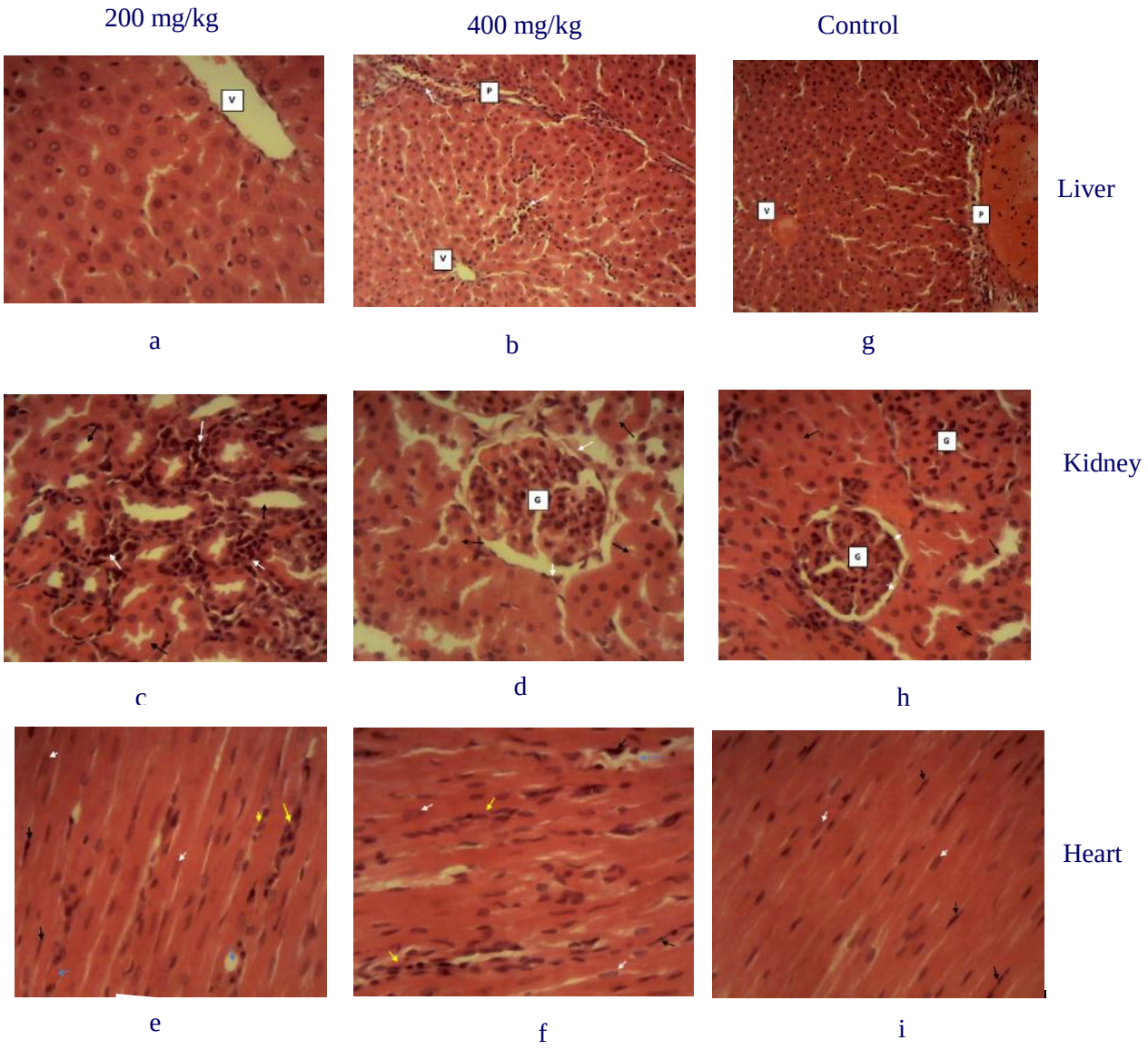

Fig. 5: Effects of the 200 mg/kg and 400 mg/kg of *M. pudica* extracts on various rat organs histomorphologies in sub chronic oral toxicity study, (a), (b) and (g): Liver; (c), (d) and (h): Kidney; (e), (f) and (i): Heart.

Legend: Glomerulus (G); Bowman's capsule (white arrow), Nucleus of cardiomyocyte (white arrow); Pericyte (black arrow), Blood vessel (blue arrow). Inflammatory cells in between myofibres (yellow arrow) renal tubules (black arrow, The nucleus of cardiomyocyte (white arrow); Pericyte (black arrow), Blood vessel (blue arrow). Inflammatory cells in between myofibres (yellow arrow). Magnification x400

4. Discussion

Oral administration of a dose of 500 mg/ kg of ethanolic extracts of the leaf of *M. pudica* showed acute toxicity on the tested albino rats. The LD_{50} of the extracts was found to be 3808 mg/kg.bw. The toxicity of *M. pudica* may be because of the mimosine constituent of the plant. Mimosine is a toxic amino acid that is a main constituent in the tropical to subtropical cultivated *M. pudica*. Mimosine and its metabolites 3-hydroxy-4- (14)- Pyridone (DHP), are toxic (Mauldin and Peter-Kennedy, 2016).

The effect of the ethanol leaf extracts of *M. pudica* on the body weight serves as a sensitive indication of the general health status of the animals. However, weight gains was observed in all animals administered with 200 mg/kg, 400 mg/kg of *M. pudica* in measured weekly weights of the albino rats for 28 days. However there was decrease in weekly weights of treated groups of albino rats when compared to control groups. It can be stated that *M. pudica* extract did interfere a little with the normal metabolism of

animals as corroborated by the significant difference from animals in the control group but not with treated groups. The treated group did not show any significant alteration in water or food consumption (data not shown). The weekly gain in body weight in treated groups could be as a result of increment in food and water intake. Loss of appetite is often synonymous with weight loss due to disturbance in the metabolism of protein, fat or carbohydrate (Ezeonwumelu *et al.,* 2011). Therefore, the increase in weights observed in treated groups is as a result of the normal food and water intake. This result is in consonance with earlier findings of Kwan *et al*., (2013), who carried out an investigation on the acute and sub chronic toxicity study of *Euphorbia hirta* L. methanol extracts in rats. They observed that increment in food and water intake is responsible for the body weight gain. Also they attributed increase in weekly body weight of the treated groups to be possibly the nutritional value of *M. pudica* extracts (Kwan *et al*., 2013; Ezeonwumelu *et al.,* 2011; Duke, 1997).

In evaluating the toxicity of drugs and plant extracts the assessment of the liver and kidney is a very important index, as both are necessary for the survival of the organism (Olurunisola *et al.,* 2012). Liver and renal kidney function indices evaluated in this study were TP, ALB, ALT, AST, ALP, urea and creatinine. Elevation in liver enzymes (ALT, AST, ALP) activity outcome are reported in liver diseases or hepatotoxicity (Brautbar and Williams, 2002) or liver injury.

The result of the effect of ethanol leaf extract of *M. pudica* on liver and renal parameter in albino rats showed that there were significant differences in the liver and renal function of the rats compared to control group. Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), urea and creatinine activities significantly increased ($P<0.05$) in a dose-dependent fashion when compared to the control group. Total protein (TP) activity was higher in control group than in treated groups. The rats that received 200 mg/kg and 400 mg/kg doses had a higher albumin (ALB) and Aspartate aminotransferase activities compared to the control group.

The significant increase in AST with *M. pudica* suggests that administration of higher doses of these extracts may induce the destruction of the liver cells. Also the kidney function indices evaluation for *M. pudica* correlates with the findings of Muhammad *et al.,* (2011), who carried out an investigation on the acute and sub-chronic toxicity of kernel extract of *Sclerocanya birrea* in rats. They reported that a significant increase in urea and creatinine was observed when the experimental rats received higher doses of the kernel extract of *Sclerocanyabirrea* ranging from 3000 to 4000 mg/kg body weight.

To determine the extent of the deleterious effect of *M. pudica* extract on the blood of the albino rats, the evaluation of the hematological parameters was used. And also to explain blood related functions of this plant extract or its products (Yakubu *et al.,* 2007). Such analysis is very important in risk evaluation as changes in the hematopoietic system have higher predictive value for human toxicity when the data generated are translated from animal studies (Olson *et al*., 2000). The effect of the ethanol leaf extracts of *M. pudica* on hematology profile in albino rats showed that there was no significant difference on hematological profile in albino rats. We observed that the concentration of the hemoglobin (Hb), white blood

cell (WBC), red blood cell (RBC), platelets and packed cell volume (PCV) significantly decreased ($P>0.05$) with increase in dosage. The concentration of these parameters was high in control group except for hemoglobin (HB). We also observed that white blood cell concentration was higher in 200 mg/kg dose than in 400 mg/kg dose in treated group with *M. pudica* but was highest in control group. Administration of the ethanol leaf extracts of *M. pudica*, showed no significant effect ($P>0.05$) in the hematological profile of rats suggesting that *M. pudica* may not be toxic to the blood system. But significant decrease ($P>0.05$) in hematological profile as the dose of extracts increased from 200mg/kg to 400mg/kg may be toxic to the blood system

The liver, Kidney and heart did not show any significant difference in weight. These insignificant increase and decrease in body weight could have been as a result of variation in size of internal organs (Chunlaratthaphorn *et al*., 2007), it may also be as a result of toxicity induced by the extracts.

The first hand indication of toxicity of chemical or biological substances is the hypertrophy of the organs. The microscopic examination of the liver, kidney and heart of the rats treated with various doses of *M. pudica* showed changes in colour compared to control. Hypertrophy observed in organs of rats treated

with *M. pudica*. The histopathological examination of the kidney and heart in experimental rats that were administered with 200 mg/kg body weight of the ethanol extracts of *M. pudica* revealed mild inflammation and necrosis of the kidney and heart sections respectively. Also the examination of the liver and heart in the experimental rats, that were administered with 400 kg/mg body weight of the ethanol extracts of *M. pudica* revealed mild inflammation of inflammatory cells into the periportal areas of the liver and into the highly vascularized myocardial connective tissue matrix of the heart.

Hepatotoxic chemicals/substances can cause necrosis which occur within distinct zones in the liver, either distributed massively or occur diffusely. Zonal necrosis is produced by many chemicals, necrosis that is confined to a specific zone of the hepatic acinus (Roberts *et al*., 2003). As observed in this study significant increase in serum Aspartate aminotransferase (AST) and Alkaline phosphate (ALP) may be due to hepatic necrosis. However, the liver has the ability to regenerate itself and this makes it able to withstand moderate zonal or diffuse necrosis. Over a period of several days, necrotic cells are removed and replaced with new cells and normal hepatic architecture and function were restored (Robert *et al*., 2003).

The lymphocytic infiltration in organs has been attributed to the presence of glycosides as reported by Adedapo *et al*., (2003). The result of this study is consistent with the findings of Builders *et al.*, (2012), who investigated the toxicity of *Parkia biglobosa* stem bark extract in rats and reported that the toxicity of some of the herbal medications might be a result of phytochemical constituents. Also Muhammad *et al*., (2011) reported that large tannin intake may cause kidney and liver damage.

5. Conclusion

These findings conclude that the ethanol leaf extracts of *M. pudica* is safe but the intake of high doses and also prolonged use cause organ toxicity.

Conflict of Interests

The authors of this paper have no conflict of interests

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