wjpmr, 2017,3(9), 08-13

SJIF Impact Factor: 4.103

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

BIOCHEMICAL EFFECTS OF ETHANOL LEAF EXTRACT OF *MIMOSA PUDICA* IN THIOACETAMIDE-INDUCED HEPATIC AND NEPHROTIC INJURY IN RATS

Dr. Sule O. J.*[1], Arhoghro E. M.[1] and Erigbali P.[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

[2]Department of Physiology, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

***Corresponding Author: Dr. Sule O. J.**

Department of Biochemistry, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Article Received on 31/07/2017 **Article Revised on 20/08/2017** **Article Accepted on 10/08/2017**

ABSTRACT

The study was to investigate the biochemical effect of ethanol extract of *Mimosa pudica* leaf in thioacetamide-induced albino rats by using liver and kidney serum marker enzymes. After the treatment scheduled period of 21 days, the extent of nephrotoxicity and hepatotoxicity with thioacetamide (400 mg/kg bwt.) was determined. Nephroprotective and hepatoprotective activity of ethanol extract of *Mimosa pudica* leaf was estimated from serum samples. In this study, we assess the serum creatinine, urea, bilirubin, AST, ALT and ALP using various standard kits. Administration of thioacetamide causes an increase in the level of creatinine, urea, bilirubin, ALT, AST and ALP, which are considered as the selective biomarkers of kidney and liver injury when compared with the normal control. The present study showed decrease in the elevated levels of these enzymes. Treatment with extract of *Nimosa pudica* at 200 and 400 mg/kg bwt. showed a dose dependent, significant decrease in creatinine, urea, bilirubin, ALT, AST and ALP ($P<0.05$) when compared with the thioacetamide control group. The histopathology findings supported the results. In conclusion, the results of this study showed that ethanol leaf extract of *Mimosa pudica* had nephroprotective and hepatoprotective activity against thioacetamide-induced nephrotic and hepatic injury in rats.

KEYWORDS: *Mimosa pudica,* nephrotoxicity, hepatoprotective, thioacetamide, creatinine.

1.0 INTRODUCTION

Mimosa pudica is native to South America and Central America. It is regarded as an invasive species in Tanzania, South Asia, South East Asia and many Pacific Islands. It has also been introduced to Nigeria, Seychelles, Mauritius and East Asia but is not regarded as invasive in those places.[1] *Mimosa pudica* is usually a short prickly plant with its branches growing close to ground. It grows up to a height of about 0.5 m and spreads up to 0.3m. The stem of mimosa is erect, slender, prickly and well branched. Leaves are bipinnate, fern like and pale green in colour with a tendency of closing when disturbed.[2] Flowers of this plant are axillary in position and lilac pink in colour usually occurring in globose heads. Flowering occurs from August to October in Indian conditions. Fruits of mimosa are pods, 1.5 to 2.5 cm long, falcate and closely prickly on sutures.[1] The preliminary phytochemical screening of the *M. pudica* leaf extract showed the presence of bioactive components such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins.[3,4,5] *Mimosa pudica* is known for its anti-hyperglycemic, anti-hepatotoxic, antidiarrhoeal, anti-convulsant and cytotoxic properties. This plant has been used in diseases arising from, fever, piles, jaundice, leprosy, ulcers and small pox.[6] Thioacetamide is an organosulfur compound with the formula C_2H_5NS. This white crystalline solid is soluble in water and serves as a source of sulfide ions in the synthesis of organic and inorganic compounds. Thioacetamide (TAA) has long been known as a hepatotoxicant since its biotransformation to thioacetamide sulfoxide (TAAS) occurs along the Cytochrome P-450 (CYP)-dependent pathway, which is a toxic reactive metabolite. This reactive metabolite covalently binds to liver macromolecules and dramatically increases the production of reactive oxygen species which then induce acute centrilobular liver necrosis.[7] The use of a variety of natural and synthetic antioxidants to prevent thioacetamide's toxicity has been considered and the combinations of thioacetamide with agents capable of blocking its reactive oxygen species mediated toxicity effect has been investigated.[8] The present study was carried out to investigate the biochemical effect of ethanol extract of *Mimosa pudica* leaf in thioacetamide-

induced albino rats by using liver and kidney serum marker enzymes.

MATERIALS AND METHODS

2.1 Chemicals/Reagents

The AST, ALT, ALP, Urea, Creatinine and Bilirubin kits used were purchased from Randox Laboratories limited, 55 Diamond Road, county Antrim, BT29 4QY, United Kingdom. All other reagents/chemicals obtained from standard supplies were of analytical grade.

2.2 Experimental Animals

Twenty (24) male Wistar rats weighing 150-200grams were used for this study. The animals were obtained from the Animal House in the Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Science, Niger Delta University, Bayelsa State, Nigeria. They were housed in standard cages and allowed free access to standard feds (pellets growers mash) and water for a period of one weeks for acclimatization. All the protocol was performed in accordance with the Institute Animal Ethical Committee (IAEC) as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCEA).

2. 3 Preparation of Ethanol Leaf Extract of Mimosa Pudica Leaf

The leaf of *Mimosa pudica* was used for this study. The plant was identified and confirmed by a botanist Prof Ajibeshin kolawole, Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University. The leaves were cleaned and made free from sand and shade dried for ten days. The dried leaves were ground into powdery form using a blender. 400grams of the powder was soaked in 4 litres of ethanol (95% v/v) which serve as solvent of extraction in a plastic bucket which was stirred in every four hours for 24hours before filtering. The filtrate was turned into beakers and evaporated to dryness at 37^0 C for 72hours. Appropriate weight (40g) of the residue was prepared in normal saline to obtain various concentrations of the extract that was administered orally to the rats for the 21days.

2.4 Experimental Designs.

Group 1 (Normal Control): Received pelleted growers mash and normal saline.
Group 2 (Positive Control): Received pelleted growers mash and normal saline.
Group 3 (Test Group): Received 200mg/kg bwt. of Mimosa pudica
Group 4 (Test Group): Received 400mg/kg bwt. of Mimosa pudica.

Animals in groups 2-4 were administered 400mg/kg bwt of thioacetamide i.p on the 18^{th} day.

Animals were sacrificed on the 22^{nd} day of study. Blood was collected into plain bottles for various biochemical analyses. The liver and kidney were harvested and fixed in 10% formalin for histological study.

2.5 Sample Collection

Biochemical analysis

Animals were sacrificed on the 22^{nd} day of study, using chloroform inhalation method and blood samples were collected from the animals through the jugular vein into plain bottles and allowed to clot and centrifuged at 3000rpm for 15 minutes. The resultant sera were used for biochemical analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Urea, Creatinine and Bilirubin according to appropriate standardized procedures using commercially available kits. The liver and kidney were excised using a midline abdominal incision, weighed and transferred into 10% neutral buffered formalin for histopathological examination.

2.6 Biochemical Assay

Serum transaminase (ALT and AST) was determined by method of,[9] ALP by Phenolphthalein monophosphate method.[10] Bilirubin was estimated by colorimetric method.[11] Serum Urea was estimated by[12] method, and serum creatinine by.[13]

2.7 Statistical Analysis

All data were expressed as Mean ± Standard deviation. The data obtained were analyzed using Two-way Analysis of Variance (ANOVA) using SPSS (Statistical Package for Social Sciences) Version 20. The means were separated and compared by post-Hoc and Turkey method. $P< 0.05$ was considered as statistical significant.

3.0 RESULTS

Effect of ethanol extract of *Mimosa pudica* leaf on Thioacetamide-Induced Albino Rats

Biochemical study

Serum Liver Markers: AST, ALT, Bilirubin

Administration of thioacetamide causes an increase in level of ALT, AST, ALP and Bilirubin, which are considered as the selective biomarkers of liver damage when compared with the normal control. The present study showed decrease in the elevated levels of these enzymes. Treatment with extract of *Nimosa pudica* at 200 and 400 mg/kg bwt. showed a dose dependent, significant decrease in ALT, AST, ALP and Bilirubin ($P<0.05$) when compared with the thioacetamide control group. (Table 1).

Serum Kidney Markers: Urea, Creatinine

Positive control group, showed elevated levels of Urea and Creatinine, which are important biomarkers of kidney damage when compared with the normal control. This study, indicated that extract treated groups (*Nimosa pudica* at 200 and 400mg/kg) significantly decrease the level of urea and creatinine in a dose dependent manner, ($P<0.05$), when compared with the thioacetamide control group. (Table 1).

Mean body weight, Mean Liver weight and Mean kidney weight

There was a decrease in mean body weight of rats in thioacetamide group compared with normal control. Although, no significant difference was obtained in the mean body weight of extract treated and positive control groups.

There was no significant difference in the mean liver weight of positive control compared with the normal control groups. However, treatment with extracts of *Nimosa pudica* significantly increases the mean liver weight of the test group3 and 4, compared with the thioacetamide group. ($p<0.05$). Mean kidney weight also decrease significantly in thioacetamide positive control, compared with the normal control. However, mean kidney weight of test group 3 significantly increase compared with the positive control ($p<0.05$). There was no significant difference in the mean kidney weight of test group 4, compared with thioacetamide treated group (Table 2).

Table 1: Effect of *Mimosa pudica* on thioacetamide-induced Liver and Kidney injury in rats.

	Group1	Group 2	Group 3	Group 4
	Normal Control	Positive Control (TAA)	200mk/kg Extract + 400mg/kg TAA	400mk/kg Extract +400mg/kg TAA
AST (U/L)	24.6 ± 59^{a}	48.7 ± 11.3^{c}	35.0 ± 3.20^{b}	27.4 ± 4.70^{a}
ALT (U/L)	32.6 ± 7.9^{a}	57.7 ± 4.4^{c}	47.6 ± 4.50^{b}	39.4 ± 4.80^{a}
ALP (U/L)	258.4 ± 30.9^{a}	346.5 ± 15.8^{c}	301 ± 18.90^{b}	250 ± 10.90^{a}
BIL (µmol/L)	1.10 ± 0.10^{a}	2.00 ± 0.40^{c}	1.26 ± 0.20^{b}	1.12 ± 0.10^{a}
Urea (Mmol/L)	48.63 ± 0.39^{a}	61.02 ± 1.49^{c}	55.72 ± 3.32^{b}	50.03 ± 2.52^{a}
Creatinine (Mg/dl)	3.40 ± 0.20^{a}	4.38 ± 0.16^{c}	3.99 ± 0.13^{b}	3.29 ± 0.11^{a}
Values are represented as Mean ± SD, n=6. Value with different superscripts (on the rows) from control are statistically different at $p<0.05$.				

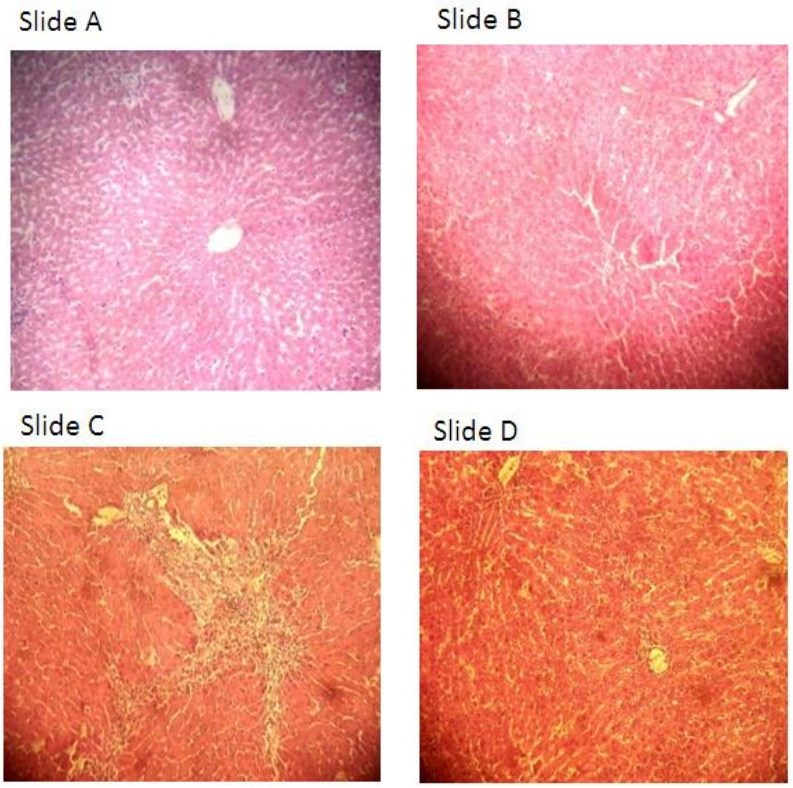
Table 2: Effect of *Mimosa pudica* on Mean Weights of Liver and Kidyney In thioacetamide-induced Liver and Kidney injury in albino rats.

Groups	Mean wt(g) rats day 1	Mean wt(g) rats day 21	Mean wt(g) rats liver	Mean wt(g) rats kidney
G1 Normal Control	183 ± 9.7^{a}	202.0 ± 14.8^{b}	6.27 ± 4.6^{a}	1.68 ± 2.6^{a}
G2 Positive Control	178.8 ± 25.5^{a}	175.0 ± 10.0^{a}	6.38 ± 2.2^{a}	1.42 ± 1.2^{b}
G3 400mk/kg Extract 200mk/kg of TAA	193.3 ± 11.3^{a}	186.7 ± 11.5^{a}	7.15 ± 3.2^{b}	1.51 ± 2.2^{a}
G4 400mg/kg Extract +400mg/kg of TAA	$17I.0\pm11.9^{a}$	172 ± 10.9^{a}	6.70 ± 4.1^{b}	1.36 ± 1.1^{b}
Values are represented as Mean ± SD, n=6. Value with different superscripts from control are statistically different at $p<0.05$.				

Histopathological changes on Doxorubicin-induced Albino Rats

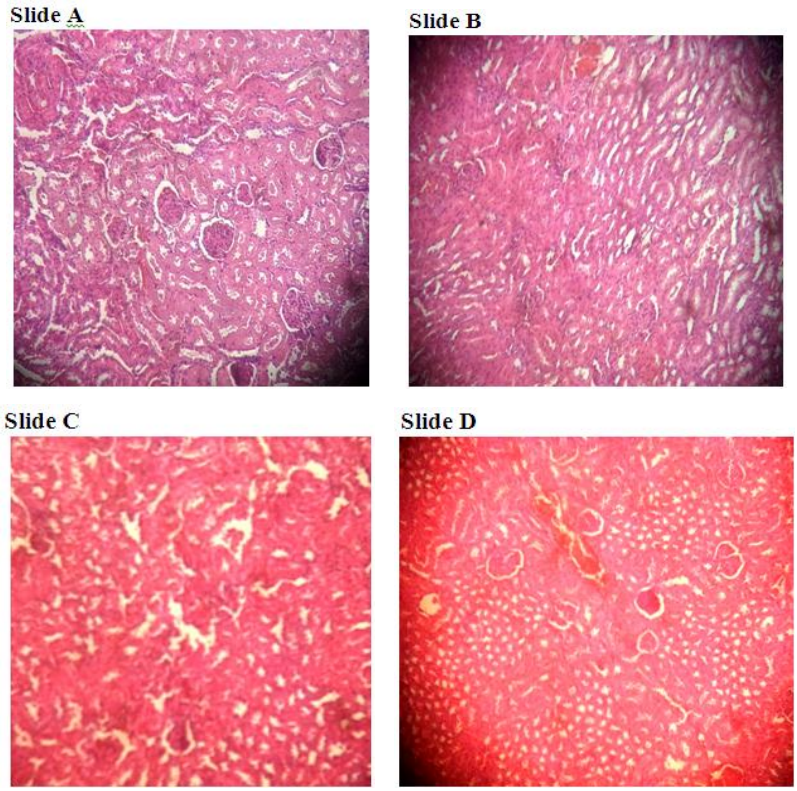
Figure 1 demonstrates the effects of *Mimosa pudica* leaf extract on histological features of livers of experimental rats: Histopathology of the liver in normal control and test groups showed normal hepatic architecture while the positive control showed enlarged hepatocyte sheets, hence localized closure of sinusoid.

Effects of *Mimosa pudica* leaf extract on histological features of kidney of experimental rats as shown in Figure 1. Histopathology of the kidney in normal control and test groups revealed normal kidney stroma with Bowman's capsule with glomerulus (renal corpuscle) and renal tubules while positive control showed intense distortion of tissue architecture.



Haematoxylin and Eosin, X100.

Fig. 1: Histopathological images of liver pretreated with ethanol extract of *Mimosa pudica* in thioacetamide-induced albino rats. A: Normal control, B: Positive control, C: Test group – 200 mg/kg, D: Test group – 400 mg/kg.



Haematoxylin and Eosin, X100.

Fig. 2: Histopathological images of kidney pretreated with ethanol extract of *Mimosa pudica* in thioacetamide-induced albino rats. A: Normal control, B: Positive control, C: Test group – 200 mg/kg, D:Test group – 400 mg/kg.

DISCUSSION

The present study was carried out to investigate the biochemical effect of ethanol extract of *Mimosa pudica* leaf in thioacetamide-induced albino rats by using liver and kidney serum marker enzymes. Administration of thioacetamide causes an increase in the level of ALT, AST, ALP and Bilirubin, which are considered as the selective biomarkers of liver damage when compared with the normal control. The findings is in agreement with[14] who reported that high doses of thioacetamide causes significant increases in AST, ALT, ALP activities and plasma bilirubin levels in wistar and Lewis rats, with maximum levels observed 48hrs after thioacetamide administration. Thioacetamide (TAA) has long been known as a hepatotoxicant since its biotransformation to thioacetamide sulfoxide (TAAS) occurs along the Cytochrome P-450-dependent pathway, which is a toxic reactive metabolite. This reactive metabolite covalently binds to liver macromolecules and dramatically increases the production of reactive oxygen species which then induce acute centrilobular liver necrosis.[7] The findings of this study is further supported by another study which indicates that the release of transaminases (AST and ALT) from the cell cytosol can occur secondary to cellular necrosis and the activity of AST and ALT is significantly increased in such cases and escapes to the plasma from the injured hepatic cells.[15] The present study showed decrease in the elevated levels of these enzymes. Animal treated with extracts of *Mimosa pudica* at 200 and 400 mg/kg bwt. showed dose dependent, significant decrease in ALT, AST, ALP and Bilirubin when compared with the thioacetamide control group. This result agrees with that of[16] and,[17] which shows that the methanolic extract of *Nimosa pudica* leaves have hepatoprotective and anti-ulcer properties against thioacetamide/ethanol induced ulcers in rats.[18] also, reported that the ethanol extract of *Mimosa pudica* leaves have hepatoprotective effect against carbon tetrachloride-induced liver damage in wistar albino rats.

The presence of antioxidant constituents such as flavonoids and tannins might be responsible for the free radical scavenging and antioxidant activity of the *Mimosa pudica*, which have been responsible for its inhibitory activity.[19] The preliminary phytochemical screening of the *Mimosa pudica* leaf extract showed the presence of bioactive components such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins.[3,4,5] Thioacetamide control, showed elevated levels of Urea and Creatinine, which are important biomarkers of kidney damage when compared with the normal control. Renal diseases which diminish the glomerular filtration lead to urea retention and decrease in urea excretion is seen in severe liver disease with destruction of cells leadings to impairment of the urea cycle.[20] Creatinine is synthesized in the liver, passes into the circulation and is taken up by skeletal muscle. Its retention in the blood is evidence of kidney impairment. This study, indicated that extract treated groups (*Nimosa pudica* at 200 and 400mg/kg) significantly decrease the level of urea and creatinine in a dose dependent manner, ($P<0.05$), when compared with the thioacetamide control. The presence of antioxidant constituents such as flavonoids and tannins might be responsible for the nephroprotectivity of the extract. This is also in agreement with,[21] who reported that the ethanolic extract of *Mimosa pudica* leaf showed significant protective and curative effect against gentamicin-induced nephrotoxicity.

The effects of *Mimosa pudica* leaf extract on histological features of liver of experimental rats indicates that the normal control and test groups showed normal hepatic architecture while the positive control showed enlarged hepatocyte sheets and localized closure of sinusoid. *Mimosa pudica* leaf extract on histological features of kidney of experimental rats also showed that the normal control and test groups are normal kidney stroma with Bowman's capsule with glomerulus (renal corpuscle) and renal tubules while positive control showed intense distortion of tissue architecture.

In conclusion, the results of this study showed that ethanol leaf extract of *Mimosa pudica* had nephroprotective, and hepatoprotective activity. This was due to the antioxidant activity of the extract, but further investigations are essential to find out the exact mechanism of nephroprotection and hepatoprotection activity of *Mimosa pudica* against thioacetamide-induced hepatotoxicity.

REFERNCES

1. Saraswat, R. and Pokharkar, R. GC-MS Studies of *Mimosa pudica*. Inter*national Journal of Pharmacological Technological Research*, 2012; 4(1): 93-98.
2. Amador-Vegas, J. and Dominguez, A. "Leaf-folding response of a sensitive plant shows context-dependent behavioral plasticity". *Plant Ecology*, 2014; 4: 4-7.
3. Wesley, J. C. and Nadar, C. S. Chidambaranathan. Pharmacognosy and Phytochemistry Mimosa pudica (Linn) in male wistar rats. *International Journal of Pharmaceutical Innovations*, 2013; 3(4): 41-46.
4. Jha, N. K. *Mimosa pudica*: Lajjalu. *Phytopharmacology*, 2007; 8: 3-8.
5. Pande, M. and Pathak, A. Preliminary pharmacognostic evaluations and phytochemical Studies on roots of *Mimosa pudica* (Laajvanti). *International Journal Pharmacology Science Review Restriction*, 2010; 1: 50-52.
6. Ghani, A. Medicinal plants of Bangladesh with chemical constituents and uses. *Asiatic Society Bangladesh, Dhaka*, 2003; 66-434.
7. Koen, Y.M., Sarma, D., Hajovsky, H., Galeva, N., Wiliams, T., Staudinger, J. and Hanzlik, R. Proteintargets of thioacetamide metabolites in rat hepatocytes. *Chemical Restrials Toxicology*, 2013; 26: 564-574.

8. Chegaev, K., Riganti, C., Rolando, B., Lazzarato, L., Gazzano, E., Guglie., Stefano., Ghigo., Fruttero, R. and Gasco, A. Doxorubicin-antioxidant Co-drugs. Bioorganic and Medicinal Chemistry Letters. *ELSEVIER*, 2013; 23, 5307-5310.
9. Reitman, S., and Frankel, s. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Mannal. *American Journal Of Clinical Pathology*, 1957; 28: 56.
10. Babson, L.A. Estimation of Alkaline Phosphatase Activity. Clin. Chem., 1965; 11: 789.
11. Jendrassik, L. and Grof. P. Bilirubin Manual (BIL). *Biochem. Z*, 1938; 297: 81.
12. Natelson S, Scott ML and Beffa, CA. Rapid method for the estimation of urea in biologic fluids. *Am. J. Clin. Pathol.*, 1951; 21: 275.
13. Jelliffe RW. Estimation of creatinine clearance when urine cannot be collected. *Lancet*, 1971; 1:875.
14. Koblihova, E., Mrazova, I., Vernerova, Z., and Ryska, M. Acute liver failure induced by thioacetamide: Selection os optimal dosage in Wistar and Lewis rats. *Physiological Restrition*, 2013; 63: 491–503.
15. Gaskill, C.L., Miller, L.M., Matton, J.S., Hoffmann, W.E., Burton, S.A., Gelens, H.C.J.,Ihle, S.L., Miller, J.B., Shaw, D.H., Cribb, A.E. Liver histopatholoy and liver serum alamine aminostranderase and alkaline phosphates activities in epileptic dogs receiving phenobabital. *Veterianary Pathology*, 2005; 42: 147-60.
16. Sanaye, M. M., Joglekar C. S. and Pagare, N. P. *Mimosa*- A brief overview. *Journal of Pharmacognosy and phytochemistry*, 2015; 4(2): 182-187.
17. Elango, V., Carolin, O. and Raghu, P. S. Antiulcer activity of the Leaf ethanolic extract of *Mimosa pudica* in Rats. *Hygiene Journal Dietary Medicine*, 2012; 4(1): 34-40.
18. Muthukumaran, P., Pattabiraman, K. and Kalaiyarasan, P. Hepatoprotective and antioxidant activity of *mimosa pudica* on carbon tetrachloride-induced hepatic damage in rats. *International Journal of Current Research*, 2010; 10: 046-053.
19. Cean Socorro M. Alaba and Christine L. Chichioco-Hernandez. 15-Lipoxygenase inhibition of Commelina benghalensis, Tradescantia fluminensis, Tradescantia zebrina. *Asian Pac J Trop Biomed*, 2014 Mar; 4(3): 184–188. / doi: 10.1016/S2221-1691(14)60229-X.
20. Ranjna C. Practical Clinical Biochemistry Methods and Interpretation, 1999; 2nd Edn: 117.
21. Geetha Karra., Nadendla Ramarao., Sindhu, B. and Umamaheshwera Rao, V. Nephroprotective, Nephrocurative activity of Mimosa pudica root against gentamicin-Induced Nephrotoxicity. *International Journal of Pharmacy and Pharmceutical Sciences*, 2015; 7(4):173-177.