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Research Article

Hepatoprotective Activity of *Asparagus retrofractus* (Family- *Asparagaceae)* Roots against Thioacetamide Induced Liver Damage in Rats

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Abstract

The present study was aimed at evaluation of hepatoprotective activity of ethanol extracts of *Asparagus retrofractus* roots against thioacetamide-induced liver damage in rats. Hepatotoxicity was induced by thioacetamide and biochemical parameters such as SGPT, SGOT, ALP, total bilirubin and also pentobarbitone induced sleeping time, ascorbic acid excretion in rat urine, bromsulphalein (BSP) uptake and histopathological changes in liver were studied along with silymarin as standard hepatoprotective agents. The thioacetamide intoxicated rat shows biochemical and histological deterioration. Pretreatment with ethanolic extract of *Asparagus retrofractus* roots reduced the elevated level of serum glutamate oxaloactetate transaminase SGOT, serum glutamate pyruvate transaminase SGPT, serum alkaline phosphatase ALP, total bilirubin TB and reduces in pentobarbitone induced sleeping time, preventing induced reduction in ascorbic acid excretion in rat urine, increased bromsulphalein (BSP) uptake and also reversed the hepatic damage towards normal which further supports the hepatoprotective activity of root of *Asparagus retrofractus*.It has been concluded that the ethanolic extract of roots of Asparagus *retrofractus* at dose of 300mg/kg and 600mg/kg have significant effect on liver of thioacetamide induced hepatotoxicity model in rats.

1. INTRODUCTION

Liver is the most important organ concerned with the biochemical activities in the human body. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. There is an ever increasing need of an agent which could protect it from such damage. In view of severe Undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity [1].

It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity[2]. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders[3].In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell [4]. There are however, members of drugs employed in traditional system of medicine for liver affections[5]. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem.

Some species of *Asparagus* genus like *Asparagus racemosus* is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers as galatogogue and nervine tonic. The decoction of root has been used in blood diseases; diarrhoea, dysentery, cough, bronchitis and general debility[6-8].Reports indicate that the pharmacological activities of root extracts include antiulcer [9], antitussive[10], antioxidant [11] and antibacterial activities [12].

The *Asparagus* genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant[13] *Asparagus retrofractus* plant from same genus and family found in growing wild in tropical and sub-tropical region of India[14]. Southern and eastern Africa .Traditionally, roots and rhizomes are eaten by turkeys and roots were added into bread to feed cows, goats and seeps.[15]. But still no scientific and methodical investigation has so far been reported in literature regarding its action on liver. Therefore, the present investigation has been designed to study the possible mechanism of ethanolic extract of *Asparagus retrofractus* root on the different parameter against Thioacetamide (TA) induced hepatic damage in albino rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Roots of *Asparagus retrofractus* was collected in the month of July 2011 from the forest of Girnar, Junagadh District, Gujarat (India). The plant material was identified and authenticated by Mr. Vinod Kumar, Department of Botany, Rajasthan University, Jaipur, Rajasthan (Herbarium No. RUBL21132).

2.2 Preparation of Extract

Roots of *Asparagus retrofractus* were washed thoroughly in tap water, shade dried and powdered. This powder was packed into Soxhlet column and extracted with water (70 - 80°C) and with ethanol (68 - 78°C) for 36 h. The extracts were concentrated on water bath (50°C). After concentrated preparation, the dried powder extract was stored at 4°C. The yield of the aquous extract and ethanolic extract were found to be 6.63 % (w/w) and 13.87% (w/w) respectively. Ethanolic extract were used for the experimental study.

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2.3 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 ± 2^0C. 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.

2.4 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 *Asparagus retrofractus* roots. The dose of 5, 50, 300 and 2000 mg/kg were selected based on the fixed doses as per the method of CPCSEA. The animals were continuously observed for 24 h to detect changes in autonomic or behavioral responses. Mortality in each group was observed for 7 days.

In the acute toxicity study ethanolic extract of Roots of *Asparagus retrofractus* were found to be toxic (2/3 rats died) at a dose of 1600 mg/kg, intraperitoneally. Hence, LD cut off value of ethanolic extract was fixed as 1600 mg/kg body weight. So, that 1/5th and 1/3rd of the LD_{50} cut off value that is 300mg/kg and 600 mg/kg body weight were selected as screening dose for hepatoprotective activity.

2.5 Assessment of Hepatoprotective Activity

2.5.1 *Biochemical Analysis*

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 p.o.). 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 300 and 600 mg/kg ethanolic extract respectively. Group V animals were treated with Silymarin (100 mg/kg, p.o.) which served as standard group. The vehicle or drug treatment was carried out p.o. from 1st day to 5th day with con-current 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.*

The animals of all the groups were sacrificed by light ether anesthesia on 6th day. 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 min. The serum was used to estimate serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloactetate transaminase (SGOT)[16] total bilirubin[17] and serum alkaline phosphatase (ALP).[18] Livers were removed and preserved in 10% formalin solution for histopathological studies.

2.5.2 *Pentobarbitone Induced Sleeping Time*

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 300 and 600 mg/kg ethanolic extract respectively. Group V animals were treated with Silymarin (100 mg/kg, p.o.) 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[19 - 22].

2.5.3 *Ascorbic acid content in urine*

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, p.o.) and after one hour were challenge with thioacedamide (400 mg/kg). The 24 h urine samples were collected at 7th day for all the groups and the sample were analyzed for ascorbic acid[23].

2.5.4 *Bromsulphalein clearance test*

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 thioacetamide 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, $MgSO_4$: 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[24].

2.5.6 *Histopathological observation*

Liver tissue collected were used for the preparation of histopathological slides by using microtome and were suitably stained and observed under microscope for architectural changes seen during Thioacetamide challenge in ethanolic extract of *Asparagus retrofractus* treated and control groups.

2.5.7 *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).

3. RESULTS AND DISCUSSION

3.1 Biochemical Analysis

Effect of ethanolic extract of *Asparagus retrofractus* on thioacetamide induced liver damage in rats with reference to biochemical changes in serum are shown in Table 1. At the end of the 5th day treatment, blood sample of thioacetamide treated control animals showed significant increase in the level of SGPT, SGOT and ALP compare to normal control. Pretreatment with *Asparagus retrofractus* extract at 300 and 600 mg/kg showed marked decreased of SGPT, SGOT and ALP as compared to the thioacetamide treated group. The maximum protection was shown by ethanolic extract at the dose of 600 mg/kg body weight (Table 1).

Total Bilirubin levels are shown in Table 1. The rats exposed to thioacetamide showed significant increased levels of bilirubin as compare to control. Pretreatment with *Asparagus retrofractus* extract showed significant ($P < 0.01$) decreased level of total and direct bilirubin to the near normal which is comparable to the values registered in the standard drug treated (Silymarin) group of animals, indicating the protection of hepatic cells.

The percentage decrease of biochemicals i.e. SGPT, SGOT, bilirubin and ALP by *Asparagus retrofractus* ethanolic extract (300mg/kg and 500mg/kg) and silymarin (100mg/kg) in thioacetamide induced hepatotoxicity in male Wistar rats is shown in Fig 1

3.2 Pentobarbitone Induced Sleeping Time

Thioacetamide treated rats showed significant increased in sleeping time as compared to normal control. Pretreatment with *Asparagus retrofractus* extract showed significant ($P < 0.001$) decreased in sleeping time as to the near normal which is comparable to the

values of the standard drug (Silymarin) treated group of animals, (Table 2). Indicating the protection of hepatic cells.
The percentage reduction in sleeping time is shown in Figure 2.

3.3 Ascorbic Acid Content in Urine

Thioacetamide treated rats showed significant reduction in daily excretion of ascorbic acid in urine compared to normal control. Pretreatment with *Asparagus retrofractus* extract showed significant ($P < 0.001$) prevention in induced reduction in daily excretion of ascorbic acid in urine near to the normal control Which is comparable to the values of the standard drug (Silymarin) treated group of animals (Table 3) indicating the protection of hepatic cells.
The percentage prevention in induced reduction in daily excretion of ascorbic acid in urine is shown in Figure 3.

3.4 Bromsulphalein Clearance Test

Liver slices of animal treated with Ethanolic extract of *Asparagus retrofractus* showed extremely statistical significant ($P<0.001$) hepatoprotective activity by BSP uptake per gm of liver tissue compared to thioacetamide control (Table 4).
The percentage uptake of BSP in liver tissue is shown in Figure 4.

Table 1: Effects of ethanolic extract of roots of *Asparagus retrofractus* on certain serum biochemical parameters in Thioacetamide induced hepatotoxicity in rats

GROUPS	BIOCHEMICAL PARAMETERS			
	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,SC)	37.33±0.95	72.33±0.95	1.04±0.03	296.5±1.708
ASPARAGUS (300mg/kg) p.o.+TA	22.83±0.47** (67.44%)	44.16±0.60** (66.80%)	0.82±0.004 ** (68.75%)	213.33±1.20** (44.47%)
ASPARAGUS (600mg/kg) p.o.+ TA	19.33±0.49 ** (83.72%)	35.83±0.60 ** (86.55%)	0.79±0.006** (78.12%)	175.33±0.88** (64.79%)
SILYMARIN (100mg/kg) p.o. +TA	16.33±1.11 ** (97.67%)	31.15±0.66** (97.65%)	0.74±0.019** (93.75%)	114.3±1.64** (97.43%)
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 *Asparagus retrofractus* and silymarin on biochemical estimation of SGPT, SGOT, Bilirubin and ALP of thioacetamide induced toxicity in male Wistar rats.

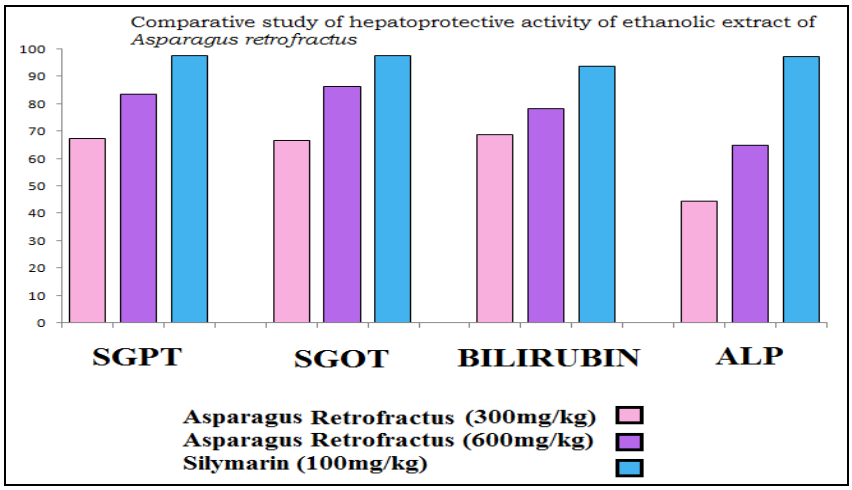

Table 2: Effects of ethanolic extract of roots of *Asparagus retrofractus* on Pentobarbitone induced sleeping time in Thioacetamide induced hepatotoxicity in rats.

Group	Onset of time	Duration of sleep
NORMAL GROUP+pento(40mg/kg)	18.83±1.86	73.16±3.60
TA control (400mg/kg,SC)+pento(40mg/kg)	8±0.96	199.33±8.16
ASPARAGUS (300mg/kg) p.o. +TA+pento(40mg/kg)	11±1.03	147.33±6.66** (41.21%)
ASPARAGUS (600mg/kg) p.o.+ TA+pento(40mg/kg)	13.5±1.47	111.33±.6.04** (69.74%)
SILYMARIN 100mg/kg p.o. +TA+pento(40mg/kg)	16.16±1.40	84.50±4.59 (91.01%)
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: Effects of ethanolic extract of roots of *Asparagus retrofractus* on Pentobarbitone induced sleeping time in Thioacetamide induced hepatotoxicity in rats

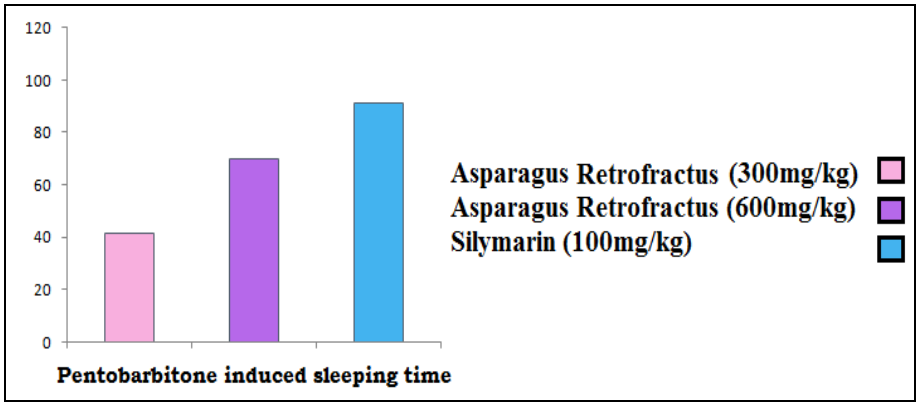

Table 3: Effects of ethanolic extract of roots of Asparagus *retrofractus* on ascorbic acid content in urine in Thioacetamide induced hepatotoxicity in rats.

Group	Before treatment (µg/ml)	After treatment (µg/ml)
THIOACETAMIDE(400mg/kg) SC	133.66±4.58	80.50±2.81
ASPARAGUS (300mg/kg) p.o.+TA	121±3.77	117.50±1.47** (69.60%)
ASPARAGUS (600mg/kg) p.o.+ TA	139.33±1.20	122.16±2.52** (78.36%)
SILYMARIN (100mg/kg) p.o. +TA	142.33±1.99	139±0.96** (110.04%)
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: Effects of ethanolic extract of roots of Asparagus *retrofractus* on ascorbic acid content in urine in Thioacetamide induced hepatotoxicity in rats.

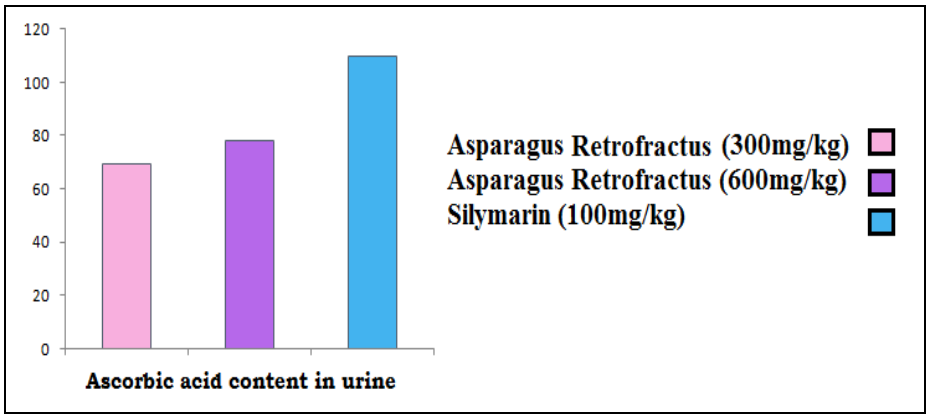

Table 4: Effects of ethanolic extract of roots of *Asparagus retrofractus* on Bromsulphalein clearance test in Thioacetamide induced hepatotoxicity in rat liver

Group	BSP UPTAKE (µg/gm of liver tissue)
NORMAL GROUP	104.10±2.20
TA control (400mg/kg,SC)	60.19±3.70
ASPARAGUS (300mg/kg) p.o. +TA)	76.95±3.05 (38.09%)
ASPARAGUS (600mg/kg p.o.+ TA)	85.09±2.21 (56.59%)
SILYMARIN 100mg/kg p.o.	99.52±4.02 89.38%)
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. 4: Effects of ethanolic extract of roots of *Asparagus retrofractus* on Bromsulphalein clearance test in Thioacetamide induced hepatotoxicity in rat.

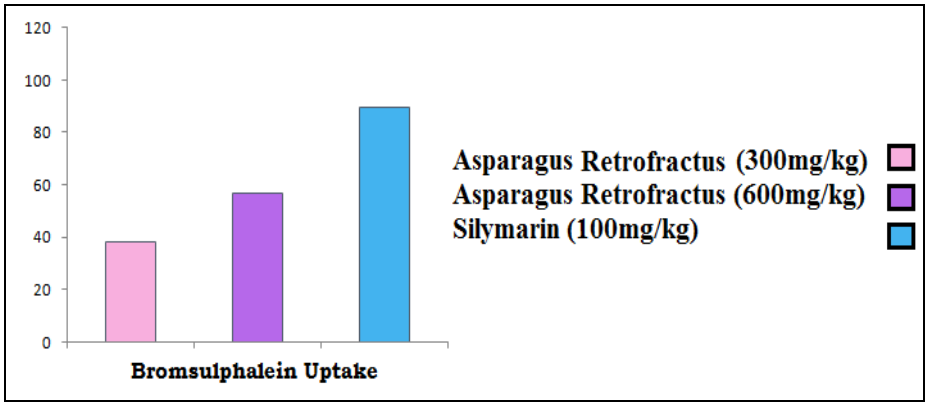

Figure 5: Histopathological photographs of the liver from the normal control and treated, group of rats.

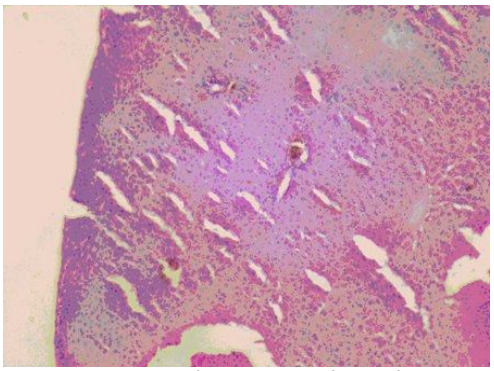

Figure A. Normal control

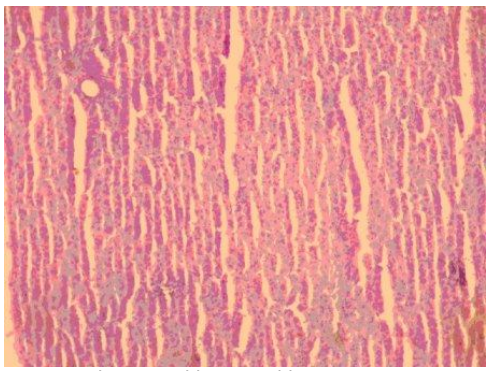

Figure B. Thioacetamide

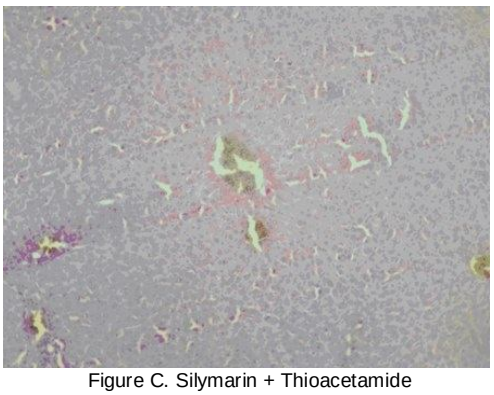

Figure C. Silymarin + Thioacetamide

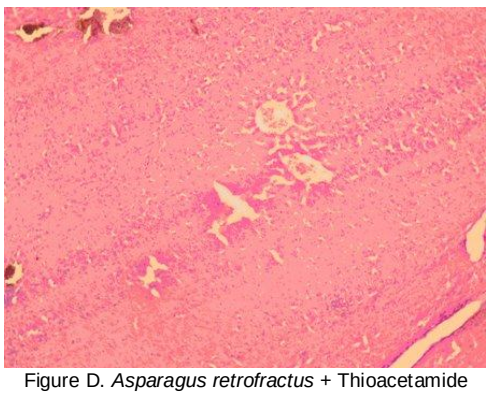

Figure D. *Asparagus retrofractus* + Thioacetamide

In histopathological examination of liver sections of normal control group, (Figure 5.A) no specific change was observed. In the thioacetamide intoxicated group (Figure 5.B), lymphocyte sinusoidal inflammation, Kupffer cell hyperplasic, congested-dilated blood vessels non necrosis fibrosis observed. In the histopathological profile of Silymarin treated group (Figure 5.C), no specific change observed. In the histopathological profile of *Asparagus retrofractus* (Figure 5.D) no specific change observed. *Asparagus retrofractus* was able to control this necrotic change that was comparable to that of thioacetamide. Thus biochemical observation, Pentobarbitone induced sleeping time, Ascorbic acid content in urine, and Bromsulphalein uptake test result co-relates well with the histopathology results of the liver samples. These above observations confirmed the potent hepatoprotective activity of *Asparagus retrofractus.*

3. CONCLUSION

On the basis of results obtained, it can be concluded that the ethanolic extract of *Asparagus retrofractus* roots seems to possess hepatoprotective activity in rats.

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