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HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA* AGAINST THIOACETAMIDE INDUCED TOXICITY IN THE LIVER OF ALBINO RATS

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ABSTRACT: The present work was carried out to check hepatoprotective potency of ethanolic extract of *Andrographis paniculata*. The whole plant extract of *A. paniculata* was used for the treatment of toxicity induced in the liver of albino rat, *Rattus norvegicus* by using thioacetamide (TAA). Thioacetamide toxicity in the animal was manifested by the significant increase in the level of serum bilirubin, ALP, SGPT, and SGOT while significant decrease in liver protein. Administration of ethanolic extract of *A. paniculata* at different doses(200 mg and 250 mg/kg b. w. showed significant decrease in the level of serum bilirubin, ALP, SGPT, and SGOT but increase in the level of protein in the liver when compared with induced rats. These results thus proved the potential hepatoprotective effect of ethanolic extract of *A. paniculata*.

KEYWORDS: Thioacetamide, Hepatotoxicity, *A. paniculata*, Protective effect.

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1.INTRODUCTION

Hepatic disease is a term used for collection of conditions, diseases and infections that affect the cells, tissues, structures or functions of the liver. There are about 20,000 deaths have been reported every year due to liver diseases [1]. The consumption of alcohol, exposure to environmental toxins, over drug consumption lead the liver in various ailments like hepatitis, cirrhosis etc. [2]. Thioacetamide is one of such toxic chemical used in synthesis of organic compounds such as rubber chemicals, curing agents, cross linking agents, metallurgy, pesticides, and pharmaceuticals [3]. It is widely used in the study of underlying hepatic fibrogensis and therapeutic effects of potential

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antifibrosis drugs. It is water soluble and easily administrated orally by dissolved in drinking water [4]. It causes severe centrilobular necrosis and also induces apoptasis and periportal inflammatory cell infiltration in the liver. The initiation of the hepatotoxic effect of TAA requires metabolic activation [5] [6] [7]. The use of folk medicine for disease management is increasing globally because of their easy access, low risk, low cost and minimum side effects. There are about 80% of world population today depend on herbal medicines for their health needs [8]. The rural populations especially the people from low income group mostly rely on use of herbal medicines. Number of medicinal preparations has also been mentioned in ayurveda for the treatment of number of diseases including liver disorders [9]. *A. paniculata* is one of the most popular medicinal herbs. It is commonly known as 'king of bitter' which is a annual herb similar in appearance and in taste as that of Neem, *Azadirachtra indica*. It is used in several traditional system of medicine in all over the world [10]. It is used in treatment of gastrointestinal tract, upper respiratory infection, herpes, fever, hepatitis, sore throat and various other chronic and infectious diseases [11]. Therefore, the present study was designed to investigate the effect of ethanolic extract of whole plant of *A. paniculata* against thioacetamide induced hepatotoxicity in albino rats of either sex.

2. MATERIALS AND METHODS

2.1 Animals

The healthy adult Wistar rats (130 – 150gm) were procured from Hindustan Antibiotic Ltd., Pune and were acclimatized in laboratory conditions at Rajarambapu College of Pharmacy, Kasegaon for two weeks (Regi. No. 209/CPCSEA). They were fed with Amrut rat feed obtained from Pranav Agro Industries, Pvt. Ltd., Sangli and water ad libitum. All the investigational measures were carried out in accordance to the guidelines of Institutional Animal Ethics Committee.

2.2 Plant Material

A. paniculata is annual herbaceous plant. It grows straight to a height of 30-110 cm. in wet shady places. The root is tap root. The stem is slender, dark green in colour and squared. The leaves are simple, glabrous, lanceolate, hairless blades measuring about 4 – 12 cm. long and 1 – 3 cm wide. The flowers are small, white with rose purple spot on the petals. The flowers are scattering axillary and terminal racemes. The fruits are capsule about 2 cm. long and it contains yellow brown seed. Flowering and fruiting time is between May to October.

2.3 Preparation of Injectable solution of drug

Injectable solution of TAA (Sigma Aldrich, Switzerland) was prepared freshly by dissolving TAA crystals in sterile, distilled water with constant stirring until all crystals were dissolved. The injectable TAA solution (200 mg / kg body weight) was administered intraperitoneally (i.p.) to rats thrice a week for about 8 weeks [12].

2.4 Collection of plant material and extraction

2.4.1 Collection of plant material

A. paniculata plant was obtained from the Botanical garden of Krishna Mahavidyalaya Rethare BK. It was authenticated by Dr. C. B. Salunkhe, Department of Botany, Krishna Mahavidyalaya Rethare BK. The voucher specimen (Collection No. KMR 1719) has been kept in our laboratory for future reference.

2.4.2 Preparation of extracts

Freshly collected whole plant material of *A. paniculata* was washed and shed dried for about 45 days and was subjected to maceration to form crude powder which was used for extraction. The powdered plant material was extracted with 50%ethanol by using Soxhlet Apparatus. The extracts were concentrated and dried at 60^0c and kept at 4^0c for further studies. Fresh solution of *A. paniculata* was prepared at the time of experimentation.

2.5 Experimental design

Three to five months old albino rats of either sex of approximately 130 – 150 gm weight were randomly divided into four groups, having eight rats in each groups.

I: Control group: Rats received intraperitoneal injection of distilled water (0.5 ml) for eight weeks.

II: Induced group: Rats which were given intraperitoneal injection of freshly prepared thioacetamide solution at a dose of 200 mg/kg body weight three times a week for eight weeks.

III: Treated group - I: Induced Rats which were given ethanolic extract of *A. paniculata* orally at a dose of 200 mg/kg body weight three times a week for eight weeks.

IV: Treated group - II: Induced Rats which were given ethanolic extract of *A. paniculata* orally at a dose 250 mg/kg body weight three times a week for eight weeks.

2.6 Blood sample collection

After experimentation, rats were fasted for 12 hrs. and sacrificed by cervical dislocation. Blood sample was directly collected from heart i. e. left ventricle and was allowed to clot at room temperature. After clotting the supernant was collected carefully and was centrifused at 3000 rpm for about 15 minutes and serum obtained at the top of tube was collected for further experiment.

2.6 Biochemical methods

i) Total protein content from liver tissue was estimated by Lowry method [13].

ii) Serum SGOT, SGPT, ALP and total bilirubin was estimated by using commercial Kits.

3. RESULTS AND DISCUSSION

The results obtained in the present investigation are shown in table and are illustrated graphically in the figs.1, 2, 3 and 4. The level of SGOT, SGPT, ALP and bilirubin in control group was 106.3± 5.981 IU/L, 38.92± 2.096 IU/L, 122.4±4.5084 IU/L and 0.28±0.08366 IU/L respectively which was found increased moderately in induced group upto 558.94±6.499 IU/L, 95.62±1.9422 IU/L, 219±5 IU/L and 0.82±0.0836 IU/L respectively. The level of liver protein in control group was 246.6± 6.348

mg/gm weight of wet tissue which was found decreased upto 117 ± 10.44 mg/gm weight of wet tissue after intoxication with TAA where the decrease was highly significant. The elevated level of SGOT, SGPT, ALP and bilirubin in TAA induced rat was found decreased with increase in dose of ethanolic extract of *A. paniculata* where the values were 335.4±6.455 IU/L, 55.08±2.984 IU/L, 174±3.542 IU/L and 0.53±0.04358 IU/L respectively at a dose of 200 mg/kg of b. w. and 290.7±6.422 IU/L, 47.23±2.463 IU/L, 137±2.1213 IU/L and 0.24±0.06374 IU/L respectively at a dose of 250 mg/kg of b. w. The level of liver protein which was found decreased (246.6± 6.348 mg/gm to 117 ± 10.44) in the animals from induced group in comparison with control group was found recovered and elevated (117 ± 10.44mg/gm to 158.7±4.256 mg/gm weight of wet tissue at a dose of 200 mg/kg b. w. and to 224.2± 3.193 mg/gm weight of wet tissue at a dose of 250 mg/kg b. w.) after the administration of ethanolic extract of *A. paniculata*.

Table : Showing the effect of ethanolic extract of *A. paniculata* on the level of protein, SGOT, SGPT, ALP and bilirubin in different groups of rat.

Parameter	Animal Groups			
	Control	Induced	Treated (*A. paniculata)*	
			200 mg/kg BW	250 mg/kg BW
Protein mg/gm	246.6± 6.348	117 ± 10.44***	158.7±4.256***	224.2± 3.193***
SGOT IU/L	106.3± 5.981	558.94±6.499**	335.4±6.455**	290.7±6.422**
SGPT IU/L	38.92± 2.096	95.62±1.9422**	55.08±2.984*	47.23±2.463*
ALP IU/L	122.4±4.5084	219±5**	174±3.542**	137±2.1213**
Bilirubin IU/L	0.28±0.08366	0.82±0.0836**	0.53±0.04358**	0.24±0.06374 **

Each value is the mean of 6 individual determinations, ± indicates SD

*** P < 0.001 highly significant, ** P < 0.01moderately significant, * P < 0.05 significant,

^{NS} P>0.05 Non significant

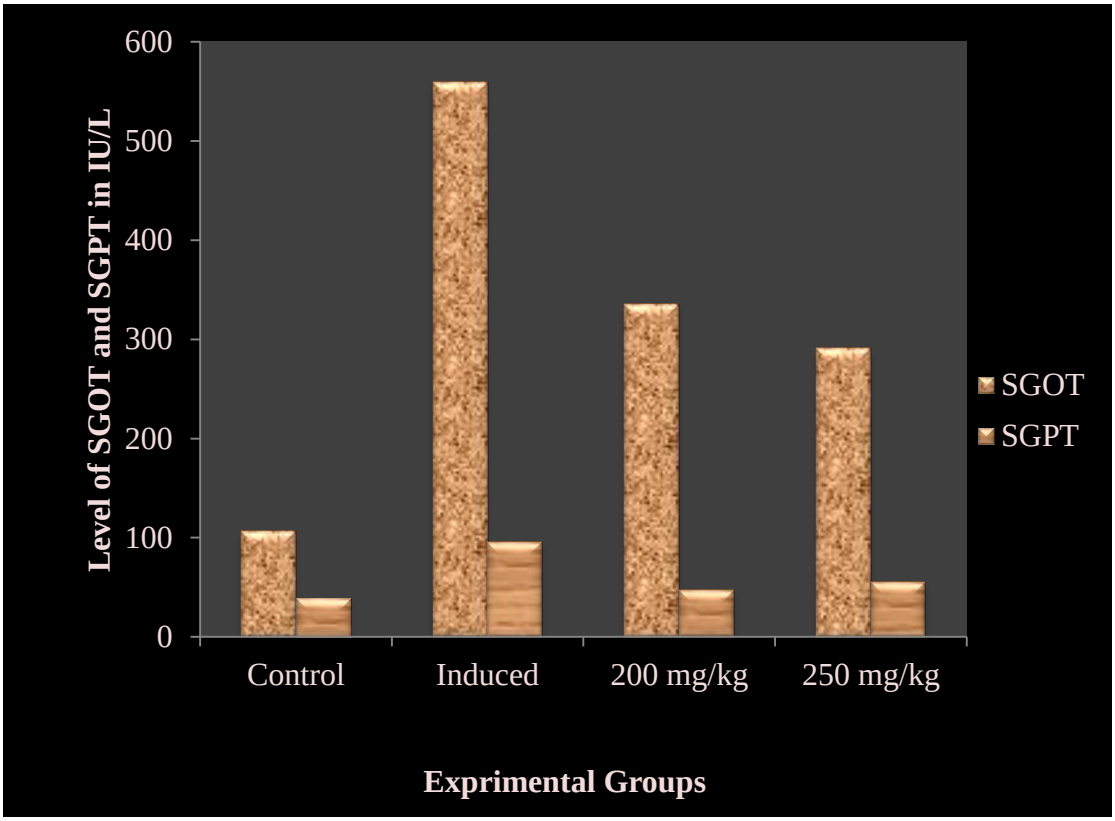

Figure 1: Showing level of SGOT and SGPT in different groups of rat

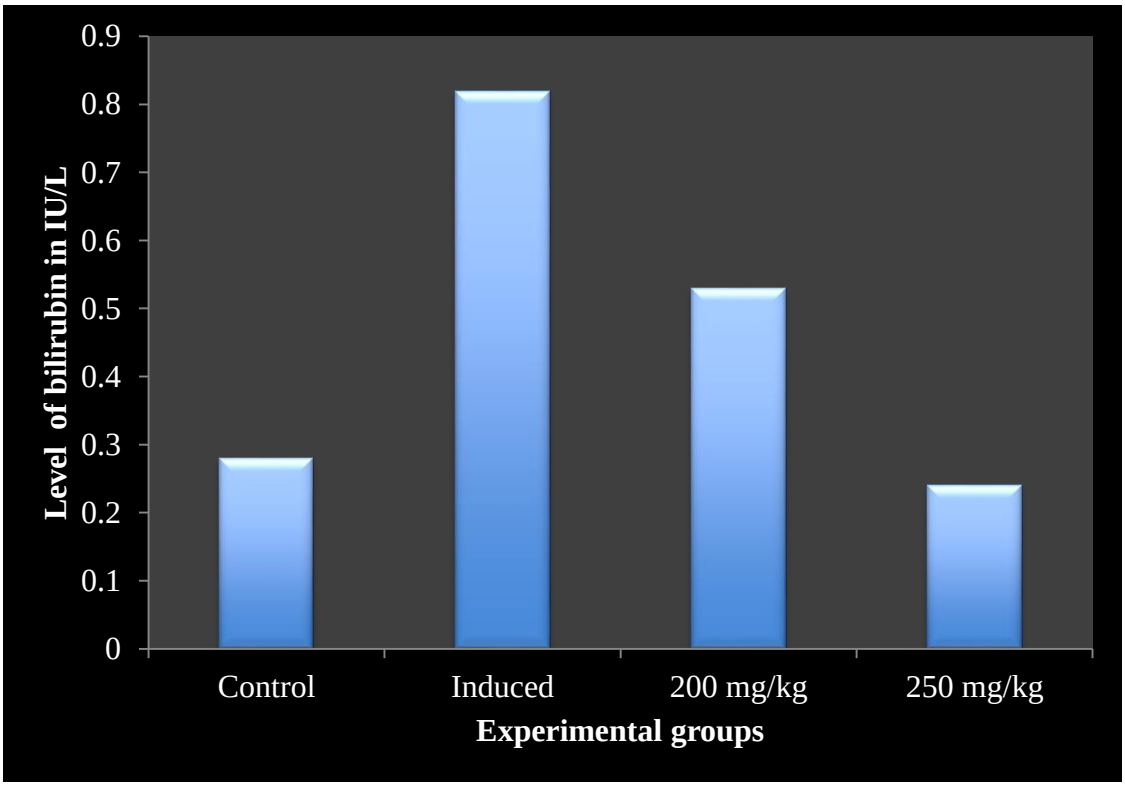

Figure 2: Showing level of bilirubin in different groups of rat

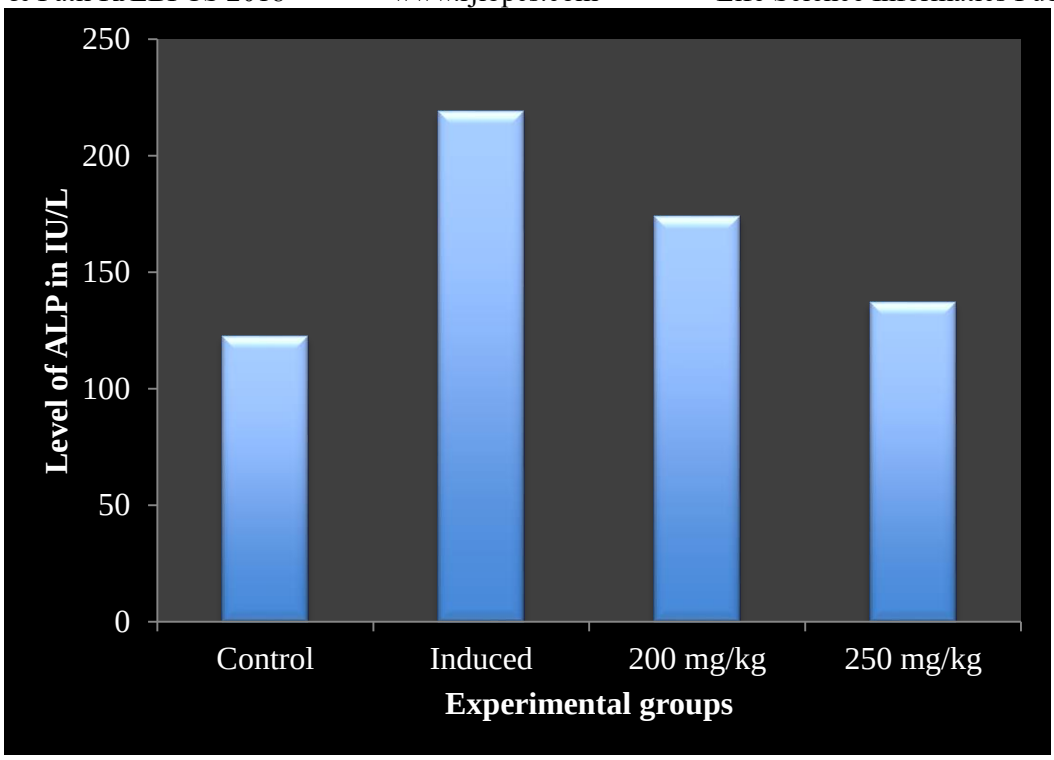

Figure 3: showing level of ALP in different groups of rat

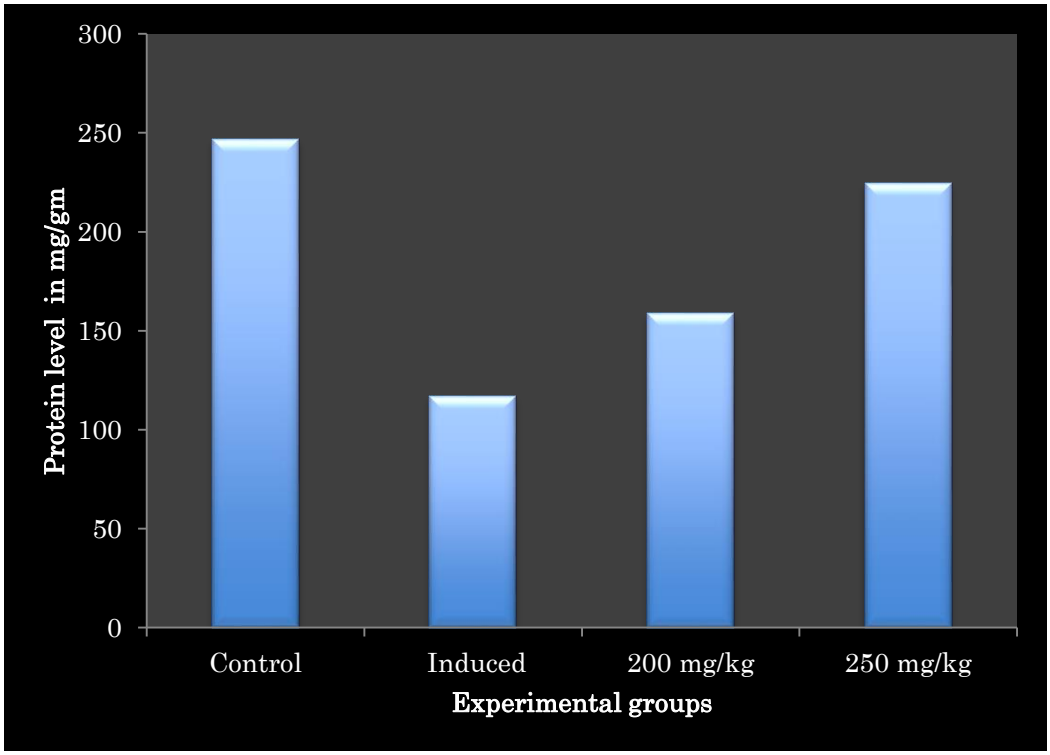

Figure 4: showing level of protein in different groups of rat

DISCUSSION

In the present study, hepatoprotective effect of ethanolic extract of *A. paniculata* was tested against thioacetamide induced hepatotoxicity in white rats. Liver function tests were considered in order to know the hepatoprotective effect of plant. The assessment of liver function can be evaluated by determining serum enzymes [14]. He further stated that hepatoprotective activity can be determined by measuring the activity of liver function enzymes such as AST, ALT, ALP, and biochemical

parameters like bilirubin and total protein in albino rats. TAA is most commonly used toxin used to induce hepatotoxicity in the experimental animals in order to induce various grade of liver damages such as nodular cirrhosis, liver cell proliferation, production of pseudolobules and parenchymal cell necrosis [15]. Sun et al. [16], Madani et al.[17] and Mohammed et al. [18] also stated that TAA is used as hepatotoxin that induces liver necrosis by formation of free radicals. According to Ambrose et al. [19] and Neal and Halpert [20] the toxicity of TAA is due to the formation of thioacetamide-5-oxide which is responsible for to change in cell permeability, increased intracellular concentration of Ca^{++}, increase in nuclear volume and enlargement of nucleoli and inhibition of mitochondrial activity that finally leads to death of cell. In most of the studies levels of the enzymes such as SGOT, SGPT, ALP and catalase are considered as liver function test. As per Drotman and Lawhorn [21] increased level of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. TAA causes significant increase in the level of serum SGOT, SGPT, ALP and Bilirubin which forms liver toxicity [22]. Achliya et al. [23] have rightly reported that bilirubin is the conventional indicator of liver diseases. According to Rajesh et al. [24] increase in the level of bilirubin is very sensitive test to substantiate the functional integrity of the liver and severity of necrosis. Increased level of bilirubin observed in the present investigation might be as per the view put forwarded by Rajesh et al. Zaragoza et al. [25] have reported that AST and bilirubin are the serum liver marker enzymes increased in TAA intoxicated rats Bassi et al. [26] also found significant increase in ALT, AST and LDH in thioacetamide administered rats. Elevated levels of AST and lipid peroxidation have also been reported in TAA treated rats by Sun et al. [27]. In the present investigation significant increase in SGOT, SGPT, ALP and bilirubin was noticed in rat after the intoxication with TAA when compared with control rats. Increase in the level of serum AST, ALT and GGT reflect loss of structural integrity of liver [28]. According to them these enzymes are released into the blood stream in presence of hepatocellular degeneration and necrotic changes resulting into the elevation of the levels of these enzymes in the blood serum. In the present study significant elevation observed in the level of above enzymes might be due to formation of pseudolobules and necrotic changes produced in the hepatocytes of liver in TAA intoxicated rats.Ramasamy [29] has reported that proteins are the important organic constituents playing vital role in the animal cell in the process of interaction between intra and extracellular media. According to him the depletion observed in the level of protein in cadmium treated rats might be because of their metabolism to liberate energy during intoxication. Protein plays a major role in the synthesis of microsomal detoxifying enzymes that helps to detoxify the toxicants entering into the animal body. The decreased level of protein in the liver of TAA intoxicated rats have also been noticed in present investigation that might be because of their role in the synthesis of detoxifying enzymes in the liver cells. According to Abiola et al. [30] reduction in protein synthesis in the liver of acetaminophen induced Wistar rats was found increased significantly in the rats pretreated with *Momordica*

charantia. In the previous studies carried out by most of the investigators it is mentioned that elevated levels of most of liver enzymes in rats intoxicated with certain toxic material was found decreased after the treatment with extracts of certain plant material whereas decreased level of protein get elevated. Kumar et al. [31] have reported that administration of *Trianthema portulacastrum* against thioacetamide treated rats showed a significant decrease in the serum AST, ALT and bilirubin levels when compared with thioacetamide treated rats. According to Muthulingam et al. [32] the decreased level of protein in TAA treated rats has increased after the oral administration of extract of *Pleurotus florida.* Reduction in the elevated level of transaminase have been reported by Muthulingam et al. [33] in allaxon treated rats after the oral administration of extract of *Asteracantha longifolia* .Madubuike et al. [34] have reported significant decrease in the levels of serum ALP, AST and ALTin CCl_4 induced albino rats after the treatment with ethanolic extract of *Jateropha tanjorensis.* Gogoi et al. [35] also reported similar results in albino rats administrated with CCl_4. According to them treatment of the rats with the fruit rinded extract of *Garsina morella* results into decrease in to the level of AST, ALT and ALP significantly and increase in the production of level of total protein in dose dependent manner. The decrease in the level of liver enzymes and bilirubin and increase in the level of protein in the liver observed in the present investigation after the oral administration of ethanolic extract of *A. paniculata* are in accordance with the reports mentioned by earlier workers in such type of studies. The ethanolic extract of *A. paniculata* might have contains the flavonides and alkaloids which might have possess the hepatoprotective activity.

4. CONCLUSION

The results obtained in the present investigation clearly indicated the hepatoprotective effect of ethanolic extract of *A. paniculata* against thioacetamide induced hepatotoxicity in white rat.

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CONFLICT OF INTEREST

The authors declare no any conflict of interest.

REFERENCES

1. Bhardwaj A, Khatri P, Soni ML. Potent Herbal Hepatoprotective Drugs: A Review. Journal of Advanced Scientific Research.2011;2(2): 15-20.
2. Sharma A, Charkraborti KK and Handa SS, Antihepatotoxic activity of some Indian herbal formulations as compared to silymarin. Fitoterapia. 1991, 62,229-235.
3. Kadir FA, Othman F, Abdulla MA, Hussan F, Hassandarvish P. Effect of *Tinospora crispa* on thioacetamide-induced liver cirrhosis in rats. Indian J Pharmacol . 2011;43(1):64.
4. Salguero Palacios R, Roderfeld M, Hemmann S, Rath T, Atanasova S,Tschuschner A, Gressner OA, Weiskirchen R, Graf J, Roeb E. Activation of hepatic stellate cells is associated with

cytokine expression in thioacetamide-induced hepatic fibrosis in mice. Laboratory investigations. 2008; 88,1192-1203.

5. Wang T, Shankar K, Ronis M, Mehendale H. Enhanced hepatotoxicity and toxic outcome of thioacetamide in streptozotocin induced diabetic rats. Toxicology and applied pharmacology. 2000; 166: 92-100.
6. Ramaiah S, Apte U, Mehendale H. Cytochrome P4502E1 induction increases thioacetamide liver injury in diet-restricted rats. Drug Metabolism and Dispostion. 2001; 29:1088-95.
7. Chilakapati J, Korrapati MC, Hill RA, Warbritton A, Latendresse JR. Toxicokinetics and toxicity of thioacetamide sulfoxide: a metabolite of thioacetamide. Toxicology. 2007; 230: 105-116.
8. Polasa K, Nirmala K, Ginger. Its role in xenobiotic metabolism. ICMR Bull. 2003;33 (6):57-63.
9. Kumar, T., Chandrashekar, K. S., Tripathi, D. K., Nagori, K., Pure, S., Agrawal, S., & Ansari, T. J. Standardization of Gokshuradichurna": An Ayurvedic polyherbal formulation. Journal of Chemical and Pharmaceutical Research. 2011. *3*(3), 742-749.
10. Niranjan, A., Tewari, S., Lehri, A. Biological activities of Kalmegh (*Andrographis paniculata* Nees) and its active principles-A review. Indian Journal of Natural Product and resources. 2010;1(2):125-135.
11. Chopra, R., Nayar, S. and Chopra, I. Glossary of Indian Medicinal Plants. NISCOM, CSIR, New Delhi, India 18. 1956.
12. Mohammed A, Alshawsh M, Ameen A, Salmah I, Zahra A. Hepatoprotective effects of *Orthosiphon stamineus* extract on thioacetamide-induced liver cirrhosis in rats. Malaysia Grant, 2009; 182:1-14.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Bio Chem. 1951;193: 265-275.
14. Gogai N, Gogoi A, Neog B, Baruah D, Singh KD. Antioxidant and hepatoprotective activity of fruit rind extract of *Garcinia Morella* (Gaertn.) Desr. Indian Journal of Natural Product and Resources. 2017; Vol. 8(2),132-139.
15. Mitra SK. Protective effect of HD-03, a herbal formulatin, against various hepatotoxic agents in rats. J. Ethnopharmacol. 1998;63: 181-186.
16. Sun F, Hayami S, Ogiri Y. Evaluation of oxidative stress based on lipid hydroperoxide, vitamin c and vitamin e during apoptosis and necrosis caused by thioacetamide in rat liver. Biochimica et BiophysicaActa. 2000;1500(2):181–185.
17. Madani H, Asgari S, Naderi G, Talebol M. Hepatoprotective effect of *Chichorium intybue* L. on liver toxicity in rat. In Persian. J Med. Plant. 2006;5: 38-32.
18. Mohammed A, Alshawsh M, Ameen A, Salmah Ismail and Zahra A. Hepatoprotective effects of *Orthosiphon stamineus* extract on Thioacetamide-induced liver cirrhosis in rats. Malaysia Grant. 2009;182: 1- 14.

19. Ambrose AM, DeEds F, Rather LJ. Further studies on toxicity of thioacetamide in rat. ProcSocEXpBiol Med. 1950;74: 134-140.
20. Neal RA, Halpert J. Toxicology of thiono-sulfur compounds. Annu Rec Pharmacol Toxicol. 1982;22: 321-339.
21. Drotman R, Lawhorn G.Serum enzymes are indicators of chemical induced liver damage. Drug ChemToxicol. 1978;1: 163-171.
22. Das K, Kathiriya A, Kumar EP, Benson MK, Einstein J. Evaluation of hepatoprotective activity of aqueous and ethanolic extract of *Oxalis corniculata* against intoxication of thioacetamide induced rats. 2011; 22(2): 412-417
23. Achliya GS, Wadodkar SG, Dorle AK. Evaluation of hepatoprotective effect of *Amalkadi ghrita* against carbon tetrachloride induced hepatic damage in rats. Journal of Ethnopharmacology. 2004;90: 229- 232.
24. Rajesh MG, Latha MS. Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. Journal of Ethanopharmacology. 2004; 91:99-104.
25. Zaragoza A, Andres D, Sarrion D, Cascales M. Potentiation of thioacetamide hepatotoxicity by Phenobarbital pretreatment in rats. Inducibility of FAD monooxygenase system and age effect. Chemical and Biological Interaction. 2005;124: 87-101.
26. Bassi AM, Canepa C, Maloberti G, Casu A, Nanni G. Effect of a load of vitamin A after acute thioacetamide intoxication on dolichol, isoprenoids and retinal content in isolated rat liver cells. Toxicology. 2004;199: 97-107.
27. Sun F, Hayami S, Ogiri Y. Evaluation of oxidative stress based on lipid hydroperoxide, vitamin c and vitamin e during apoptosis and necrosis caused by thioacetamide in rat liver. Biochimica et BiophysicaActa. 2000;1500(2):181–185.
28. Nakae D, Konishi Y, Farber JL. A role for oxygen radicals in the hepatotoxicity of aflatoxin B1 and dimethyl nitrosamine. Proc Jap Cancer Assoc. 1987;38.44.
29. Ramasamy M . Effect of sevin on blood free amino acids of the fish *Sarotherodon mossambicus*. Environment and Ecology.1987; 5(4): 633-637.
30. Abiola T, Akinyode OA, Oduwole R, Egbeolu o. Assessment of the prophylactic activity of the ethanolic extract of *Momordica charantia* leaves against acetaminophen-induced liver impairment in Wistar rats. BiochemPharmacol. 2017:6:3.
31. Kumar G, Sharmila G, VanithaPappa P, Sundararajan M, Rajasekara Pandian M. Hepatoprotective activity of *Trianthema portulacastrum* L. against paracetamol and thioacetamide intoxication in albino rats. Journal of Ethanopharmacology. 2004;92: 37-40.
32. Muthulingam M, Savio P D, Seeli T S, Indra N, Sethupathy S. Therapeutic role of edible mushroom *Pleurotus florida* (mont.) on thioacetamide induced hepatotoxicity in rats. International Journal of Current Research. 2010;5: 041-046.

33. Muthulingam M. Antidiabetic efficacy of leaf extracts of *Asteracantha longifolia* (Linn.) Nees. on alloxan induced diabetics in male albino Wistar rats. International Journal of Pharmaceutical and Biomedical Research. 2010;1(2): 28 - 34.
34. Gideon MK, Omeh YN, Matthew IA. Hepatoprotective activity of methanolic extract of *Jatropha tanjorensis* in carbontetrachloride induced hepatotoxicity. Archives of Applied Science research.2015; 7(5): 45-48.
35. Gogai N, Gogoi A, Neog B, Baruah D, Singh KD. Antioxidant and hepatoprotective activity of fruit rind extract of *Garcinia Morella* (Gaertn.) Desr. Indian Journal of Natural Product and Resources. 2017; Vol. 8(2),132-139.