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HEPATOPROTECTIVE ACTIVITY OF *BACOPA MONNIERI* EXTRACT IN ETHANOL INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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ABSTRACT

To evaluate the hepatoprotective activity of *Bacopa monnieri* extract in ethanol induced hepatotoxicity in albino wistar rats. Six groups, each group containing of six rats allotted to different treatment groups. Group 1 (control) is treated with normal saline (10ml/kg bw po) as vehicle only. All other groups received ethanol (10ml/kg bw po) with group 2 serving as ethanol treated control. After ethanol administration, group 3, 4 and 5 also received *Bacopa monnieri* at different doses (100mg/kg, 200mg/kg, 400mg/kg bw po) respectively. As for group 6, after ethanol administration rats receive silymarin (140mg/kg bw po). At the 7th, 14th, 21st and 28th day, animals were anaesthetized with ether and blood was collected from the retro orbital plexus and serum is separated by centrifugation. To study the liver function, the transaminase enzymes (AST, ALT, and ALP) were measured in the serum of respective groups. Histopathological study was also conducted to measure the action of *Bacopa monnieri* on parameters such as hepatic fatty degeneration and centrizonal necrosis of respective groups. Present study suggested that ethanol administration increased the levels of transaminase enzymes. However, the extract of *Bacopa monnieri* significantly reduced the elevated serum levels of transaminase enzymes in ethanol intoxicated rats. Apart from that, ethanol also causes hepatic fatty degeneration and centrizonal necrosis in ethanol intoxicated rats. Histopathological studies showed administration of aqueous extract of *Bacopa monnieri* offered significant protection from these damaging actions as study showed marked reduction in fatty degeneration and centrizonal necrosis in ethanol intoxicated rats. Present study suggests that extract of *Bacopa monnieri* has significant hepatoprotective activity against ethanol intoxicated rats.

Key words: *Bacopa monnieri*, Ethanol, Hepatoprotective, Transaminase enzymes, Histopathological study.

INTRODUCTION

The role of free radical reactions in disease pathology is well established. It suggests that these reactions are necessary for normal metabolism but can be detrimental to health as well including outcome of various diseases like diabetes, immunosuppressant, neurodegenerative diseases and others. Free radicals lead to cellular necrosis, which is implicated in some membrane pathophysiological conditions, including atherosclerosis, rheumatoid arthritis as well as toxicity of many xenobiotics.

Liver is the largest glandular organ of the body. It weighs about 3 lb (1.36 kg) and reddish brown in color and is divided into four lobes of unequal size and shape.

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The liver lies on the right side of the abdominal cavity beneath (Leung AY 1996) the diaphragm. Blood is carried to the liver via two large vessels called the hepatic artery and the portal vein. The hepatic artery carries oxygen-rich blood from the aorta. The portal vein carries blood containing digested food from the small intestine. These blood vessels subdivide in the liver repeatedly, terminating in very small capillaries. Each capillary leads to a lobule. Liver tissue is composed of thousands of lobules, and each lobule is made up of hepatic cells, the basic metabolic cells of the liver. The liver has many functions. Some of the functions are, to produce substances that break down fats, convert glucose to glycogen, produce urea, make certain amino acids, filter harmful substances from the blood, storage of vitamins and minerals (vitamins A, D, K and B_{12}) and maintain a proper level or glucose (Enz A *et al*., 1993).

The liver is also responsible for producing cholesterol, also it is known to produces about 80% of the cholesterol in your body. Several diseases states can affect the liver. Some of the diseases are Wilson's disease, hepatitis (an inflammation of the liver), liver cancer, and cirrhosis (a chronic inflammation that progresses ultimately to organ failure). It alters the alcohol metabolism of the liver, which can have overall detrimental effects if alcohol is taken over long periods. Hemochromatosis is one of the cause of liver problems.

During alcohol intake, it is absorbed into the bloodstream from the stomach and intestines. All blood from the stomach and intestines first goes through the liver before circulating around the whole body (Bilodeau M, 2003) therefore; the highest concentration of alcohol is in the blood flowing through the liver. The alcohol dehydrogenase enzyme breaks down alcohol by removing hydrogen in two steps that is, alcohol dehydrogenase oxidizes alcohol to acetaldehyde and second acetaldehyde dehydrogenase oxidizes the acetaldehyde (Tripathi YB *et al*., 1996) to acetyl co-enzyme. These reactions produce hydrogen ions (acid). The B vitamin niacin (in its role as the coenzyme NAD) picks up these hydrogen ions (becoming NADH) thus, once alcohol metabolized, NAD diminishes and NADH increases. During its metabolism, NAD becomes unavailable for the many other vital body processes for which it is needed, including glycolysis, the TCA cycle and the electron transport chain. Without NAD, the energy pathway is blocked, and alternative routes are taken, with serious physical (Bilodeau M, 2003) consequences. The accumulation of hydrogen atoms shifts the body's balance toward acid. The accumulation of NADH slows the TCA cycle, resulting in a buildup of pyruvate and acetyl CoA. Excess acetyl CoA results in fatty acid synthesis and fat begins to clog (Thurman RG, 2009) the liver.

An accumulation of fat in the liver can observe after only a single night of heavy drinking. With moderate drinking, the liver can process alcohol safely however, heavy drinking overtaxes the liver resulting in serious consequences. A liver clogged with fat causes liver cells to become less efficient at performing their necessary tasks, resulting in impairment of a person's nutritional health. Fatty liver is the first stage of liver deterioration in heavy drinkers, and interferes with the distribution of oxygen and nutrients to the liver's cells. If the condition persists long enough, the liver cells will die, forming fibrous scar tissue (the second stage of liver deterioration, or fibrosis (Victor J *et al*., 2006) some liver cells can regenerate with good nutrition and abstinence, however in the last stage of deterioration, or cirrhosis, the damage to the liver cells is the least reversible.

Drugs continue to be taken off the market due to late discovery (Ghosh T *et al*., 2005) of hepatotoxicity. Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury from drugs and other substances. Seventy five percent of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins, which bring drugs and xenobiotics in near-undiluted form several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of oxidants, which, in turn, injure hepatic cells (Tripathi YB *et al*., 1996) activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also leads to oxidative stress injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside liver this promotes further liver damage. Non-parenchymal cells such as Kupffer cells, fat storing stellate cells, and leukocytes also have role (Kapoor LD, 1990) in the mechanism. Liver blood tests are some of the most commonly performed blood tests. These tests can assess liver functions or liver injury.

An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood under normal circumstances; these enzymes reside within the cells of the liver. However, when the liver is injured for any reason, these enzymes are spilled into the blood stream (Enz A *et al*., 1993) enzymes are proteins that are present throughout the body, each with a unique function enzymes help to speed up (catalyze) routine and necessary chemical reactions in the body. Among the most sensitive and widely used liver enzymes are the aminotransferases.They include aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). These enzymes are normally contained within liver cells. If the liver is injured

or damaged, the liver cells spill these enzymes into the blood, raising the enzyme levels in the blood and signaling disease. Other blood tests pertaining to the liver are measurements of some of the other enzymes found the liver. In addition to AST and ALT, alkaline phosphatase, 5' nucleotidase, and gamma-glutamyl transpeptidase (GGT) are other enzymes located in the liver. The focus of this article is mainly on the most common (Nandave M *et al*., 2007) liver enzymes, AST and Alanine transferase.

Bacopa monnieri a member of the Scrophulariaceae family is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers. In India and the tropics it grows naturally (Sumathi T, 2008) in wet soil, shallow water, and marshes. This herb is found at elevations from sea level to altitudes of 4, 400 feet, and is easily cultivated if adequate water is available. Flowers and fruit appear in summer and the entire plant is used medicinally compounds which is responsible for the pharmacological effects of Bacopa include alkaloids, saponins and sterols (Ghosh T, 2005). Many active constituents,the alkaloids Brahmine and herpestine, saponins d-mannitol andhersaponin, acid A, and monnierin. *Bacopa monnieri* other active constituents (Willianson EM, 1996) including betulic acid, stigmastarol, beta-sitosterol, as well as numerousbacosides and bacopasaponins. This constituents responsible for Bacopa's cognitive (Bhattacharya SK *et al*., 2000) effects are bacosides A and B (Handa SS,1998) Brahmi has been valued as a cardiac, nerve and brain tonic and widely used by students for improving mental clarity, confidence, intelligence, concentration and memory recall. Research on the brahmi plant identified 2 active molecules which is Bacoside. A that assists in the release of nitric oxide allowing relaxation of the aorta and veins and blood to flow more smoothly through the body and aids circulation; and Bacoside B, a protein valued for nourishing (Marletta MA,1989) nerve cells in the brain.

Studies have confirmed that indeed oxidative stress plays an important role in the initiation (Singh HK, 1997) and progression of liver disease. A free radical is an unstable chemical fragment, which can cause havoc by damaging DNA, corroding cell membranes and destroying cells (Yoshiki K, 1998) research shows the value of antioxidants in preventing and also in reversing many forms of cancer, heart disease, atherosclerosis, adult diabetes, lung diseases, cataracts and a host of other diseases. All of these diseases, as well as premature aging, are caused by free radical oxidation of healthy tissues. As *B. monnieri* contains large amounts of saponins it is thought worthwhile to investigate the antioxidant and hepatoprotective activity of the aerial parts of *Bacopa monnieri* in scientific manner. Along with the more familiar antioxidants, Bsitosterol, a powerful fatty acid in brahmi, acts to relieve many degenerative conditions.

In summary, brahmi can help support and improve all aspects of mental function, and also as antioxidant in hepatoprotective activity.Therefore this study has been conducted to evaluate the hepatoprotective activity of aqueous extract *Bacopa monnieri* of on ethanol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant material

The plant material of *Bacopa monnieri* extract was purchased from India in the form of dried powder (Handa SS, 1998). Further identification has also been done.

Chemicals

All reagents used in the study (Budavari S, 1996) were of high purity. All chemicals such as Ethanol, Formalin, Xylene and DMSO were purchased from Sigma Aldrich Chemical (Malaysia). Silymarin purchased from local pharmaceutical company were also used in the experiments.

Experimental animals

Experiments were carried out on healthy adult male albino wistar rats weighing 200 ± 20 grams. They were raised in the animal house at the Faculty of Pharmacy of the Masterskill University College of Health Sciences. Animals were housed in polypropylene cages with stainless steel grill top at 25 ± 2^{o}C with 12:12 hours light and dark cycle was followed. They were fed a standard diet of pellets and tapped water ad libitum. Rats were routinely acclimatized to laboratory conditions for 7 days prior to experiments (Bhattacharya SK *et al*., 2000). After acclimation, the animals will be subjected to a gross observation, to ensure that the selected animals are in good state of health. Animals were then randomly selected for final allotment to the study. Prior authorization for the use of laboratory animals in this study was obtained from the University College Animal Ethical Committee (Reg. MUCH/AEC/HS/2012/15). The experimental procedures were carried out in strict compliance with the Animal Ethics Committee's rules and regulations followed in this institute.

Acute toxicity study

Acute toxicity studies (Nolan JP, 1980) were carried on rats as per the guidelines (OECD NO: 423) given by the Organization for Economic Co-operation and Development. Overnight-fasted Albino wistar rats (200 ± 20g) of male sex were used for the study. The animals were divided into five groups of three animals each. The extracts were administered separately to the all the three

animals in each group at starting single dose of 5mg/kg. Animals were observed for the period of 1 h, occasionally for 3 h for severity of any toxic sign and mortality. If no mortality is observed at this dose, the same procedure will be repeated for dose level of 50, 400, 2000mg/kg of extracts (Channa S *et al*., 2003) of separate newer groups. The LD_{50} was thus determine, which was selected for the hepatoprotective animals study. The animals were observed up to 7 days after drug administration to find out for any delayed mortality.

Experimental design

A total of 36 rats were used in this study. Six groups, each group containing of six rats allotted to different treatment groups. Group 1 (control) is treated with normal saline (10ml/kg bw po) as vehicle only. All other groups received ethanol (10ml/kg bw po) with group 2 serving as ethanol treated control. After ethanol administration, group 3, 4 and 5 also received *Bacopa monnieri* at different doses (100mg/kg, 200mg/kg, 400mg/kg bw po) respectively. As for group 6, after ethanol administration rats receive silymarin (140mg/kg bw po). This study was carried out continuously for 28 days.

Collection of blood

At the 7th, 14th, 21st and 28th day, animals were anaesthetized with ether and blood was collected from the retro orbital plexus and serum is separated by centrifugation.

Biochemical estimation

To study the liver function, the transaminase enzymes (AST, ALT, and ALP) levels in the serum were essayed spectrophotometrically using Sigma Diagnostic kits (USA).

Histopathological studies

Livers from animals from different groups were isolated and fixed in 10% phosphate buffered formalin for at least 24 h. Then the paraffin sections were prepared and cut into 5 µm thick section in a rotary microtome and mounted on the slide. The sections were then stained with haemotoxylin-eosin dye. After staining, the sections were observed under light microscope for histopathological changes, i.e. necrosis, fatty degeneration, lymphocytes and Kupffer cells infiltration, and photographs were taken (Handa SS, 1998).

Statistical Analysis

Data were expressed as the mean ± standard error of mean (SEM). The data were analyzed using one way analysis of variance (ANOVA) followed by Turkey's test as post hoc test for multiple comparisons (Mohanan PV., 1997) Data were considered significant at different level of P value; $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS

Acute toxicity studies

All the rats that received aqueous extract of *Bacopa monnieri* either at high dose up to 2000 mg/kg or low dose were found to be safe. No mortality or toxic symptoms were observed during the entire duration of the study. Aqueous extract of *Bacopa monnieri* showed a stable compliance towards the rats and proved to be safe.

Table 1. Effect of aqueous extract of *Bacopa monnieri* on liver enzymes in ethanol induced hepatotoxicity in wistar albino rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	52.270±21.098	38.688±11.229	19.965±5.287
Ethanol	260.660±43..952 [a]	194.59±56.095 [a]	92.106±32.963 [a]
BM 100mg	255.097±53.792 [a]	180.708±45.757 [a]	86.126±24.443 [a]
BM 200mg	244.415±56.858 [a]	163.583±42.411 [a]	83.300±3.323 [a]
BM 400mg	151.610±35.837 [b,c]	98.316±16.371 [c]	43.683±7.322 [c]
Silymarin	107.723±16.032 [c]	59.200±16.364 [c]	43.058±7.322 [c]

The results are expressed as the Mean ± SD of six rats/group; One way ANOVA followed by Turkey's multiple test.
a = Results significantly different from Control group, P < 0.001
b = Results significantly different from Control group, P < 0.01
c = Results significantly different from ethanol group, P < 0.001

Effect of aqueous extract of *Bacopa monnieri* on serum levels

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

Figure 1 showed the effect of *B. monnieri* on AST level in serum of ethanol induced hepatotoxicity in male wistar albino rats. The control had shown the AST level in serum of 52.270±21.098 IU/L but after ethanol treatment, it increased to 260.660±43..952 IU/L. Whereas

after administration of *B. monnieri* at the doses of 100mg/kg, 200mg/kg, and 400mg/kg bw po in ethanol intoxicated rats, the AST level reduced to 255.097±53.792 IU/L, and 244.415±56.858 IU/L 151.610±35.837 IU/L respectively.

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

Figure 2 showed the effect of *B. monnieri* on ALT level in serum of ethanol induced hepatotoxicity in male wistar albino rats. The control had shown the ALT level in serum of 38.688±11.229 IU/L but after ethanol treatment, it increased to194.59±56.095 IU/L. Whereas after administration of *B. monnieri* at the doses of 100mg/kg, 200mg/kg, and 400mg/kg bw po in ethanol intoxicated rats, the ALT level reduced to 180.708±45.757 IU/L, 163.583±42.411 IU/L, 98.316±16.371 IU/L, respectively.

Figure 1. Effect of aqueous extract of *Bacopa monnieri* on serum AST level in ethanol induced hepatotoxicity in wistar albino rats

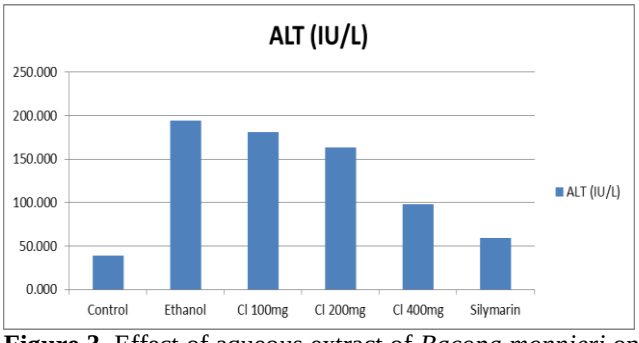


Figure 2. Effect of aqueous extract of *Bacopa monnieri* on serum ALT level in ethanol induced hepatotoxicity in wistar albino rats

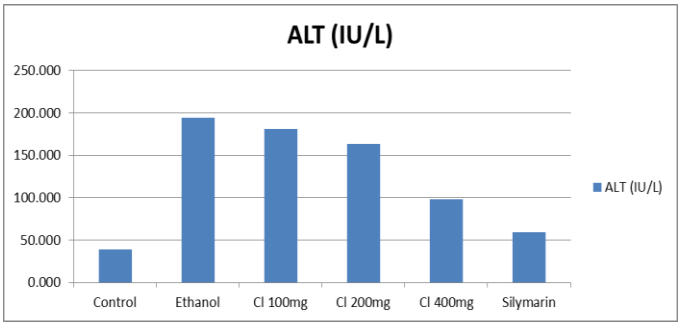


Figure 3. Effect of aqueous extract of *Bacopa monnieri* on serum ALT level in ethanol induced hepatotoxicity in wistar albino rats

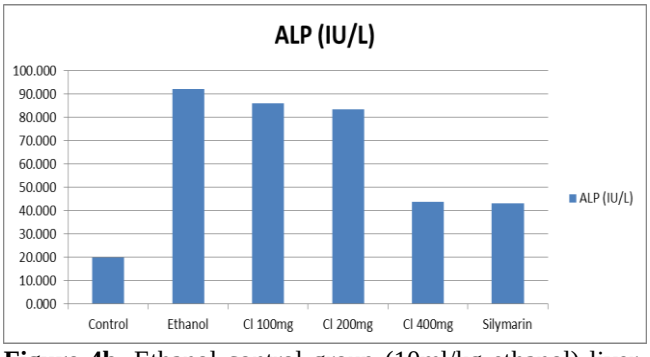


Figure 4a. Control group (10ml/kg normal saline) liver section revealing normal hepatic parenchyma with a central vein at the top corner. 100 xs

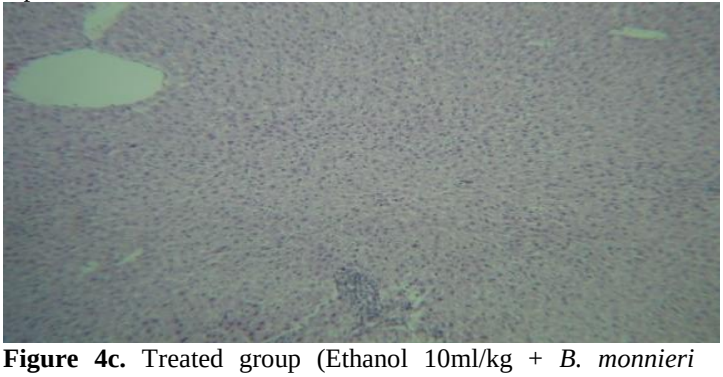


Figure 4b. Ethanol control group (10ml/kg ethanol) liver section showed intense necrotic hepatitis in the diseased control revealing nuclear pyknosis, karyolysis/karyorhexis and intense cellular infiltration. 400 xs

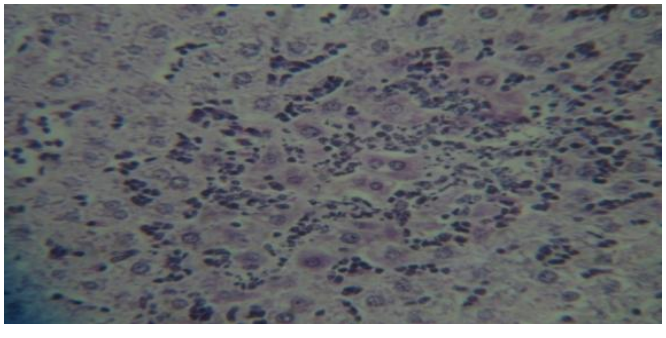


Figure 4c. Treated group (Ethanol 10ml/kg + *B. monnieri* 100mg/kg) liver section revealing swollen hepatocytes with decreased sinusoidal spaces. 400 xs

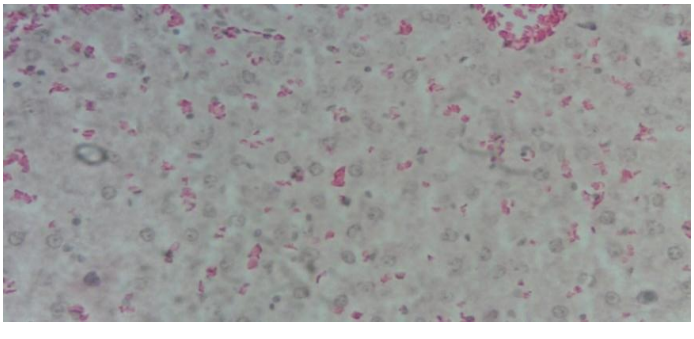


Figure 4d. Treated group (Ethanol 10ml/kg + *B. monnieri* 200mg/kg) liver section revealing swollen hepatocytes with decreased sinusoidal spaces. 400 xs

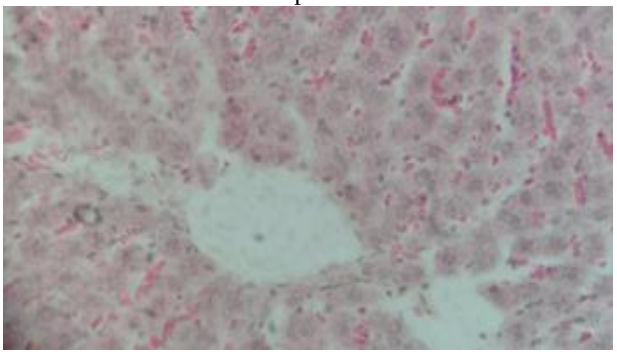


Figure 4e. Treated group (Ethanol 10ml/kg + *B. monnieri* 400mg/kg) liver section revealing comparatively normal hepatic parenchyma with a single focus of spotty necrosis. 400 xs

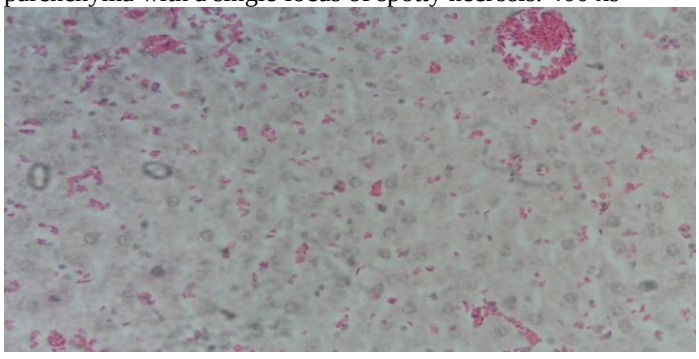
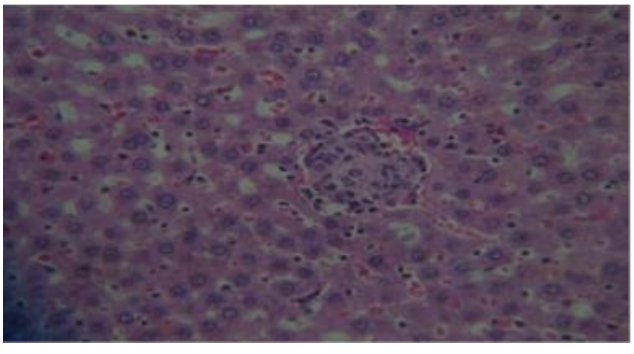


Figure 4f. Treated group (Silymarin 140mg/kg + *B. monnieri* 400mg/kg) liver section revealing comparatively normal hepatic parenchyma with a single focus of spotty necrosis. 400 xs



Effect of aqueous extract of *Bacopa monnieri* on serum ALP level in ethanol induced hepatotoxicity in wistar albino rats

Figure 3 showed the effect of *B. monnieri* on ALP level in serum of ethanol induced hepatotoxicity in male wistar albino rats. The control had shown the ALP level in serum of19.965±5.287 IU/L after ethanol treatment, it increased to 92.106±32.963 IU/L. Whereas after administration of *B. monnieri* at the doses of 100mg/kg, 200mg/kg, and 400mg/kg bw po in ethanol intoxicated rats, the ALP level reduced to 86.126±24.443 IU/L, 83.300±3.323 IU/L, 43.683±7.322 IU/L respectively.

Histopathological studies provided supportive evidence for the biochemical analysis

Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure 4a). Group II animals exhibited disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration (Figure 4b). The liver sections of the rats treated with aqueous extract of *B. monnieri* followed by ethanol intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure 4c, 4d and 4e). The liver sections of the rats treated with silymarin followed by ethanol intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure 4f).

DISCUSSION

This study was undertaken to evaluate the hepatoprotective activity of aqueous extract of *B. monnieri* on ethanol induced hepatotoxic in male albino wistar rats. Addition to this, this study was also to compare the hepatoprotective activity of aqueous extract of *B. monnieri* against standard drug silymarin and determining the dose that is producing almost similar activity to silymarin. The LD_{50} of *B. monnieri* was found to be safe up to 2000 mg/kg. Thus, it would be safe to use this extract *(B. monnieri)* as a hepatoprotective agent.

The significant ($P < 0.01$) increase in levels of serum AST (260.660±43.952), ALT (194.59±56.095) and ALP (92.106±32.963) confirmed the hepatotoxicity in the group of rats administered with ethanol as shown in table 1. Pretreatment of group of rats with aqueous extract of *B. monnieri* at dose level of 100 and 200 mg/kg showed significant ($P < 0.001$) different from control group, which proves at this doses the extract has hepatoprotective activity but not sufficient activity by restoring at the levels of AST (255.097±53.792 IU/L, and 244.415±56.858), ALT (180.708±45.757 IU/L, 163.583±42.411 IU/L) and ALP (86.126±24.443 IU/L, 83.300±3.323 IU/L) respectively.

Groups of rats pretreated with aqueous extract of *B. monnieri* at dose level of 400 mg/kg showed more significant ($P < 0.001$) different from ethanol control group proved by improvement in levels of the AST (151.610±35.837), ALT (98.316±16.371) and ALP

(43.683±7.322) respectively. The animals pretreated with the silymarin (140 mg/kg) showed slightly higher significant ($P < 0.001$) reduction in rise in the serum enzymes level, AST (107.723±16.032), ALT 59.200±16.364 () and ALP (43.058±7.322) in comparison to ethanol control group.

The aqueous extract of *B. monnieri* used in the study preserved the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided as compared to the enzyme levels in ethanol control group rats. The aqueous extract of *B. monnieri* at dose level 400 mg/kg showed prominent hepatoprotection in comparison to the ethanol control group and silymarin pretreated group rats.

CONCLUSION

In this study, hepatoprotective activity of the aqueous extract of *Bacopa monnieri* was studied. The aqueous extract of *Bacopa monnieri* at the dose of 400mg/kg showed very prominent and similar to silymarin hepatoprotective activity as demonstrated by significant ($P<0.001$) decrease in transaminase enzyme levels and preserved the structural integrity of the hepatocellular membrane as evident from the protection provided as compared to the ethanol control group rats.

Identification of natural compound of plant will help to develop new therapeutically agents. The results obtained from this study shows that this plant is a good natural source for hepatoprotecticve activity. Since this plant is easily available and the aqueous extract is showing better activity, this suggests that this plant is a cost effective natural treatment available in market. B. monnieri is found phenolic compound in significant amount, which attributes to its rationality of possessing antioxidant activity. Further clinical trials should be done in order to develop a prominent formulation that will be useful for public.

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Declaration of interest

The authors report no conflicts of interest.

Authors' contributions

YA conceived the study and wrote the first draft of the paper. All the authors participated in the data collection, data analysis and interpretation of data. VS did the critical revision of the draft. All authors read and approved the final manuscript.

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