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Research Article

HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF NEURACANTHUS *SPHAEROSTACHYUS* FAMILY *ACANTHACEAE* (*RUELLIA* FAMILY) AGAINST HEPATOTOXICITY INDUCED BY THIOACETAMIDE

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ABSTRACT

Objective: Hepatoprotective activity of ethanolic extract of leaves of *Neuracanthus Sphaerostachyus* family *Acanthaceae* (*ruellia* family) against hepatotoxicity induced by thioacetamide.

Method: The present study is done to find out the LD_{50} and hepatoprotective activity of the plant. The leaves were successively extracted with water, chloroform and ethanol. The ethanolic extract was used for the present study. The animals were divided in five groups of six rats in each. All the animals of group II to V received thioacetamide 400mg/kg (S.C.), Group II animals were maintained as thioacetamide control without any drug treatment. Group III and IV were treated with 100 and 200 mg/kg ethanolic extract respectively. Group V animals were treated with Silymarin (100 mg/kg, *p.o*.) which served as standard group. On the sixth day all animals were sacrificed, blood samples were collected and evaluated for glutamate pyruvate transaminase (SGPT), serum glutamate oxaloactetate transaminase (SGOT), total bilirubin and serum alkaline phosphatase (ALP). Livers were removed and preserved in 10% formalin solution for histopathological studies. Pentobarbitone induced sleeping time, ascorbic acid content in urine and bromsulphalein clearance test parameters were done to study the hepatoprotective activity of plant.

Result: Group III and IV shows significant reduction in SGPT, SGOT, total bilirubin and ALP with no specific changes in histopathology of liver when compare to thioacetamide group. Reduction in Pentobarbitone induced sleeping time, and significantly increased in ascorbic acid content in urine, Bromsulphalein uptake in animal treated with ethanolic extract of the plant.

Conclusion: It is concluded from the study that the ethanolic extract of leaves of *Neuracanthus Sphaerostachyus* shows significant hepatoprotective activity.

Keywords: *Neuracanthus Sphaerostachyus,* SGPT, SGOT, ALP, Total bilirubin, $LD_{50,}$ Thioacetamide Pentobarbitone induced sleeping time, Ascorbic acid content in urine, Bromsulphalein clearance**.**

INTRODUCTION

Liver is one of the largest organs in human body and involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, and energy provision[1]. It plays a major role in detoxification and excretion of many endogenous and exogenous compounds. Any injury to it or impairment of its functions may lead to implications on one's health[2]. Thus the disorders associated with this organ are numerous and varied[3]. Management of liver diseases is still a challenge to modern medicine. Modern medicine has little to offer for alleviation of hepatic ailments.

Most of the hepato-protective agents now available are expensive and hence a genuine need is felt to devise some cost effective drugs based on plant principles in this regard[4]. Numerous medicinal plants and various formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicine in India. Many plants possess hepato-protective activity against carbon tetrachloride, ethanol, paracetamol, anti tubercular drugs, galactosamine and thioacetamide induced liver damage in albino rats and hence a similar study mentioned is presented in this study[5].

Neuracanthus sphaerostachyus is found in Western Ghats, Deccan and Gujarat. Traditionally its root paste is applied in ring worm infection[6].

The ash of whole plant is mixed with either jaggery or honey and given orally 2 - 3 times a day to cure cough and asthma[7].

MATERIALS AND METHODS

Plant material

The leaves of *Neuracanthus sphaerostachyus* were collected in the month of Aug 2011 from Forest of Girnar, in the Junagadh district, Gujarat, India. The plant material was identified and authenticated by Mr. Vinod Kumar, Department of Botany, Rajasthan University, Jaipur, Rajasthan. (Herbarium No.RUBL221135)

Preparation of extract

The leaves of *Neuracanthus sphaerostachyus* were washed thoroughly in tap water, shade dried and powdered. This powder was packed into Soxhlet column and extracted with water (70 – 80^{0}C) for 36 h. The same marc was successively extracted with chloroform (50 - 60^{0}C) and later with ethanol (68 - 78^{0}C) for 24 h. The extracts were concentrated on water bath (50^{0}C). After concentrated preparation, the dried powder extract was stored at 4^{0}C. The yield of the aqueous extract, chloroform extract and ethanolic extract were found to be 5.64%(w/w), 1.80 % (w/w) and 7.59% (w/w) respectively. Ethanolic extract were used for the experimental study.

Animals

Wister male Albino rats (150 - 200 g) were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 26 ± 2^{0}C. They were fed with standard diet supplied by Pranav agro industries Ltd. Sangli. All the animal experiments are conducted in accordance with the guidelines of the CPCSEA (Reg No. 1239/a/08/CPCSEA), guide for care and use of laboratory animals. After procuring the animals were acclimatized for 10 days under standard husbandry conditions as: Relative humidity 45 - 55%, and 12 h light and dark cycle.

Acute toxicity study

The male Wistar rats of 150 - 200g body weight were selected to find out the acute toxicity study of ethanolic extract of *Neuracanthus sphaerostachyus* leaves. The dose of 5, 50, 300 and 2000 mg/kg were selected based on the fixed dose method as per method of CPCSEA. The animals were continuously observed for 24 h to detect changes in autonomic or behavioral responses.

In the acute toxicity study ethanolic extract of leaves of *Neuracanthus sphaerostachyus* were found to be toxic (2/3 rats died) at a dose of 600 mg/kg, intraperitoneally. Hence, LD_{50} cut off value of ethanolic extract was fixed as 600 mg/kg body weight. So, that 1/7th and 1/5th of the LD_{50} cut off value that is, 100 and 200 mg/kg body weight were selected as screening dose for hepatoprotective activity.

Experimental design

Male albino rats of Wistar strain were selected and divided into five groups of 6 animals each. They should be treated for six days as follows:

Group-I = Normal control (Normal saline 5 ml/kg)

Group-II = Thioacetamide 400 mg/kg[8].

Group-III = Ethanolic extract (100 mg/kg)

Group-IV = Ethanolic extract 200 mg/kg

Group-V = Silymarin (100 mg/kg, *p.o.)[9].*

From 1st day to 5th day with concurrent administration of thioacetamide on 2nd and 3rd day. During the period of drug treatment the rats were maintained under normal diet and water *ad libitum.* On the sixth day animals of all the groups were sacrificed by light ether anesthesia. The blood sample of each animal was collected separately by carotid artery into sterilized dry centrifuge tubes and allowed to coagulate for 30 min. Serum was separated by centrifugation 3000 rpm for 15 mm. The serum was used to estimate serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloactetate transaminase (SGOT), total bilirubin and serum alkaline phosphatase (ALP). Livers were removed and preserved in 10% formalin solution for histopathological studies.

Biochemical analysis

The blood samples were analyzed for (SGPT), serum glutamate oxaloactetate transaminase (SGOT), total bilirubin and serum alkaline phosphatase (ALP).

Pentobarbitone induced sleeping time [[10]-[12]]

The animals were divided into five groups of six Wistar male albino rats each. The animals were fasted for 24 h prior to Thioacetamide treatment. Group I was maintained as normal control received normal saline 5 ml/kg *po*. All the animals of group II to V received thioacetamide 400mg/kg, Group II animals were maintained as thioacetamide control without any drug treatment. Group III and IV were treated with 100 and 200 mg/kg ethanolic extract respectively. Group V animals were treated with Silymarin (100 mg/kg, po) which served as standard group.

The reduction in the sleeping time was used to evaluate the protection of rat liver against the Thioacetamide induced liver damage. On first day animals were given their respective doses. After two hours of treatment all animals were given Thioacetamide .On second day again all the rats were given their respective doses and one hour after treatment they were given pentobarbitone sodium (40 mg / kg i.p.). The onset of action and duration of sleep (loss of righting reflex) was noted.

Ascorbic acid content in urine [13]

Ascorbic acid in urine was determined by modified method by Roe and Kuether (1943). The animals were divided into four groups of six Wistar male albino rats each. They were kept in a metabolic cage for collection of urine. They supplied with standard diet and water *ad libitum*, one week before and during the experimental period. Twenty four hour urine simple were collected separately for each group for day in 5 ml of oxalic acid solution and analyzed for ascorbic acid and their average value were taken as control. Then the rat of group I, II,III,and IV were treated with thioacedamide respectively. Group II,and III, were treated with ethanolic extracts at dose of 100 and 200 mg/kg respectively and group IV animals were treated with Silymarin (100 mg/kg, po).and after one hour were challenge with thioacedamide (400mg/kg).The 24 h urine sample were collected at 7th day for all the groups and the sample were analyzed for ascorbic acid.

Bromsulphalein clearance test[14]

Bromsulphalein clearance test is the most sensitive and dependable method to assess the physiological status of liver function. The test indicates the excretory function of the liver. It is generally agreed that in the passage of bromsulphalein (BSP) from the plasma to the bile, it undergoes storage, metabolism and excretion by the liver. It is well documented that CCl_4 produces morphological and functional changes in the liver. The abnormal functional effects produced by thioacetamide are easily demonstrated by the retention of BSP. Liver slices kept in ice cold phosphate buffer (0.2 M) at pH 7.4 were incubated in media (KCl: 10 mM, MgSO4: 1 mM, NaCl: 1 mM in phosphate buffer) containing 30 µg BSP/ml at 38°C. An aliquot of reaction mixture was analyzed after 30 min to determine the concentration of BSP in the media at 580 nm.

Histopathological observation

Liver tissue collected were used for the preparation of histopathologi-cal slides by using microtome and were suitably stained and observed under microscope for architectural changes seen during thioacetamide challenge in ethanolic extract of *Neuracanthus sphaerostachyus* treated and control groups.

Figure 5 shows a magnification of the changes of liver histopathology from the normal control. The normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein were observed in the normal control group (Figure 6). However, Thioacetamide intoxicated treatment exhibited severe histopathological changes, such ascentrilobular hepatic necrosis, fatty change, kupffer cell, ballooning degeneration, and infiltrating lymphocytes (Figure 9.) extract and silymarine treated group show no specific changes.

Statistical analysis

The mean ± S.E.M. was calculated for each parameter. Total variations, present in a set of data were estimated by one way analysis of variance (ANOVA), followed by Dunnet's 't' test. $P<0.05$ was considered as statistically significant when compared to control group. The percentage of the protection is calculated as 100 X (Values of Thioacetamide control — Values of test sample) / (Values of thioacetamide control — Values of normal control)

Table 1: Effects of ethanolic extract of leaves of *Neuracanthus sphaerostachyus* on certain serum biochemical parameters in Thioacetamide induced hepatotoxicity in rats.

Biochemical parameters Groups	SGPT(IU/L)	SGOT(IU/L)	Total Bilirubin (mg/dl)	ALP(IU/L)
Normal Group	15.83±1.49	30.16±0.87	0.72±0.02	109.5±1.088
TA control (400mg/kg) s.c.	37.33±0.95	72.33±0.95	1.04±0.03	296.5±1.708
Ethanolic extract	24.5±0.76	54.5±0.75**	0.88±0.012**	185.33±1.49**
** (100mg/kg) p.o. + TA	(59.67%)	(42.28%)	(50.00%)	(59.44%)
Ethanolic extract	22.66±0.66**	51.16±0.87**	0.81±0.020**	139.16±1.68**
(200mg/kg) p.o. + TA	(68.23%)	(50.20%)	(71.87%)	(84.13%)
Silymarin	16.33±1.11**	31.66±0.66**	0.74±0.019**	114.33±1.64**
(100mg/kg) p.o. +TA	(97.67%)	(96.44%)	(93.75%)	(97.32)

Values are Mean ± SEM, (n =6 in each group). Figures in parenthesis are percent protection as compared to thioacetamide control. Thioacetamide control group was compared with normal group and all values were significantly different ($P< 0.01$). Experimental groups were compared with thioacetamide control: *$p<0.05$ and **$P< 0.01$

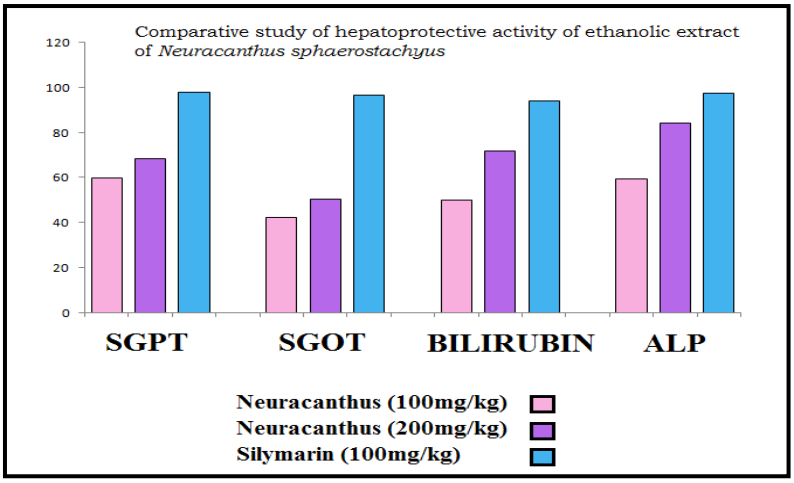

Fig. 1: Effect of ethanolic extract of *Neuracanthus sphaerostachyus* and sylmarin on biochemical estimation of SGPT, SGOT, Bilirubin and ALP of thioacetamide induced hepatotoxicity in male Wistar rats.

Table 2: Effects of ethanolic extract of leaves of *Neuracanthus sphaerostachyus* on Pentobarbitone induced sleeping time in Thioacetamide induced hepatotoxicity in rats

Pentobarbitone induced sleeping time		
Group	**Onset of time**	**Duration of sleep**
Normal Group	15.5±0.76	71.33±2.39
Pentobarbitone (40mg/kg)		
TA control (400mg/kg,SC) +pentobarbitonez (40mg/kg)	4.33±0.49	192.66±7.17**
Ethanolic extract (100mg/kg) *p.o.* +TA+pentobarbitone (40mg/kg)	6.83±0.60	127.33±4.27** (53.82%)
Ethanolic extract (200mg/kg)*p.o.* + TA+pentobarbitone (40mg/kg)	8.5±0.42	109.16±6.36** (68.82%)
Silymarin 100mg/kg p.o. +TA+pentobarbitone (40mg/kg)	12.33±0.61	80.86±2.007 (92.14%)

Values are Mean ± SEM, (n =6 in each group). Figures in parenthesis are percent protection as compared to thioacetamide control. Thioacetamide control group was compared with normal group and all values were significantly different (P< 0.01). Experimental groups were compared with thioacetamide control: *p<0.05 and **P< 0.01.

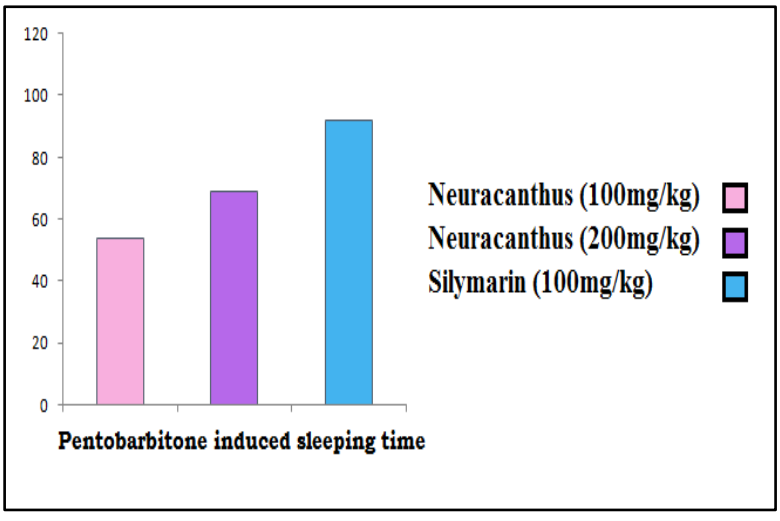

Fig. 2: Effect of ethanolic extract of *Neuracanthus sphaerostachyus* and sylmarin on pentobarbitone induced sleeping time of thioacetamide induced hepatotoxicity in male Wistar rats.

Table 3: Effects of ethanolic extract of leaves of *Neuracanthus sphaerostachyus* on ascorbic acid content in urine in Thioacetamide induced hepatotoxicity in rats.

Ascorbic acid content in urine		
Group	**Before treatment (µg/ml)**	**After treatment (µg/ml)**
THIOACETAMIDE (400mg/kg) SC	131.16±4.10	80.5±2.09
Ethaniloc extract 100mh/kg + Thioacetamide	120.66±3.81	117±1.93** (72.04%)
Ethanolic extract 200mg/kg + Thioacetamide	137.16±2.31	120±1.94** (77.97%)
Silymarine (100mg/kg) p.o.+TA	140.66±1.54	136.83±1.95** (111.92%)

Values are Mean ± SEM, (n =6 in each group). Figures in parenthesis are percent protection as compared to thioacetamide control. Thioacetamide control group was compared with normal group and all values were significantly different (P< 0.01). Experimental groups were compared with thioacetamide control: *p<0.05 and **P< 0.01.

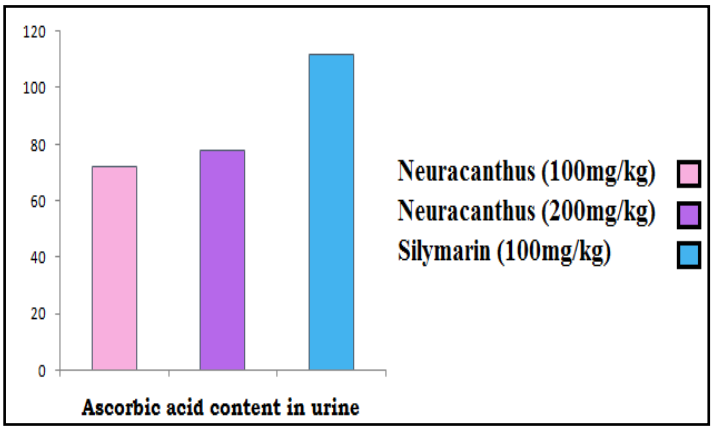

Fig. 3: Effect of ethanolic extract of *Neuracanthus sphaerostachyus* and sylmarin on ascorbic acid content in urine of thioacetamide induced hepatotoxicity in male Wistar rats.

Table 4: Effects of ethanolic extract of leaves of *Neuracanthus sphaerostachyus* on Bromsulphalein clearance test in Thioacetamide induced hepatotoxicity in rats liver

Bromsulphalein clearance test	
Group	**BSP uptake (µg/gm of liver tissue)**
NORMAL GROUP	105.6±2.76
TA control (400mg/kg,SC)	65.34±1.47
NEUROCANTHUS (100mg/kg) p.o. + TA	74.09±2.60** (21.83%)
NEUROCANTHUS (200mg/kg) p.o.+ TA	82.69±1.25** (43.09%)
Silymarine 100mg/kg *p.o.* + TA	102.13±2.61** (91.38%)

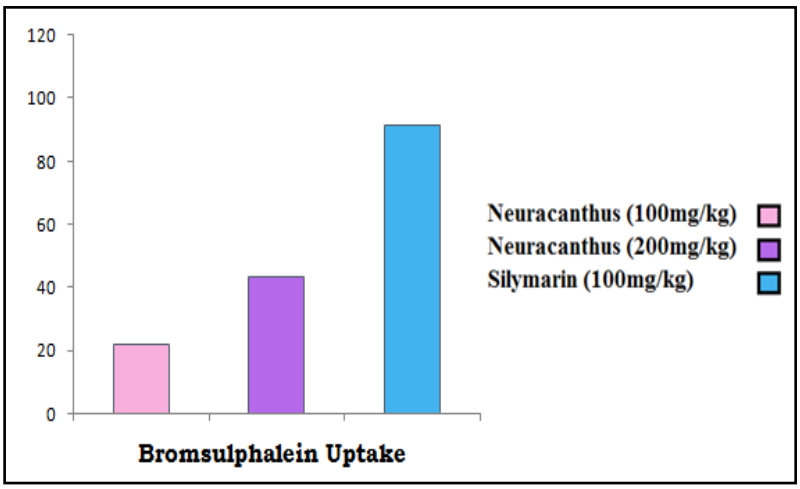

Fig. 4: Effects of ethanolic extract of leaves of *Neuracanthus sphaerostachyus* on Bromsulphalein clearance test in Thioacetamide induced hepatotoxicity in rats liver.

Histopathological study

Liver sections of 4-6 micron in thickness were stained with hematoxylin and eosin and observed under H & E x100 resolution of microscope for histopathological changes and photographed.

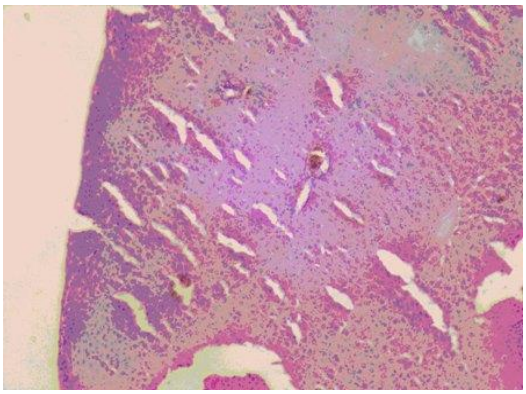

Fig. 5: Normal control

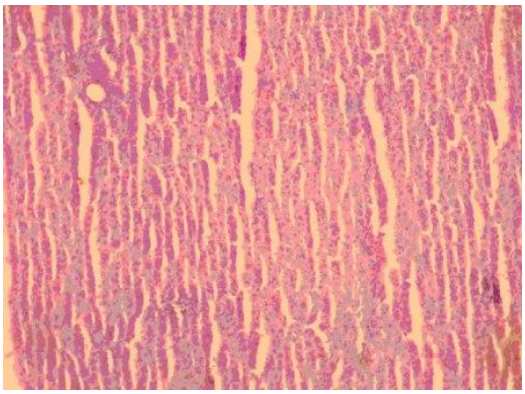

Fig. 6: Thioacetamide

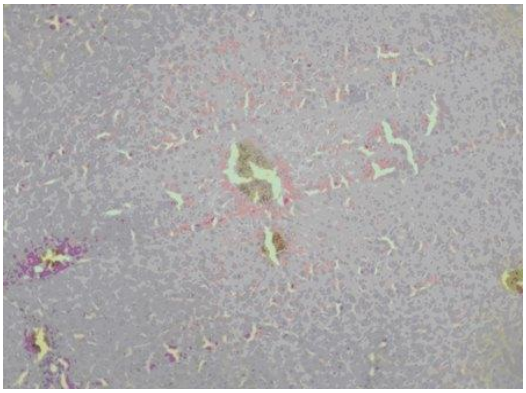

Fig. 9: Silymarine

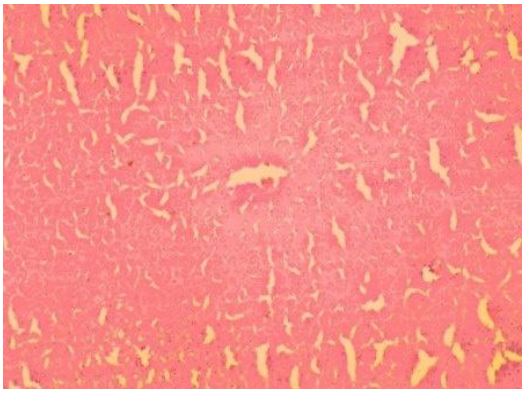

Fig. 7: Ethanolic extract 100mg/kg+TA

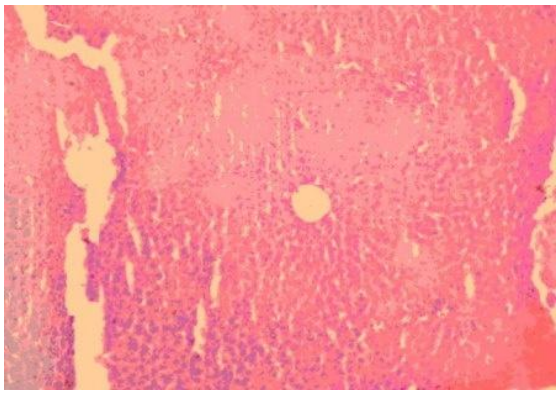

Fig. 8: Ethanolic extract 200mg/kg+TA

RESULT AND DISCUSSION

The biochemical (table 1) and histopathological (fig. 6) study reveals that Thioacetamide produces hepatotoxicity where increased in the level of SGPT, SGOT, ALP and bilirubin indicate the hepatotoxicity . Ethanolic extract of *Neuracanthus sphaerostachyus* at dose of 100mg/kg and (table1) significantly reduces the serum enzyme level. The histopathological study indicate no specific changes. The study indicate that the extract significantly protect the liver against hepatotoxicity caused by thioacetamide. Pentobarbitone sleeping time study shows that sleeping time is decrease in animal treated with ethanolic exract at dose of 100mg/kg and 200mg/kg. compare to thioacetamide group (table 2 and fig.2). Ascorbic acid content in urine is significantly increased in animal treated with ethanolic extract as compare to thiacetamide group.(table3 and fig.3). Bromsulphalein clearance test(table 4 and fig.4)indicate that thioacetamide treated group shows reduction bromsulphalein uptake where as animal treated with ethanolic extract shows significant increase in uptake of bromsulphalein.

CONCLUSION

Overall study indicate that ethanolic extract of *Neuracanthus sphaerostachyus* at dose level of 100mg/kg and 200mg/kg significantly protect the liver against hepatotoxicity induced by thioacetamide.

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