RESEARCH ARTICLE

Evaluation of Hepatoprotective Potential of *Zizyphus xylopyrus* Extract

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ABSTRACT

The present study was carried out to evaluate the hepatoprotective activity of selected plant by paracetamol induced liver injury, which are indigenous to Indian region. *Zizyphus xylopyrus* (Z. xylopyrus) is used traditionally in the treatment of variety of diseases such as obesity, urinary troubles, diabetes, skin infections, fever, diarrhoea, insomnia and digestive disorder. Ethanobotanical survey shows that various parts of plant have been used in the treatment of diseases by folk person. The aerial and root barks, leaves, and fruits of Zizyphus species used in Indian system of medicine for the treatment of various diseases such as weakness, liver complaints, obesity, diabetes, skin infections, fever, diarrhoea, insomnia and digestive disorders. It is used in Pyorrhoea and to check oogenesis. The bark is used for its astringent activity and as dental sticks for teeth cleaning. In different parts of India this plant is also used in the treatment of diarrhoea. The present work emphasizes the evaluation of hepatoprotective activity *Zizyphus xylopyrus* roots. The result revealed that methanolic extracts of plant could be useful in preventing paracetamol induced liver injury.

Keywords: *Zizyphus xylopyrus*, hepatoprotective activity

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INTRODUCTION

Liver, the largest gland in the body, a spongy mass of wedge-shaped lobes that has many metabolic and secretory functions. The liver secretes bile, a digestive fluid; metabolizes proteins, carbohydrates, and fats; stores glycogen, vitamins, and other substances; synthesizes blood-clotting factors; removes wastes and toxic matter from the blood; regulates blood volume; and destroys old red blood cells. Liver tissue consists of a mass of cells tunneled through with bile ducts and blood vessels. Hepatic cells make up about 60 percent of the tissue and perform more metabolic functions than any other group of cells in the body. A second group of cells, called Kupffer cells, line the smallest channels of the liver's vascular system and play a role in blood formation, antibody production, and ingestion of foreign particles and cellular debris. The liver cells synthesize a number of enzymes. As blood flows through the liver, both from the portal vein and from the hepatic artery, the cells and enzymes are filtered.[2] Liver is the largest and most complex internal organ in the body. It plays an important role in maintenance of internal environment with the help of its multifarious and several functions. It is connected to inter mediatory metabolism of proteins, fats, and carbohydrates as well as in the synthesis of number plasma proteins such as albumin, fibrinogen and in the production of various enzymes, formation and excretion of bile. Drug-induced liver injury is a major health problem that remonstrance not only health care profession but also pharmaceutical industry and drug regulatory agencies.[3] According to the United States Acute Liver Failure study group, drug-induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%). The most common liver diseases are various types of acute (sudden) hepatitis (inflammation), chronic (long duration) hepatitis, fatty liver, cirrhosis (scarring), and cancer. Viruses, drugs, and alcohol, as well as metabolic, immune (defence) system, and genetic (hereditary) abnormalities are the common causes of these liver diseases.[4]Many common liver diseases can cause the organ to become inflamed. This inflammation can progress to scarring, or cirrhosis. It is critical that patients with cirrhosis, due to any type of liver disease, seek help because people with cirrhosis are at an increased risk for liver cancer or liver failure. Liver cancer and liver failure can be treated by a multidisciplinary approach including radiation, medication, or surgery, including transplant. In present scenario, liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Acute liver failure refers to the rapid development of severe acute liver injury with impaired synthetic function and encephalopathy in a person who previously had a normal liver or had well compensated liver disease. Acute and chronic liver diseases can interfere with the functions of the liver and thereby cause symptoms. The liver has a hefty reserve capacity. In other words, it usually takes substantial damage to the liver before a disease interferes with the functions of the liver and causes symptoms.[5] Examples of such symptoms includes jaundice (yellow skin) that can occur when the liver is unable to properly metabolize or secrete the yellow pigment bilirubin in bile. Bleeding or easy bruising that can occur when the liver is unable to make enough of the normal blood clotting proteins.

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Table 1: Study of biochemical parameters of *Zizyphus xylopyrus* extracts

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Bilirubin level (mg/dL)	Liver weight (g)	MDA (nmol / g tissue)	SOD (µM of H2O2 produced/min/g of tissue)	Catalase (µM of H2O2 produced /min/g of tissue)
Normal control	110.16 ± 5.38	60.89 ±2.26	235.76 ± 8.97	0.35 ±0.019	7.982±0.18	169.59	75.14±1.06	133.92±3.76
Disease control	205.34 ±7.04	123.64 ±3.36	405.62 ± 7.65	0.70±0.037	8.914±0.13	380.21	42.98±1.52	65.70±2.78
Standard	113.13 ±8.19	67.92 ±2.65	270.14 ±8.79	0.38±0.016	7.867±0.11	196.49	65.12±1.25	119.56±2.23
MZX (100 mg/kg)	139.96 ±6.23	91.34 ±4.07	298.71 ±12.68	0.49±0.017	8.317±0.12	249.56	52.82±1.94	100.56±3.67
MZX (200 mg/kg)	126.96 ±10.64	72.96 ±3.21	279.48 ±9.82	0.43±0.029	7.979±0.10	210.26	62.82±1.62	110.56±3.21
AZX (100 mg/kg)	165.08 ±7.57	100.44 ±4.16	338.16 ±11.47	0.59±0.021	8.587±0.18	308.68	47.82±1.78	87.56±3.78
AZX (100 mg/kg)	146.60 ±8.47	96.44 ±3.02	313.53 ±9.32	0.55±0.013	8.429±0.17	288.53	52.82±1.67	99.56±3.67

Swelling of the legs with fluid (edema) that can occur when the liver is unable to make enough albumin and the serum albumin gets too low. Fatigue that is of unknown cause, but may be related to some impaired metabolic function of the liver.[6] *Z. xylopyrus* is a large, straggling shrub, 6-10 m tall; young shoots rusty tomentose, spines in pairs on younger branches, one straight, the other curved; nodes swollen at the leaf scars. It is known by various names in India, e.g. Sanskrit: Ghoti, Gotika; English: Jujab and in Hindi: Ghunta, Kakora. This plant is used traditionally in the treatment of variety of diseases such as obesity, urinary troubles, diabetes, skin infections, fever, diarrhoea, insomnia and digestive disorders. Ethanobotanical survey shows that various parts of plant have been used in the treatment of diseases by folk person. *Z.xylopyrus* is one of the chief hosts for the propagation of lac, most satisfactory material for the manufacture of photographic records, a highgrade insulator and used in electrical industry. Dye obtained from the fruits is also used for tanning of leather in industries. People find its place in Ayurvedic Pharmacopoeia of India, but attempts have not made to describe the complete folkloric or traditional uses, phytochemical and pharmacology of this plant.[7,8] People find its place in Ayurvedic Pharmacopoeia of India, but attempts have not made to describe the hepatoprotective activity of this plant. The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both.[9,10] The present work emphasizes the evaluation of hepatoprotective activity *Zizyphus xylopyrus* roots, to authenticate the traditional medicinal claim regarding this plant.

MATERIALS AND METHODS

Plant materials

The plants *Zizyphus xylopyrus* root was collected from the Bhopal district, M.P., India, Plant materials were air dried in the laboratory for 5 days at room temperature then grinded to powder form using an electrical mill. The powder sample was kept in an air tight container until required.

Table 2: Effect of Zizyphus xylopyrus extracts on lipid profiles

Treatment	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control	96.17±3.11	84.28±2.14	49.01±1.18	45.12±1.09	18.12±0.87
Disease control	175.04±4.15	148.32±1.76	25.13±1.13	105.16±1.68	40.23±0.92
Standard	98.05±0.93**	86.24±1.37**	45.09±0.92**	50.86±1.67**	21.02±0.68**
MZX(100 mg/kg)	110.12±1.23*	90.46±1.89**	38.84±1.12**	67.27±1.28**	28.74±1.18*
MZX (200 mg/kg)	103.37±1.17**	88.38±1.06**	42.57±1.24**	57.18±1.36**	25.62±0.23**
AZX (100 mg/kg)	110.13±1.64*	93.97±1.42**	34.38±1.28**	74.03±1.45**	31.98±1.27*
AZX (100 mg/kg)	104.47±1.49**	91.97±1.34**	29.18±1.37**	70.33±1.58**	29.97±0.35**

n=6 ± SD, Values are statistically significant at *p<0.05, more significant at **p<0.01

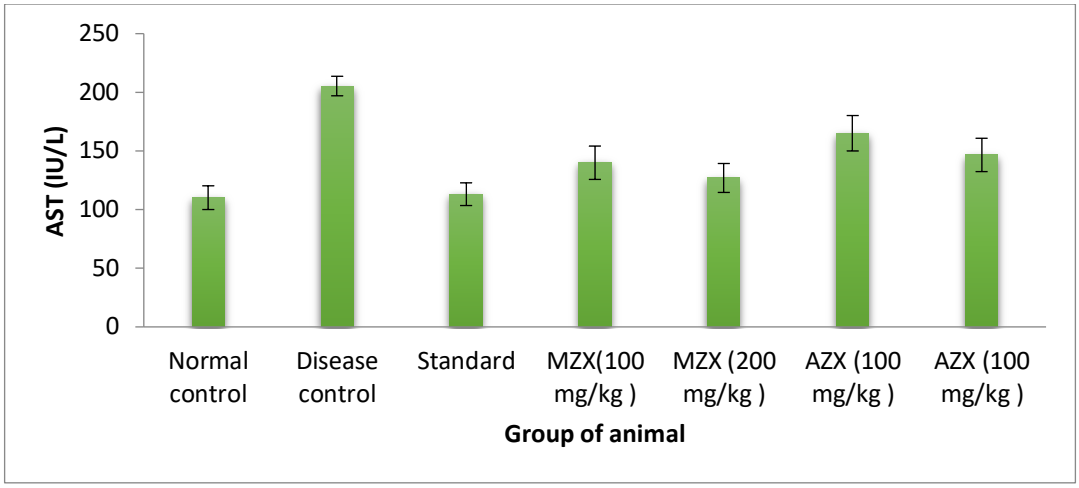

Figure 1: Effect of Zizyphus xylopyrus extracts on AST levels in blood plasma of animals

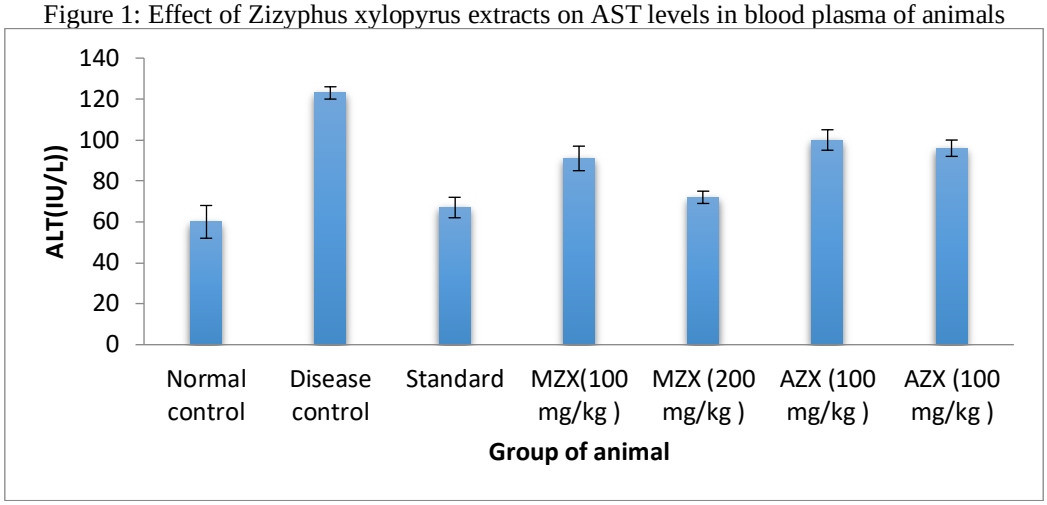

Figure 2: Effect of Zizyphus xylopyrus extracts on AST levels in blood plasma of animals

Extraction of plant material

About 100 g of plant sample was extracted with petroleum methanol 500 mL solvent by using soxhlet extractor. For aqueous extract the maceration method was used. Then both the solvents were evaporated under reduced pressure using Rota-Vapor and to obtain viscous semisolid masses. Phytochemical screening carried out for methanol and water extract plants for the presence of phyto-constituents. The methanolic extract of plant were tested for steroids, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthraquinone and amino acids. Phytochemical screening of both the extracts was carried out according to the standard methods.

Acute toxicity studies

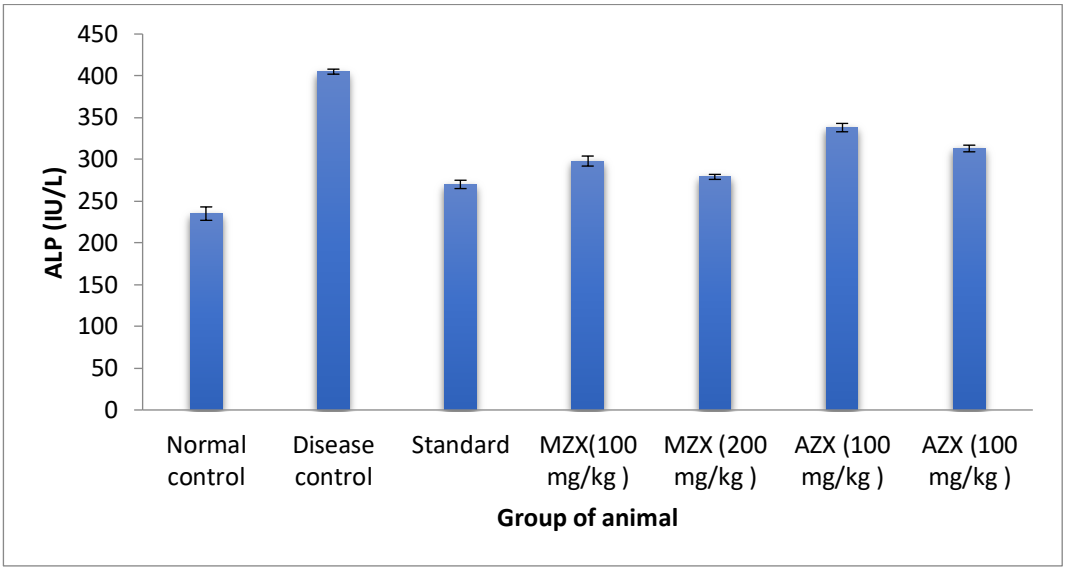

Figure 3: Summary of the Zizyphus xylopyrus extracts on ALP levels in blood plasma

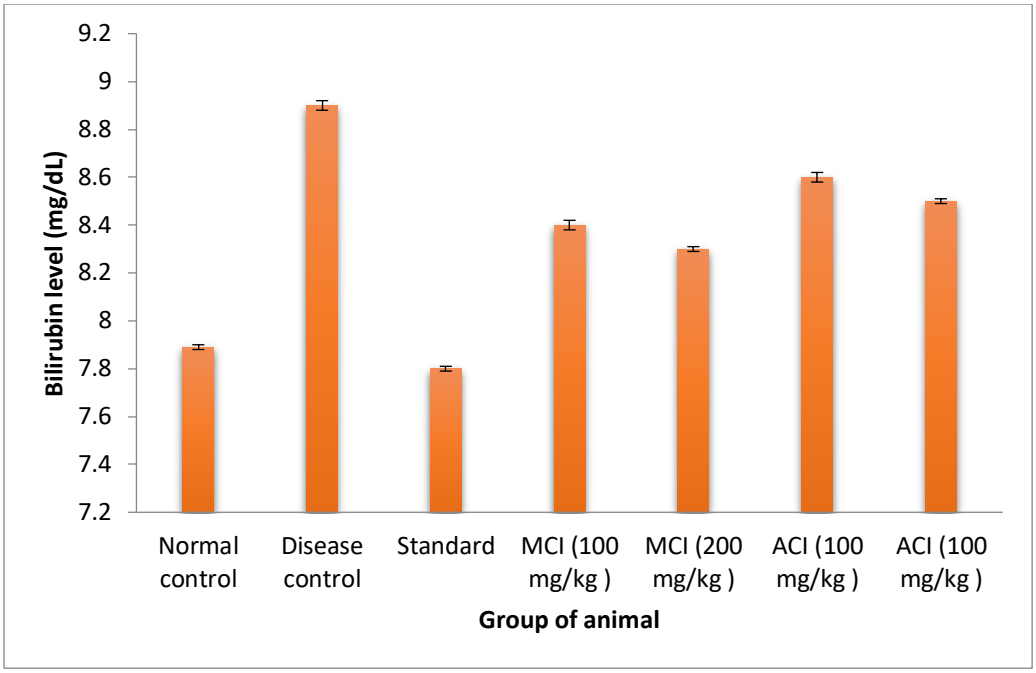

Figure 4: Bilirubin level of animals treated with Zizyphus xylopyrus extracts

The acute toxicity studies for methanolic extracts of the plant were determined on albino rat (200-220g). The animals were fasted overnight prior to the experiment. Fixed dose method as per OECD. The rats were divided in to control and test group each containing 6 animals. The test groups of mice were administrated with the dose of 500 and 2000 mg/kg of extracts. Carefully observe all the animals and any sign of toxicity in the f first four hours, after the administration of extracts and daily following that for the period of 14 days.[8]

Hepatoprotective activity

Selection of animals, caring and handling the albino rats (wistar strain 150-200 g) of either sex were used. After randomization in to various groups, animals were accustomed for a period of 10 days under standard husbandry condition. Room temperature: 23 ± 3°c Relative humidity: 50 ± 20% 12 h dark and light cycle. All the animals were feed with rodent pellet diet and water was allowed ad-libitum under strict hygienic condition.

The animals were divided in to vehicle control, toxic standard and test extract of the drug treatment was given once daily per oral.Animals of group I were treated with 1 ml/kg bw of saline (0.85%) intraperitoneally twice a week for four weeks. Rats of group II to XI were treated with (Paracetamol 1000 mg/kg p.o. for 7 days). Animals of group III serve as standard treated with silymarin suspension (10 mg/kg body weight, IP). Remaining group animals received methanol and aqueous extracts of *Zizyphus xylopyrus* root. After 24 h of the last treatment, all the animals were weighted, sacrificed, collected the blood while liver was removed, weighted and perfuse in ice-cold saline solution. Liver samples were treated with liquid nitrogen and stored at −70 °C for further studies. The vehicle control and all test extract administered orally for seven days at different dosage from level and enhance the liver damage in animals. On the 7th day after 6 hrs of

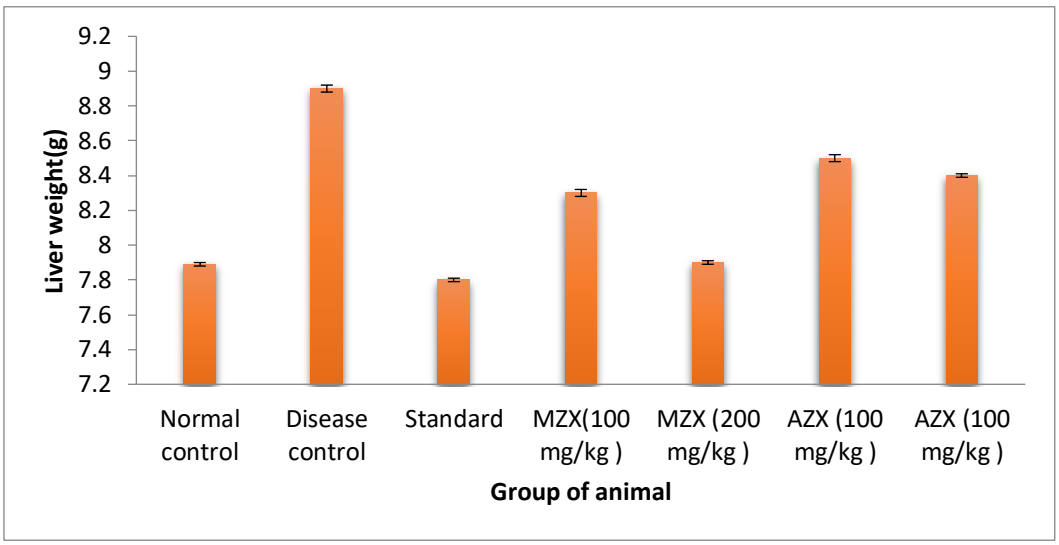

Figure 5: Effect of Zizyphus xylopyrus extracts on absolute weight of Liver

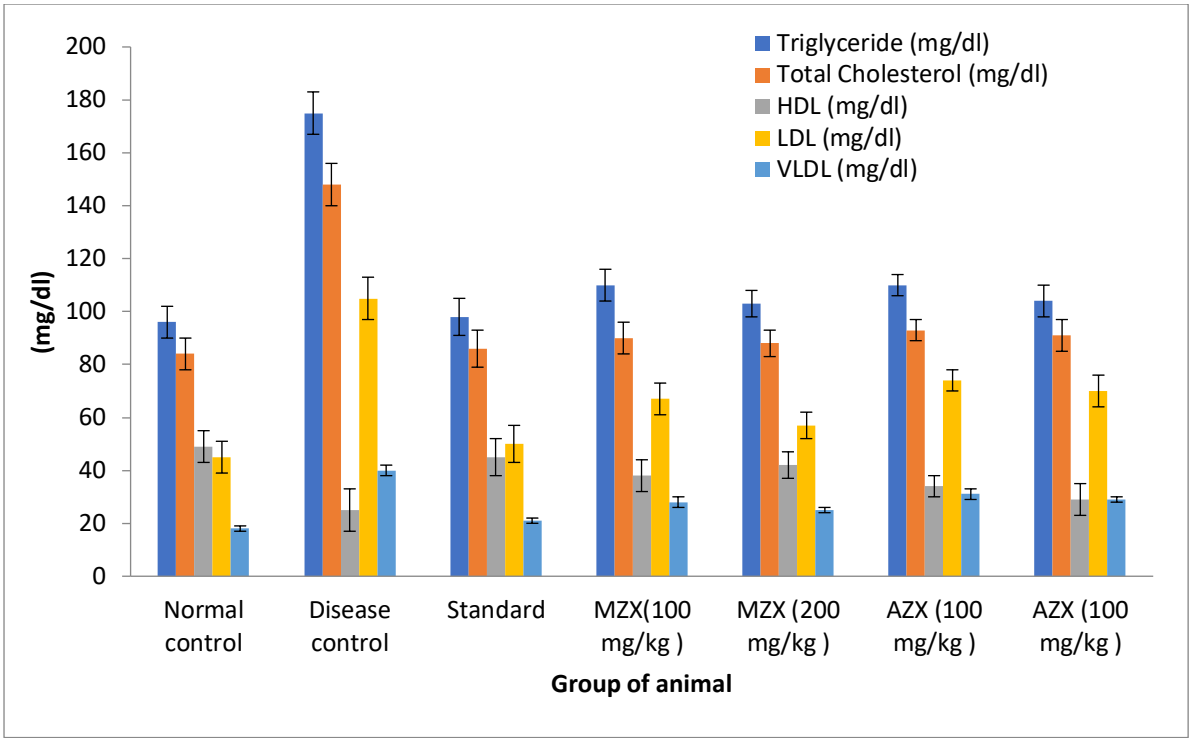

Figure 6: Effect of Zizyphus xylopyrus extracts on lipid profiles

the administration. of the drug by giving a single dose of CCl4 with liquid paraffin (1: 1 ratio). After 24 hrs blood samples were drawn from all animals by puncturing retro-orbital plexus on the day of the treatment. The blood samples were centrifuged immediately to get clear serum and subjected for estimation of various biochemical parameters namely SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase), ALP (alkaline phosphate), and TB (total bilurubin). Later the animals were sacrificed and liver tissues were collected from all groups for histopathological studies.[11-13]

Statistical analysis

Results were expressed as mean <SEM, n=6. Statistical analysis was performed with one way analysis of varience (ANOVA) by Dunnett's multiple comparisions test. P value < 0.01 was considered to be statistically with control and toxicant group as possible.

RESULTS AND DISCUSSION

Extracts of *Zizyphus xylopyrus* was evaluated for acute toxicity as per the OECD guidelines 463 for 14 days in albino rats. Righting reflex, corneal reflex and pinna reflex were found normal for single dose oral administration of 2000 mg/kg of body weight of albino rats. Clinical abnormalities like excessive defecation, urination or changes in fur were not noticed during the study period and extracts showed no abnormalities or mortality up to the single dose oral administration of 2000 mg/kg during the study period of 14 days. However slight changes in

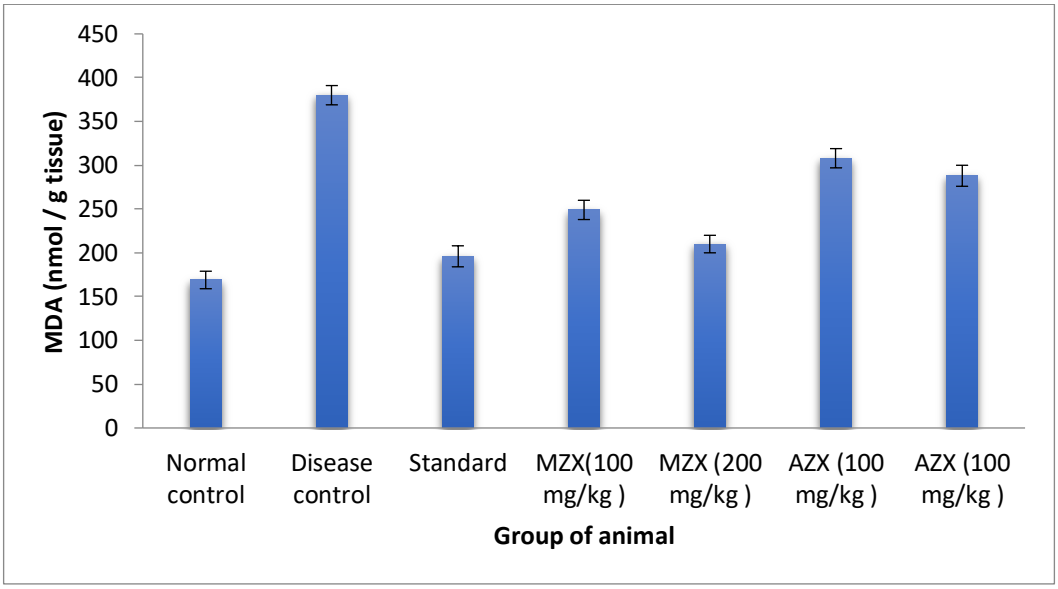

Figure 7: Effect on MDA contents in liver of animals treated with Zizyphus xylopyrus extracts

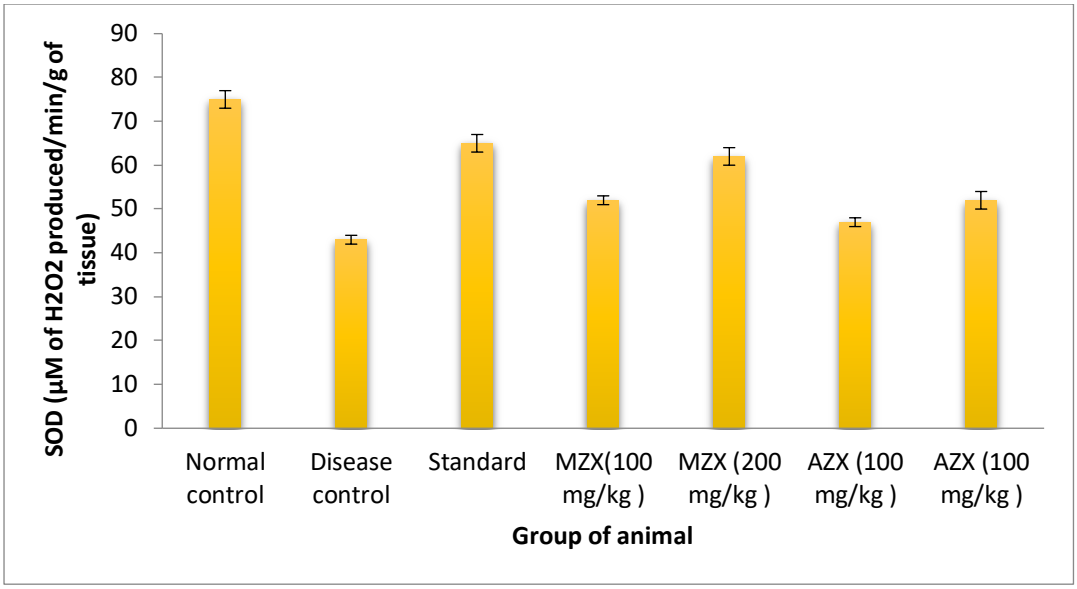

Figure 8: Effect of Zizyphus xylopyrus extracts on SOD level in liver

body weight (increased) of animals were noted during the study period. Further, no casualties and no abnormal behavioural pattern were observed in experimental animals at single dose oral administration of the extracts during 14-day study period. In the present study, hepatoprotective activity of the extracts of *Zizyphus xylopyrus* were carried out using model of paracetamol induced hepatotoxicity in experimental animals (Wistar rats). Administration of paracetamol and different extracts of *Zizyphus xylopyrus* showed no mortality or morbidity in the animals during the period of study. Cage side observations did not show any observable clinical signs related to the compound toxicity. No tremors, convulsions, salivation, diarrhoea, lethargy, or unusual behaviors were observed in extract treated animals throughout the study period.

Biochemical analysis

The serum level of transaminase enzymes are most frequently used as indicators of liver damage severity. Particularly serum concentration of ALT and AST use as a biomarker of hepatic necrosis. Both ALT and AST enzymes involve in the reductive transfer of amino acid from alanine or aspartate, respectively to alpha ketoglutarate to form pyruvate or oxaloacetate, respectively. Damaged hepatocytes release their contents, including ALT and AST into the extracellular space. Thus, the elevated level of these enzymes is considered as the symbol of liver damage. ALT is present in heart, brain, skeletal muscle and liver; however, it is present in higher amounts in liver than any other organs. On the other hand, AST is considered to have lower specificity for liver damage due its presence in other organs. The reactive species (NAPQI) produced by paracetamol overdose harm the hepatic cells by lipid peroxidation and thereby damage the cellular permeability resulting higher serum levels of ALT and AST. The reduction of serum ALT and AST is an indicator of protective effects of an agent. Methanol extract of control the AST level more effective than aqueous extract. *Zizyphus xylopyrus* methanol extract control the AST level more efficiently compared to aqueous extract treated group. Treatment with *Zizyphus xylopyrus* methanol extract control the ALT level similar to standard treated group. Hepatotoxicity causes the biliary congestion leading to the inability of excretion of the ALP

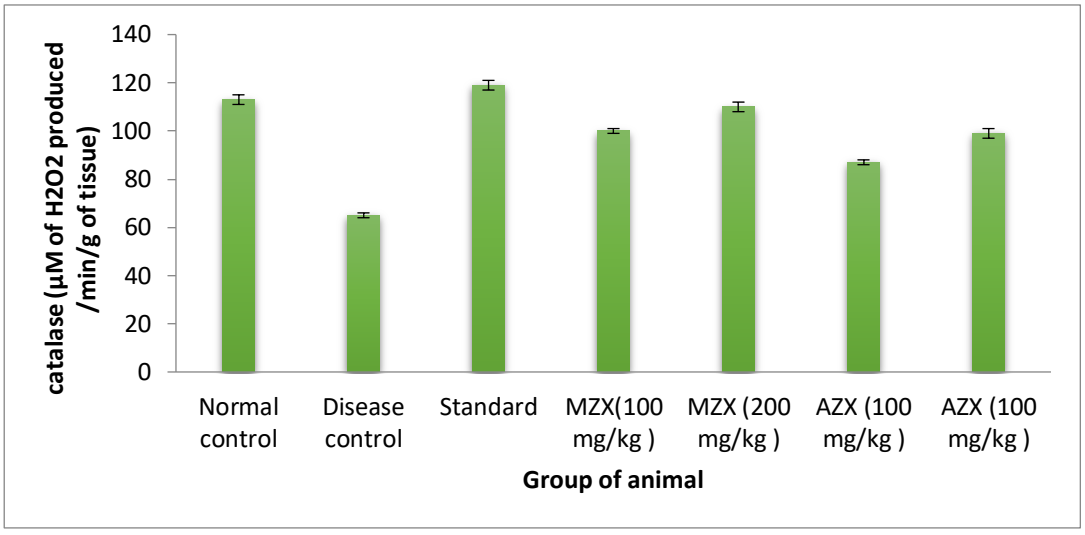

Figure 9: Effect of Zizyphus xylopyrus extracts on catalase activity

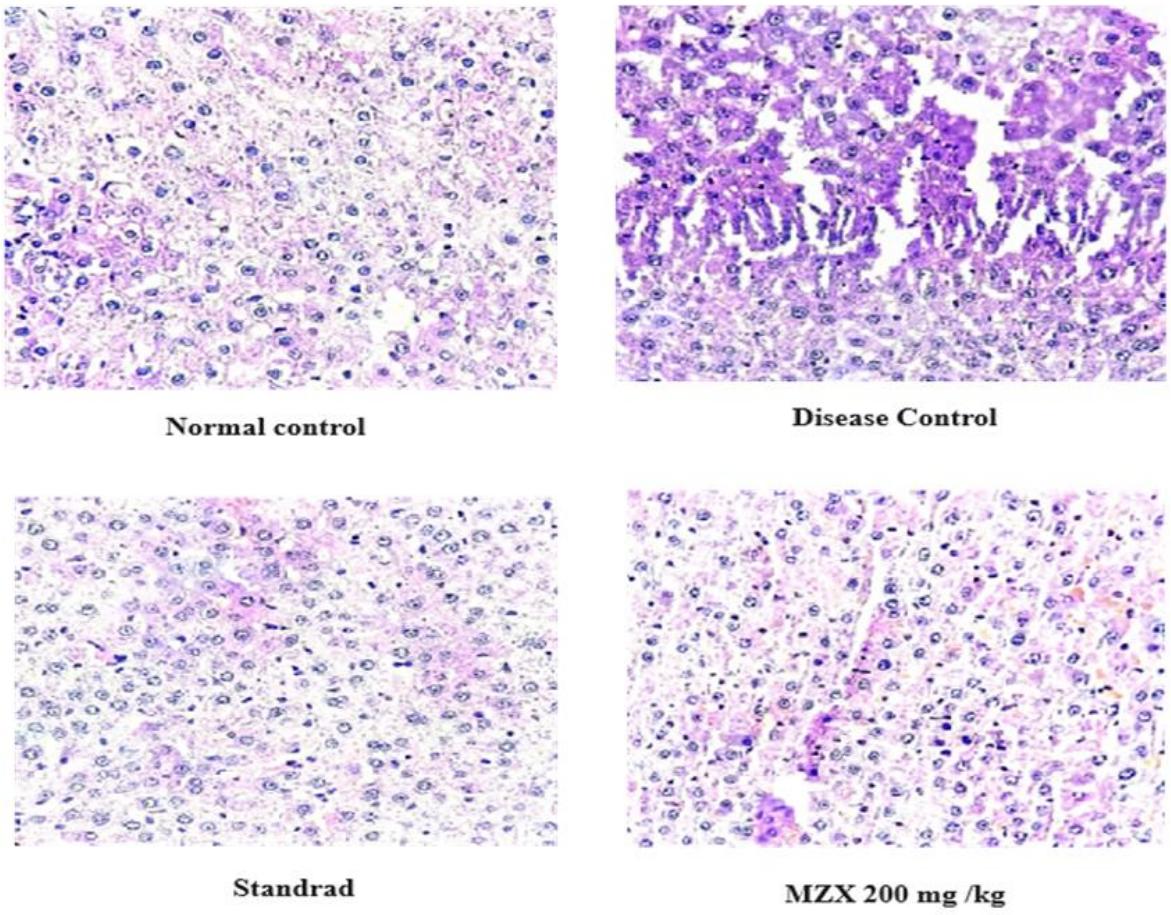

Figure 10: Histopathology of Liver of the animals treated with extracts

from the body that leads to elevation of the ALP level as seen in the vehicle group. ALP level significantly and dose dependent manner comparable to the control group. The *Zizyphus xylopyrus* extracts showed reduced levels of ALP activity. Administration of methanol extracts of *Zizyphus xylopyrus* extracts control the ALP level similar to standard treated group. *Zizyphus xylopyrus* methanol extract control the ALT level more efficiently compared to aqueous extract treated group. This result indicates that Zizyphus xylopyrus methanol extract can reduce the obstruction of bile duct induced by paracetamol overdose. The methanol extracts of *Zizyphus xylopyrus* extracts showed reduced Bilirubin level in blood plasma of animals. Administration of methanol extract of Zizyphus xylopyrus control the Bilirubin level than aqueous extract. This too suggests that the methanol extract of *Zizyphus xylopyrus* at the dose of 200 mg/kg and isolated compounds are also able to relieve the symptoms of hepatotoxicity. In group received methanol extract of *Zizyphus xylopyrus* (200mg/kg) the absolute weight of liver remained close to the absolute weight of liver in control group. Disease control group increased in liver weight comparatively to control group. The liver weight of treated group with extract was restored with treatment of plant extract. Methanol extract of *Zizyphus xylopyrus* (200mg/kg) was significantly restored the liver weight of treated group than aqueous extract. Administration of methanol extract of *Zizyphus xylopyrus* was significantly ($P<0.01$) restored the liver weight of treated groupsimilar to standard treated group.

Lipid extraction and estimation

The plasma triglyceride and total cholesterol levels were seen to be surprisingly increased in diseased control animals compared with normal control animals. The methanol extract of Zizyphus xylopyrus (200 mg/kg) significantly lowered the levels of cholesterol, triglycerides and LDL when compared with the diseased control group. Consequently, the HDL significantly increased in groups treated with extracts and standard in comparison to diseased control group. The results of the MDA content in various experimental groups, the difference between malondialdehyde content in normal and disease control was quite significant. Maximum recovery was shown by the group that received 200 mg/kg of methanol extract of *Zizyphus xylopyrus*. This is suggestive of the fact that supplementation of diet with any drug increases lipid peroxidation, and can be lowered by the methanol extract of the *Zizyphus xylopyrus*. MZX (200mg/kg) were found most effective among other extract and significantly ($P<0.01$) reduce the MDA content towards normal, similar to standard treated group. The paracetamol treated animal group significantly reduce SOD level compared with the normal control group. Standard and MZX (200mg/kg) treated group reversed the situation and significantly increases the SOD level. Methanol extract of the *Zizyphus xylopyrus* (200mg/kg)

was found most effective than aqueous extract and significantly increases the SOD level and showed SOD level similar to standard treated group. SOD catalyzes the conversion of superoxide radical to H2O2. Although H2O2 is not a radical, it is rapidly converted to highly reactive hydroxy radical through the activity of CAT. Thus, there is a mutual protective relationship between two enzymes. When the superoxide radical is produced, it is disabled by CAT, while SOD is inhibited by H2O2. Therefore, the antioxidant defense mechanism is affected by ROS thereby decrease SOD, CAT, and GSH, leading to hepatic damage. Methanol extract of the *Zizyphus xylopyrus* (200mg/kg) were found most effective among other extract and significantly increases the catalase level and similar to standard treated group. In animals fed with aqueous extracts, the catalase activity were also obtained but they found less effective than methanol extract.

Histopathology

Hepatoxicity leads to various pathological malfunctions. Perivascular leukocytic infilteration is accumulation of leukocytes in amount excess than of the normal around a vessel. Different chemical structure including medical drugs and industrial pollutants are known to induce irreversible cytoplasmic vacuolization. Histological sections of liver of normal control Group, revealed normal histology. The central vein and hepatocytes surrounding it were observed. Disease control Group, animals showed minimal focal perivascular leukocytic infiltration, focal mild hepatocellular necrosis, hepatocytes with pyknotic nuclei, condensed cytoplasm and severe cytoplasmic vacuolation over a large area. Standard treated Group did not reveal any lesion of pathological significance. Group received a dose of 200 mg/kg of methanol extracts of *Zizyphus xylopyrus* revealed normal histology.

CONCLUSION

Liver is an important organ and a central one for many of the metabolic functions of the body, decomposition of toxic and waste substances, and disposal of harmful substances from the body. Liver illnesses are still the serious problem of

human health. Making active oxygen species is an unavoidable result in aerobic organisms, and their removal is done at a basic level and with regard to the useful physiological functions and their harmful effects. This sensitive balance is for retaining the intracellular redox state which has an important role in optimizing cell functions. Excessive accumulation of free radicals or the body's inability to remove them causes the relocation of redox equilibrium for creating oxidation states in the body. The present work evaluate the hepatoprotective activity of methanol and aqueous extract of *Zizyphus xylopyrus* roots. Methanol extract of the *Zizyphus xylopyrus* (200mg/kg) were found most effective among other extract and significantly nomalize the biochemical parameter similar to standard treated group. In animals fed with aqueous extracts, the catalase activity were also obtained but they found less effective than methanol extract. In conclusion *Zizyphus xylopyrus* root methanolic extract has hepatoprotective action against paracetamol-induced hepatotoxicity in rats, according to the findings of this study.

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