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HEPATOPROTECTIVE ACTIVITY OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT

To evaluate the hepatoprotective activity of aqueous extract of *Andrographis paniculata* in ethanol induced hepatotoxicity in albino wistar rats. Animals were divided into six different groups containing of six rats each. Group I acts as control were administered single daily dose of normal saline (10 ml/kg bw po) for 28 days. Group II received ethanol for 28 days (20%v/v bw po) were administered to induce hepatotoxicity. Group IV, V and VI received *Andrographis paniculata* at three different doses (100mg/kg, 200mg/kg and 400mg/kg bw po) respectively. Group III received silymarin (50 mg/kg bw po) daily for 28 days. On the 28th day, animals were anaesthetized after 1 hour receiving ethanol under ether and the blood was collected from the retro orbital plexus. Centrifugation at 2500- 3000 rpm at 30^{0}C was done to separate the serum. The transaminase enzymes such as AST and ALT were measured in the serum of respective groups to study the liver functions. To evaluate the action of *Andrographis paniculata* histopathological study was done to measure the characteristics such as centrizonal necrosis, sinusoidal dilation and hepatic fatty degeneration. Results revealed that, there is a tremendous elevation in the liver enzymes such as ATP and ALT associated with ethanol administration. In addition to that, administration of rats caused liver tissue abnormalities such as centrizonal necrosis, sinusoidal dilation and hepatic fatty degeneration. The aqueous extract of *Andrographis paniculata* showed significant reduction in the liver tissue abnormalities and liver enzymes. In this present study, it has shown that *Andrographis paniculata* demonstrate a strong hepatoprotective activity against ethanol induced rats.

KEYWORDS

Andrographis paniculata, Ethanol, Hepatoprotective, Transaminase enzymes and Histopathological study.

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INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved almost all the biochemical pathways to growth, active against disease, nutrient supply and energy protection. Therefore the maintenance of a healthy liver is vital to overall health and well being[1]. Liver injury caused toxic chemicals and certain drugs have been recognized as a toxicological problem. Herbs play a

role in the management of various liver disorders[2]. Recent studies in animal models suggest that liver injury in chronic alcoholics is due to oxidative stress that leads to fibrosis and impaired liver functions and increased apoptosis[3]. Many research reports have linked chronic alcohol consumption and variety of pathological condition varying from simple intoxication to life threatening pathological states[4-6]. A number of plants have shown hepatoprotective property. *Andrographis paniculata* (Acanthaceae) is used extensively in the Indian traditional system of medicine as hepatoprotective and hepatostimulative agent. The aqueous extract of leaf of this plant has traditionally been used for treatment of various liver disorders. The present study aims to study the influence of the aqueous extract of *Andrographis paniculata* on severe liver damage leading to a carcinogen condition[7]. *Andrographis* was selected by the ministry of health as one of the medicinal plant to be included in "the national drug list of essential drugs A.D 1999" (list of herbal medicinal products) in Thailand[8].

MATERIAL AND METHODS

Plant material

The plant material of *Andrographis paniculata* was purchased from Natural Remedies Centre for the preclinical evaluation of the hepatoprotective activity and further identification has also been done on this plant.

Preparation of extract

The freshly collected leaves of *Andrographis paniculata* were washed thoroughly for 3 times, shade dried and size reduced into coarse powder. The coarse powder was macerated in 500ml of distilled water for 7 continuous days at room temperature. Extract were filtered and concentrated by using rotary vacuum evaporator under reduced pressure and the residues were weighed[9].

Chemicals

All the reagents used in this study were of high purity in content. Chemicals which have been used such as ethanol, formalin and chloroform were purchased from Sigma Aldrich Chemical (Malaysia). On the other hand, silymarin was purchased from a Sigma Aldrich (China).

Experimental animals

Adult albino wistar male rats weighing 180±20 grams were used in these pharmacological and toxicological studies. The animals were maintained in well ventilated room temperature 25± 2ºC with 12:12 hours light and dark cycle in polypropylene cages with stainless steel grill top[10]. The rats were fed a standard diet of pellets and tap water ad libitum. Rats were routinely acclimatized to laboratory conditions for 7 days prior to experiments. To ensure that the selected animals are in good state of health, after acclimation the animals was subjected to a gross observation. For the final allotment in the study, the animals were randomly selected. In order to use the laboratory animals, prior authorization was obtained from the University College Animal Ethical Committee.

Acute toxicity study

According to the OECD guidelines the acute toxicity in this present study[11] was followed. Fifteen male wistar rats weighing 180±20g were used for this study. The animals were divided into five different groups each consisting of 3 animals. Single dose of 5 mg/kg was administered separately to all the three rats in each group which were fasted over night with water *ad* libitum. To monitor for severity of any toxic sign and mortality the animals were observed for the period of 1 hour, occasionally for 3 hours. If there are no any signs of mortality at this dose, the dose will be increased to 50, 400 and 2000 mg/kg of extracts of the same procedure for newer groups. The animals were observed up to 7 days after drug administration to find out for any delayed mortality.

Experimental design

Animals were divided into six different groups containing of six rats each.

Group I- (control) were administered single daily dose of normal saline (10ml/kg bw po) for 28 days.

Group II - received ethanol (20%v/v/kg bw po) were administered to induce hepatotoxicity.

Group VI, V and VI - received *Andrographis paniculata* at three different doses (100mg/kg, 200mg/kg and 400mg/kg bw po) respectively.

Group III received silymarin (50mg/kg bw po) daily for 28 days.

Collection of blood

On the 28th day, animals were anaesthetized after 1 hour receiving ethanol under ether and the blood was collected from the retro orbital plexus. Centrifugation at 2500-3000 rpm at 30^0C was done to separate the serum[12]. The transaminase enzymes such as AST and ALT were measured in the serum of respective groups to study the liver function.

Biochemical estimation

To study the liver function, the transaminase enzymes such as AST and ALT levels in the serum were essayed by using spectrophotometry. The enzymes were tested using Sigma Diagnostic kits (Korea) [11].

Histopathological studies

The animals from all groups were sacrificed under ether anesthesia. The livers were detached from the sacrificed animals and preserved in 10% phosphate buffered formalin for at least 24 hours. The standard histopathological procedure was followed in which the liver tissues were prepared and 5µm thick sections were cut in a rotary microtome and mounted on the slide. The sections were stained with heamotoxylin-eosin dye [11]. The tissue sections were then observed under light microscope for the histopathalogical changes, i.e. necrosis, lymphoycytes, fatty degeneration, fibrous connective tissue proliferation, and kuppfer cells infiltration. Photographs were taken from the observed microscope.

Statistical analysis

The experimental data were indicated by mean ± S.D., and were analyzed by an analysis of variance using ANOVA followed by Duncan's test, significant difference was assumed for $p< 0.05$.

RESULTS

Acute toxicity study

High dose up to 2000 mg/kg or low doses aqueous extract of *Andrographis paniculata* were found to be safe in all the rats that received it. During the entire duration of the study no mortality or toxic symptoms were observed. Aqueous extract of *Andrographis paniculata* showed a steady compliance towards the rats and proved to be safe.

The results of biochemical parameters of liver enzymes such as AST and ALT in six different groups were recorded in Table No.1.

Effect of aqueous extract of *Andrographis paniculata* on serum AST level in ethanol induced hepatotoxicity in albino wistar rats

Figure No.1 showed the effect of *Andrographis paniculata* on AST level in serum of ethanol induced hepatotoxicity in male albino wistar rats. The serum AST level in the control group shows the reading of 65.968 ± 0.803^a IU/L, whereas after ethanol treatment, it has increased to 162.507 ± 5.804^b IU/L. The AST level starts reducing to 103.820 ± 6.585^e IU/L and 77.445 ± 8.433^c respectively at the doses of 200 mg/kg and 400 mg/kg bw po in ethanol intoxicated rats, after administration of *Andrographis paniculata*.

Effect of aqueous extract of *Andrographis paniculata* on serum ALT level in ethanol induced hepatotoxicity in albino wistar rats

Figure No.2 showed the effect of *Andrographis paniculata* on ALT level in serum of carbon tetrachloride induced hepatotoxicity in male albino wistar rats. The serum ALT level in the control group shows the reading of 23.272 ± 0.990^a IU/L, whereas after ethanol treatment, it has increased to 60.290 ± 4.907^b IU/L. The ALT level starts reducing to 40.200 ± 2.989^e IU/L and 30.672 ± 0.898^c IU/L respectively at the doses of 200 mg/kg and 400 mg/kg bw po in ethanol intoxicated rats, after administration of *Andrographis paniculata.*

Surrounded by cords of hepatocytes and normal arrangement of hepatocytes with clearly brought out nuclei, cytoplasm, central vein and portal triad.

Histopathological studies provided supportive evidence for the biochemical analysis

Histopathological profile of liver sections of control group in Figure No.3 (a) showed central vein In Figure No.3 (b) where Group II animals treated with ethanol showed cloudy swelling, sinusoidal dilatation, individual hepatocytic necrosis of hepatic cells and centrilobular fatty changes with clear space representing fatty materials or lipids. In Figure No.3 (c) the liver sections of rats treated with aqueous extract of *Andrographis paniculata* of 200 mg followed by ethanol intoxication noticed sinusoidal dilatation marked congestion with mild hepatocellular necrosis in the centrilobular area. The liver sections of the rats treated with aqueous extract of *Andrographis paniculata* of 400 mg followed by ethanol intoxication showed a sign of safety as it was apparent by the nonappearance of necrosis and vacuoles in Figure No.3 (d). The liver sections of the rats treated with silymarin showed a well brought out central vein, hepatic cell with well-preserved cytoplasm, prominent nucleus and nucleolus in Figure No.3 (e).

DISCUSSION

Andrographis paniculata is a well-known traditional medicinal plant which possesses numerous pharmacological functions and biological activity. Ethanol is a direct hepatotoxin which produces centri lobular necrosis and steatosis.

This present study has been done to evaluate the hepatoprotective activity of aqueous extract of *Andrographis paniculata* on ethanol induced hepatotoxic in male albino wistar rats. In addition to this, the hepatoprotective activity of *Andrographis paniculata* was compared against the standard drug silymarin. On the other hand, the study on different doses of *Andrographis paniculata* was done to prove a similar activity to that of silymarin.

The present data revealed that the serum AST and ALT levels were significantly ($p<0.05$) increased in rats intoxicated with ethanol in contrast with the control group as shown in Table No.1.

Rats that were treated with aqueous extract of *Andrographis paniculata* at dose level of 400 mg/kg produced significant ($p< 0.05$) difference from the control group. Based on the results obtained in this study, it shows that at 200 mg/kg dose the extract has a positive effect on hepatoprotective activity but not as sufficient as the standard drug, silymarin. On the other hand, groups that were administered with aqueous extract of *Andrographis paniculata* at dose level of 400 mg/kg indicate greater significant ($p< 0.05$) difference from the ethanol group.

In this present study also revealed that the ability of silymarin (50 mg/kg) in reducing the serum enzyme levels such as AST and ALT was more pronounced compared with that of *Andrographis paniculata* at dose level of 200 mg/kg. Silymarin also produced a slightly higher significant reduction in comparative with the 400 mg/kg dose of *Andrographis paniculata*. Thus, the dose of 400 mg/kg would be safe to use as a hepatoprotective agent. In addition, silymarin produced significant ($p< 0.05$) difference from the ethanol control group.

Table No.1: Effect of aqueous extract of *Andrographis paniculata* on liver enzymes in ethanol induced hepatotoxicity in albino wistar rats

S.No	Groups	AST (IU/L)	ALT (IU/L)
1	Control	65.968±0.803[a]	23.272±0.990[a]
2	Ethanol (20% v/v)	162.507±5.804[b]	60.290±4.907[b]
3	Silymarin (50mg/kg)	74.218±4.396[c]	30.215±1.344[c]
4	*A.Paniculata*100mg	135.250±4.919[d]	56.597±2.795[d]
5	*A.Paniculata* 200mg	103.820±6.585[e]	40.200±2.989[e]
6	*A.Paniculata* 400mg	77.445±8.433[c]	30.672±0.898[c]

Values are means ± S.D. of six rats in each group. Values that have a different superscript letter (a,b,c,d, e) differ significantly with each other ($p< 0.05$).

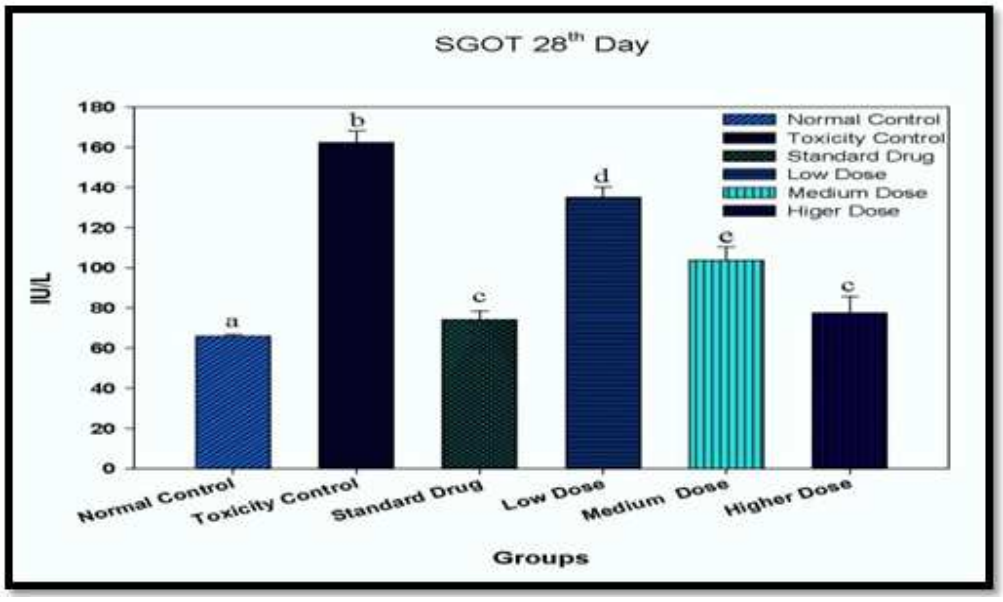

Figure No.1: Effect of aqueous extract of *Andrographis paniculata* on serum AST level in ethanol induced hepatotoxicity in albino wistar rats

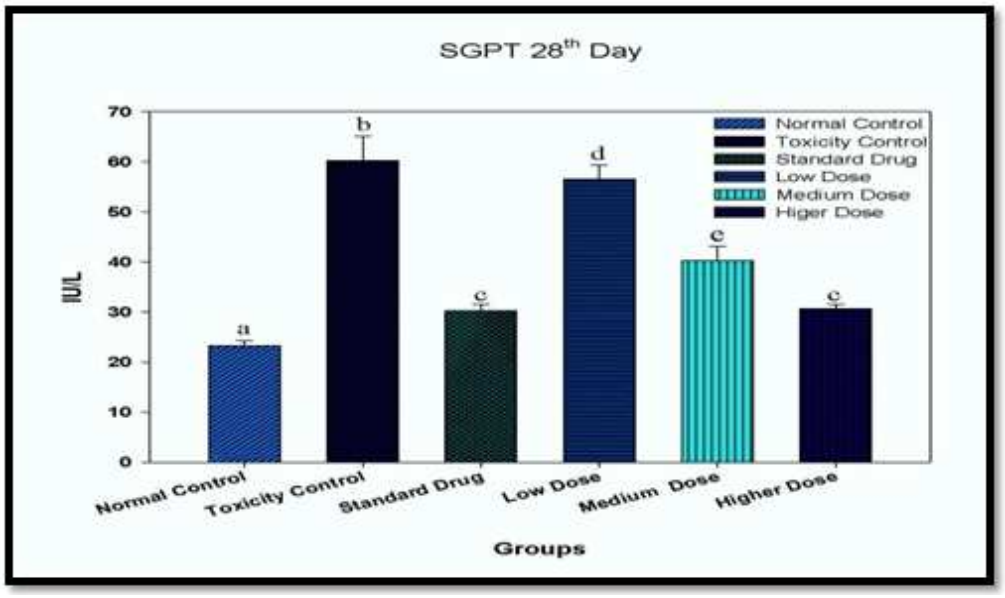

Figure No.2: Effect of aqueous extract of *Andrographis paniculata* on serum ALT level in ethanol induced hepatotoxicity in albino wistar rats

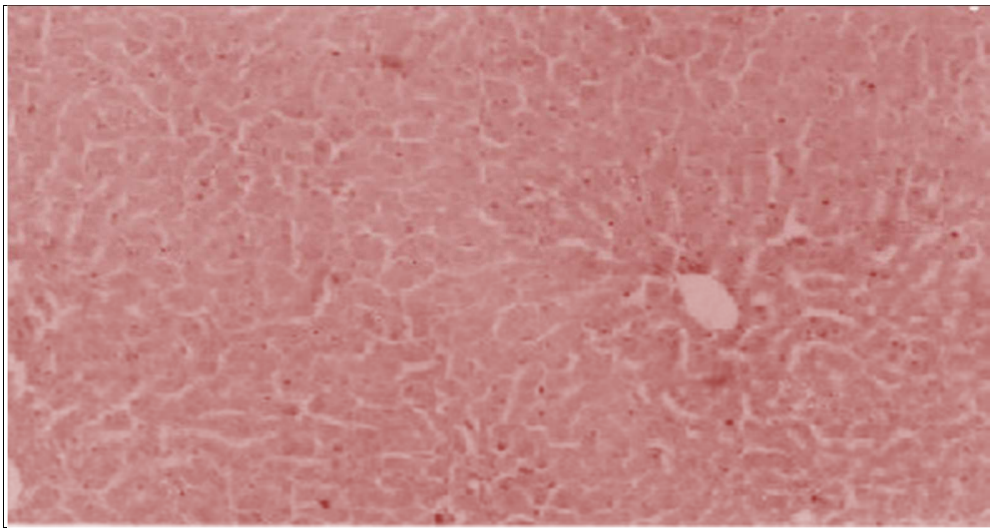

Figure No.3 (a): Control group (10ml/kg Normal saline) liver section show central vein surrounded by cords of hepatocytes and normal arrangement of hepatocytes with clearly brought out nuclei, cytoplasm, central vein and portal triad. 100x

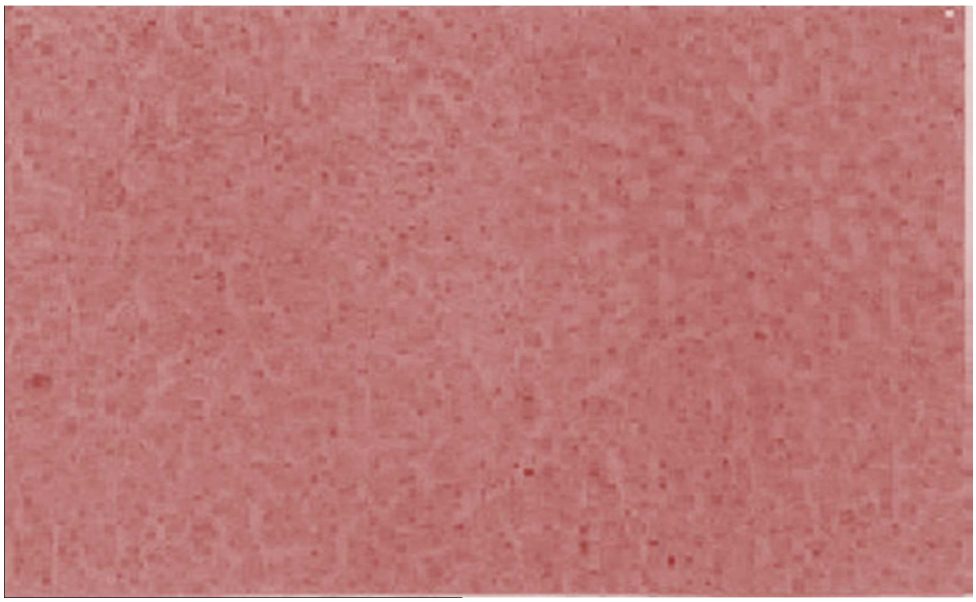

Figure No.3 (b): Ethanol control group liver section showed cloudy swelling, sinusoidal dilatation, individual hepatocytic necrosis of hepatic cells and centrilobular fatty changes with clear space representing fatty materials or lipids. 400x

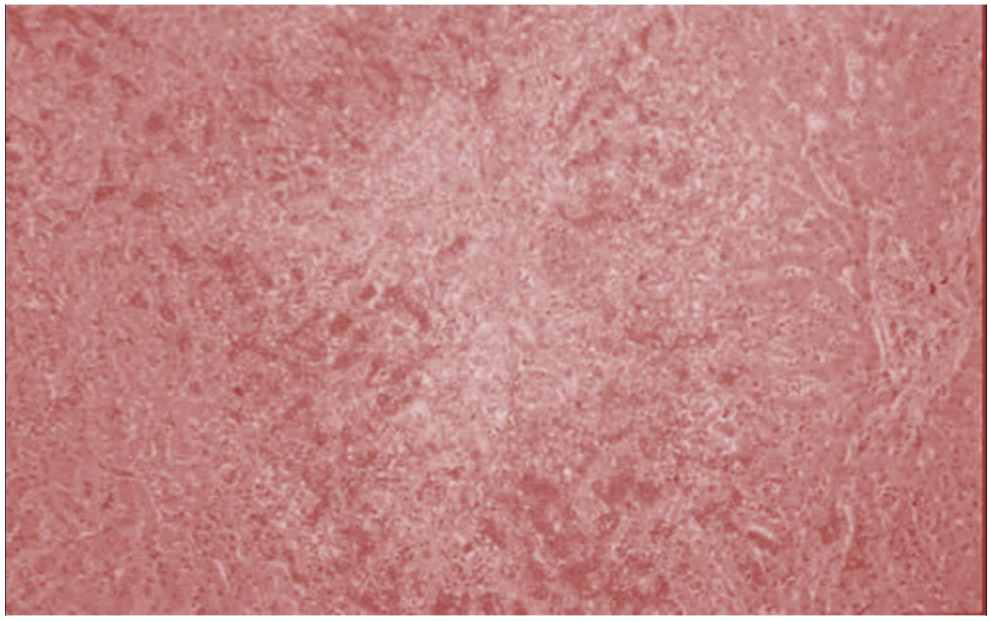

Figure No.3 (c): Treated group (Ethanol + *A.Paniculata* 200mg/kg) liver section revealed noticed sinusoidal dilatation marked congestion with mild hepatocellular necrosis in the centrolobular area. 400x

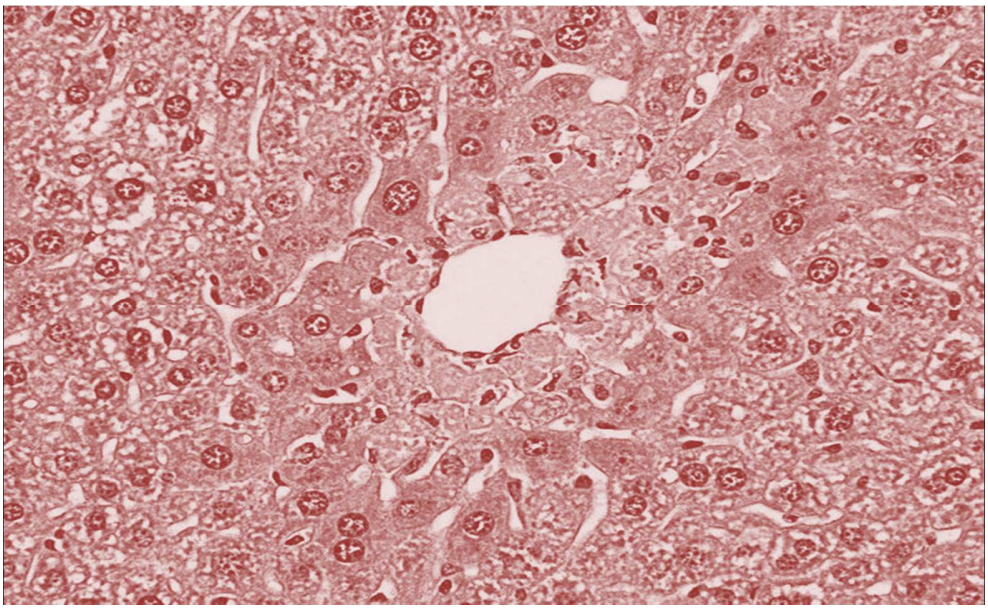

Figure No.3 (d): Treated group (Ethanol + *A.Paniculata* 400mg/kg) liver section shows mild sinusoidal dilatation and mild fatty changes. 400x

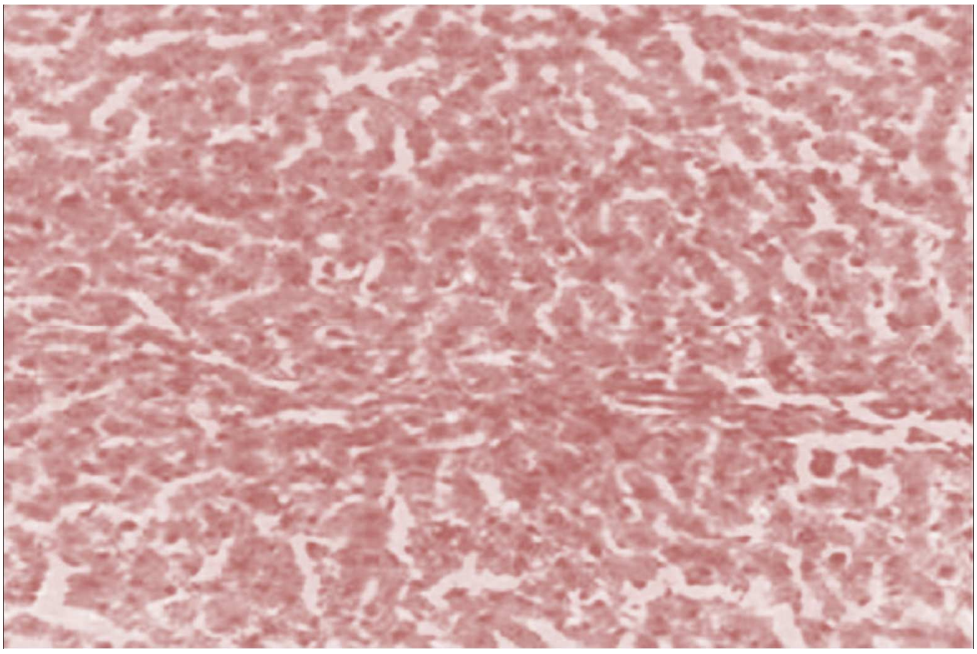

Figure No.3 (e): Group (Silymarin 50mg/kg) liver section revealing well brought out central vein, hepatic cell with well-preserved cytoplasm, prominent nucleus and nucleolus. 400x

CONCLUSION

Many reports have described that lipid peroxidation of liver cell membrane caused by reactive oxygen species (ROS) is the major reason of liver damage induced by ethanol[13-18]. The aqueous extract of *Andrographis paniculata* at dose level of 200 and 400 mg/kg were administered orally to the induced rats. In this present study, it has shown that *Andrographis paniculata* demonstrate a strong hepatoprotective activity at the dose of 400 mg/kg. The oral toxicity study of the *Andrographis paniculata* showed that the plant extract is safe to be used as a medium in different formulations. It can be concluded that the biochemical and histological alterations induced by the administration of ethanol were enhanced under the effect of *Andrographis paniculata* at 400 mg/kg. Thus, the choice of suitable plant extract in case of hepatotoxicity induced by ethanol is very important. The present study also suggests that the concept of traditional medicine has a lot of benefits and has to be revealed to the public in order for them to gain a beneficial knowledge on the importance of traditional medicine.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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