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HEPATOPROTECTIVE EFFECT OF METHANOL EXTRACT OF *CANNA INDICA* ROOT ON PARACETAMOL INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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Keywords:

Liver dysfunction, Hepatoprotective activity, *Canna indica* root, Paracetamol induced hepatotoxicity, Wistar albino rats

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ABSTRACT: 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. *Canna indica* commonly known as an Indian shot, extensively used as a nutritive agent & has a number of valuable pharmacological activities. People find its place in Ayurvedic Pharmacopoeia of India, but attempts have not made to describe the hepatoprotective activity of this plant. The present work emphasizes the comprehensive ethano-medicinal uses of *Canna indica* root to enlighten its phytochemical constituents and hepatoprotective activity of this plants. To authenticate the traditional medicinal claim of investigation has been undertaken to evaluate the hepatoprotective activities of *Canna indica* plant. Administration of paracetamol and different extracts of *Canna indica* showed no mortality or morbidity in the animals during the period of study. The values of AST, ALT, ALP, and total bilirubin were all substantially raised by paracetamol. Significant hepatoprotective effects were obtained by pretreatment with *Canna indica* methanol extract. Histopathological examination of the liver tissues of paracetamol control group represented the. Pretreatment with *Canna indica* extract reversed histopathological alterations such as presence of marked foci of mononuclear infiltration in the hepatic parenchyma tissue, necrosis, and fatty changes of hepatocytes. Maximum recovery was shown by the group that received 200 mg/kg of methanol extract of *Canna indica*.

INTRODUCTION: The liver is the largest solid organ in the body. It removes toxins from the body's blood supply, maintains healthy blood sugar levels, regulates blood clotting, and performs hundreds of other vital functions [1]. The liver is an essential organ of the body that performs over 500 vital functions.

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These include removing waste products and foreign substances from the bloodstream, regulating blood sugar levels, and creating essential nutrients [2]. As liver being the central organ of metabolism it is highly vulnerable target for injury from drugs and chemicals, the manifestations of which are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure [3].

The World Health Organization (WHO) determined that around 2.4 million deaths yearly are linked to some liver disease, and that around 800 thousand of these deaths are attributable to cirrhosis. On the other hand, epidemiological

studies conducted by the National Institute of Statistics and Geography (INEGI by its Spanish acronym) indicate that in 2013 in Mexico, over 600 thousand deaths were recorded [4]. Despite the advances in modern medicine and the development of new hepatoprotective drugs. The incidence of hepatic diseases has not decreased or stopped; on the contrary, statistics suggest that these continue to increase [5]. Paracetamol is metabolically activated by cytochrome P450 to a reactive metabolite that covalently binds to Protein.

The reactive metabolite responsible for hepatotoxicity is N-acetyl-p-benzoquinone-imine which reacts with N-acetyl cysteine [6]. The primary metabolic pathways for paracetamol are sulphation and glucuronidation, which produce unreactive metabolites. The cytochrome P-450 system subsequently activates these unreactive metabolites to cause liver damage [7]. A drug's electrophilic metabolite appears to be what causes acetaminophen's recognizable zone 3 necrosis (N-acetyl-p-benzo quinonimine, NAPQI). NAPQI is first detoxified by forming mercapturic acid by conjugation with reduced glutathione. However, NAPQI will oxidize tissue macromolecules, such as lipids or protein thiols, and change the homeostasis of calcium after depleting glutathione, leading to cell death. This occurs when the rate of NAPQI synthesis surpasses the rate of detoxication by glutathione. The damaging mechanism in liver damage brought on by acetaminophen intake has been theorized to be lipid Peroxidation [8].

Following acetaminophen treatment, liver tissue protective effects coincided with antioxidant activity, indicating that lipid peroxidation and free radical production may both contribute to this kind of drug damage mechanism. Hepatitis is nothing but inflammation of the liver, caused mainly by viral infection (viral hepatitis) but also by some liver toxins (e.g. alcoholic hepatitis), autoimmunity (autoimmune hepatitis) or hereditary conditions. According to WHO, globally 170 million people are chronically infected with hepatitis-C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis-B virus (HBV) and over 5 million are getting infected with acute HBV annually [9-10]. Herbs and their products are generally thought to act as hepatoprotective agents through different mechanisms such as immunomodulation, which means they potentiate and modulate the immune system. The following medicinal plants; *Silybum marianum* (L.) Gaertn., *Glycyrrhiza glabra*, *Phyllanthus amarus* Schumach. & Thonn., *Salvia miltiorrhiza* Bunge., *Astragalus membranaceus* (Fisch.) Bunge, *Capparis spinosa* L., *Cichorium intybus* L., *Solanum nigrum* L., *Sapindus mukorossi* Gaertn., *Ginkgo biloba* L., *Woodfordia fruticosa* (L.) Kurz, *Vitex trifolia* L., *Schisandra chinensis* (Turcz.) Baill., *Cuscuta chinensis* Lam., *Lycium barbarum*, *Angelica sinensis*, and *Litsea coreana* H. Lév reported to have hepatoprotective activity [11]. *Canna indica* commonly known as an Indian shot, the *Canna arises* from the Greek word for a cane or reed. Canna is the only genus in the family *Cannaceae* & 19 species of flowering plants. The species have large, eye-catching foliage & horticulturists have turned it into a large flowered & bright garden plant. In addition, it is a horticultural plant & is one of the world's richest starch sources. It extensively used as a nutritive agent & has a number of valuable pharmacological activities [12]. The present work highlights the comprehensive ethano-medicinal uses of *Canna indica* root and hepatoprotective activity of this plant. To authenticate the traditional medicinal claim of investigation has been undertaken to evaluate the hepatoprotective activities of *Canna indica* plant.

MATERIAL AND METHODS:

Plant Material: *Canna indica* root collected from local area of Bhopal (M P). Plant material was identified and authenticated. The plant materials were thoroughly washed with water dried in shade, powdered moderately and pass-through sieve ≠ 10.

Physicochemical Evaluation: Pharmacognostical parameters such as total Ash value, acid insoluble ash value, water soluble ash value, alcohol soluble extractive value, water soluble extractive value and loss on drying were determined for the herb.

Extraction of plant material: The extraction of drug signifies a solid from solid separation, as solid components must be extracted from a solid substance. This type of extraction is generally performed before any separation processing and should be differentiated from solid liquid extraction where the solid drug is extracted with a liquid

medium. The liquid liquid extraction is one in which any of the two liquids that are not miscible includes the substance to be extracted. Plants powders were extracted sequentially with Petroleum ether, chloroform and methanol using continuous hot extraction method i.e. Soxhlet extraction. The completely dried *Canna indica* roots were coarsely powdered and 200g were packed in Soxhlet apparatus and extracted with petroleum ether till completion of extraction. The exhausted root powder extracted with chloroform than methanol till completion of extraction. The obtained extract was concentrated under reduced pressure using rotary evaporator. The methanol exhausted root powder macerated with water, filtered and concentrated to get aqueous extract. Each time before extracting with the next solvent, the marc was dried in an air.

Phytochemical Test (Qualitative Analysis of Extracts): Phytochemical screening provides an idea about the preliminary chemical composition of the extracts. The chemical tests were performed as per the standard procedures. Various tests were performed to detect presence of various primary as well as secondary chemical constituents in the extract. Successive solvent extracts (Petroleum ether, chloroform, methanol and aqueous extract) were subjected for qualitative analysis. All extracts were subject to various qualitative analyses to detect the presence of plant constituents *i.e.* alkaloids, glycosides, saponins, carbohydrates, phytosterols, tannins and phenolic compounds (flavonoids) and proteins and free amino acids.

Hepatoprotective Activity: In the present study, hepatoprotective activity of the extracts of *Canna indica* root carried out using model of paracetamol induced hepatotoxicity in experimental animals (Wistar rats). Methanol and aqueous extract of *Canna indica* root was found to have higher amount of total phenolic and total flavanoid content, thus both type of extract used for hepatoprotective activity.

Animals Wistar rats weighing between 200-220 gm were selected for hepatoprotective activity. All the animals were segregated into groups of six animal rats each. All animals were housed in air-conditioned rooms with 10-15 air circulation cycles per hour. The relative humidity was maintained between 30-70%, temperature between 22- 25ºC and illumination cycle set to 12 hours artificial fluorescent light and 12 hours dark. In each of the polypropylene cages with stainless steel grill top (32.5cm x 21cm), facilities for food and water bottle and bedding of clean paddy husk, the animals were kept in the groups of five. Standard pelleted basal diet and purified water were provided *ad libitum* to the animals. All the animals were acclamitized to the laboratory conditions before they were used in the experiments. Experimental protocol was approved by Institutional Animal Ethics Committee and ethical norms were strictly followed during all experimental procedures

Acute Toxicity Study: The acute toxicity study was performed in order to observe undesirable side effects. OECD guidelines 423 were followed for evaluating undesirable effects or toxicity of extracts on animals. The animals of either sex satisfying the conditions of body weight, age, and non-infected/ non-wounded, showing no abnormal behaviour was included in the study. Rats of either sex were divided into the groups of 3 animals per group.

Extract of *Canna indica* root were administered orally at a dose of 500mg/kg and 2000 mg/kg body weight to each group every 24 hours. The rats were then critically observed after 30min, 1hr, 2hr, 3hr, 24 hr for clinical signs, gross behavioural changes and mortality. These observations were continued for a period of 14 days. The maximal safe dose was determined after observing mortalities and behavioural profile for the stipulated time. Further, in accordance with the OECD guidelines, the doses for the study were narrowed out.

Study Design: 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) intraperitoneally. Animals of group III serve as standard treated with silymarin suspension (10 mg/kg body weight, IP). Remaining group animals received extracts of *Blepharis edulis* leaves and seeds. 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.

Biochemical Analysis: Using capillaries, blood was drawn from retro-orbital plexus and was transferred to the in heparinised eppendorf tubes. It was then centrifuged at 3000 RPM for 10 minutes to separate plasma, which was collected using clean pipette. After collection, plasma samples were subjected to biochemical analysis. Biochemical analysis was performed on blood of animals fasted overnight. The major reason for this is the increased variability that would inevitably result from feeding and would mask more subtle effects and make interpretation difficult. The biochemical parameters from the samples of blood plasma were evaluated using automated analyzer (ERBA Diagnostc Mannheim GmbH; Model: CHEM-7). The parameters amino transaminases, which included, Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT) were analyzed. These enzymes are found mainly in liver, but are also found in red blood cells, heart cells, muscle tissue blood plasma and other organs like pancreas and kidney. Elevated levels of AST and ALT indicated disease or injury to the liver.

RESULT AND DISCUSSION: *Canna indica* L. (Cannaceae) is an ornamental, perennial herb; native of tropical regions of America but also found in other tropical countries of world widely used as a folklore medicine with beneficial effects in, hepatitis, infection, rheumatism. Roots and rhizomes of *C. indica* L. are thick, cylindrical and creamy white or pinkish in colour. Roots are about 2–5 mm in diameter with numerous root hairs. Rhizomes may be sympodial, stoloniferous or tuberous **Fig. 1.** Physicochemical parameter such as Ash values (Total ash, Acid insoluble ash and Water-soluble ash), Extractive values, Loss on drying of plant materials were performed. Ash values of crude drug provide an idea about the inorganic composition or earthy matter and other impurities present in drug. Ash values and extractive values can be used as a reliable aid to detect adulteration. These studies help in the identification of the plant material. Ash values of drug give an idea of earth matter or the inorganic composition and other impurities present along with drug **Table 2.**

TABLE 1: STUDY DESIGN FOR HEPATO-PROTECTIVE EFFECT OF *CANNA INDICA* EXTRACTS

Normal control	Normal control
Disease control	Paracetamol 1000 mg/kg p.o. for 7 days
Standard	(silymarin treated) + Paracetamol (1000 mg/kg p.o.)
MCI (100 mg/kg)	Methanol extract of *Canna indica* root(100 mg/kg)
MCI (200 mg/kg)	Methanol extract of *Canna indica* root (200 mg/kg)
ACI (100 mg/kg)	Aqueous extract of *Canna indica* (100 mg/kg)
ACI (100 mg/kg)	Aqueous extract of *Canna indica* (200 mg/kg)

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF *CANNA INDICA* ROOT

S. no.	Physicochemical parameter values	(% w/w)
1	Total ash	7.8
2	Water soluble ash	5.12
3	Acid insoluble ash	1.98
4	Loss on drying	24.2
5	Alcohol Soluble Extractive	15.2
6	Water Soluble Extractive	21.3
7	Foreign organic matter determination	1.2

The phytochemical analysis of *Canna indica* L. root ethanol extract showed that they contained various phytochemicals including alkaloids, carbohydrates, proteins, flavonoids, terpenoids, cardiac glycosides, steroids, tannins and saponins. Water extract showed the presence of alkaloids, carbohydrates, tannins, flavanoids, protein and amino acids and saponins **Table 3.**

TABLE 3: PRELIMINARY PHYTOCHEMICAL SCREENING OF *CANNA INDICA* ROOT EXTRACTS

S. no.	Phytochemical Tests	*Canna indica* extracts			
		Petroleum ether	Chloroform	Methanol	Aqueous
1	Carbohydrates	-	-	+	+
2	Proteins And Amino Acids	-	-	+	+
3	Acidic Compounds	-	-	-	-
4	Mucilage	-	-	+	+
5	Fixed Oil	-	-	-	-
6	Alkaloids	-	-	-	-
7	Glycosides	-	-	+	-

8	Sterols And Steroids	+	-	-	-
9	Anthraquinones	-	-	+	-
10	Flavonoids	-	-	+	+
11	Phenolic Compounds	-	-	+	+
12	Terpenoids	+	+	-	-
13	Saponins	-	-	-	-

TABLE 4: EFFECT OF *CANNA INDICA* EXTRACTS ON VARIOUS BIOCHEMICAL LEVELS IN BLOOD PLASMA OF ANIMALS

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	75.92±1.12	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	43.28±1.23	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	68.29±1.38	119.56±2.23
MCI (100 mg/kg)	142.96±09.23	93.34±3.27	304.71±10.85	0.50±0.014	8.421± 0.11	253.42	50.25±1.76	98.56±3.21
MCI (200 mg/kg)	130.16± 7.78	77.96±2.78	284.48±10.83	0.45±0.019	8.327± 0.12	214.18	58.42±1.28	105.56±2.09
ACI (100 mg/kg)	168.08±6.65	103.44±5.76	338.16±12.67	0.60±0.024	8.689± 0.16	312.71	43.82±1.85	85.56±2.56
ACI (100 mg/kg)	148.60±4.85	98.44±3.98	314.53±13.12	0.56±0.018	8.587± 0.21	291.72	49.57±1.64	96.56±1.93

Values are mean of 6 replicates + SD.

TABLE 5: EFFECT OF *CANNA INDICA* EXTRACT ON LIPID PROFILES

Groups	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**
MCI (100 mg/kg)	107.13±1.64*	91.97±1.42**	40.38±1.28**	65.03±1.45**	25.98±1.27*
MCI (200 mg/kg)	100.47±1.49**	90.97±1.34**	44.18±1.37**	54.33±1.58**	23.97±0.35**
ACI (100 mg/kg)	112.12±1.23*	94.46±1.89**	36.84±1.12**	70.27±1.34**	31.74±1.28*
ACI (100 mg/kg)	105.37±1.17**	92.38±1.27**	39.57±1.36**	61.18±1.79**	28.62±0.23**

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

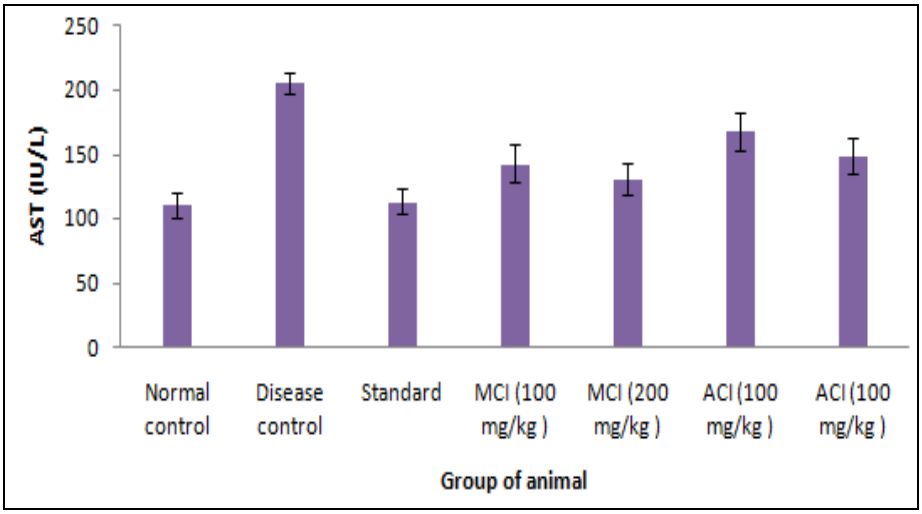

FIG. 1: EFFECT OF *CANNA INDICA* EXTRACTS ON AST LEVELS IN BLOOD PLASMA OF ANIMALS

Extracts of the *Canna indica* root was evaluated for acute toxicity as per the OECD guidelines. In the present study, hepatoprotective activity of the extracts of *Canna indica* root was carried out using model of paracetamol induced hepatotoxicity in experimental animals (Wistar rats). 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.

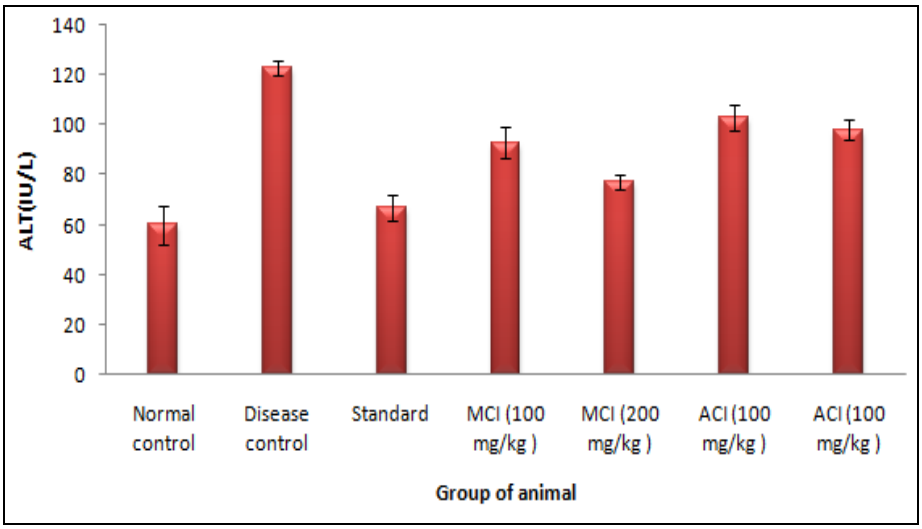

FIG. 2: EFFECT OF *CANNA INDICA* EXTRACTS ON ALT LEVELS IN BLOOD PLASMA OF ANIMALS

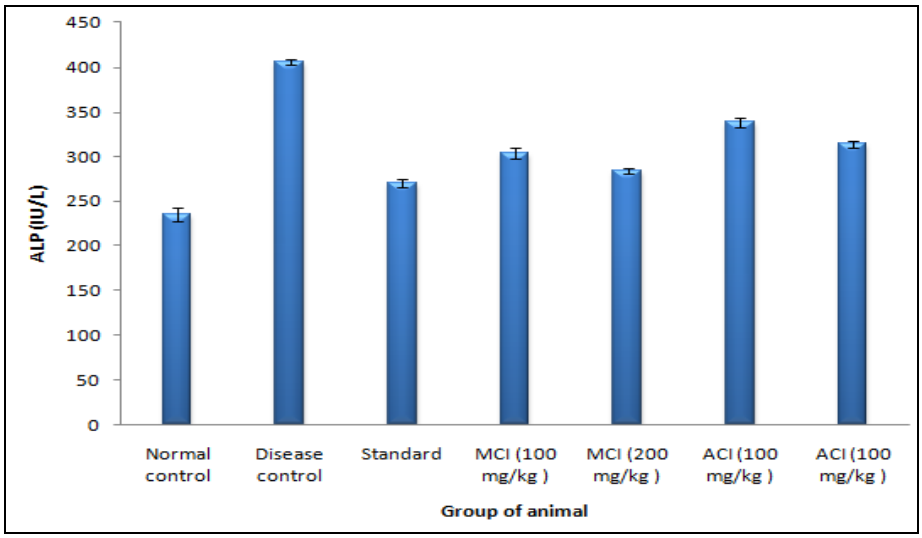

FIG. 3: SUMMARY OF THE *CANNA INDICA* EXTRACTS ON ALP LEVELS IN BLOOD PLASMA

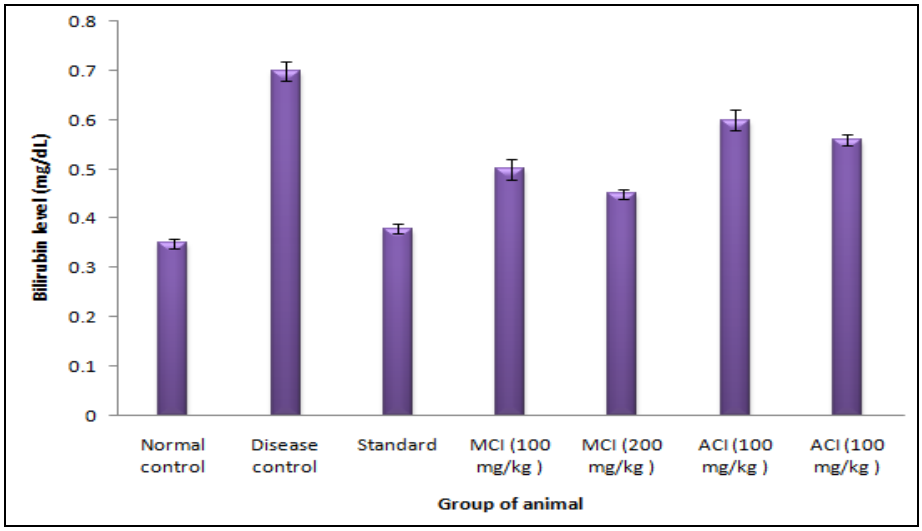

FIG. 4: BILIRUBIN LEVEL OF ANIMALS TREATED WITH *CANNA INDICA* EXTRACT

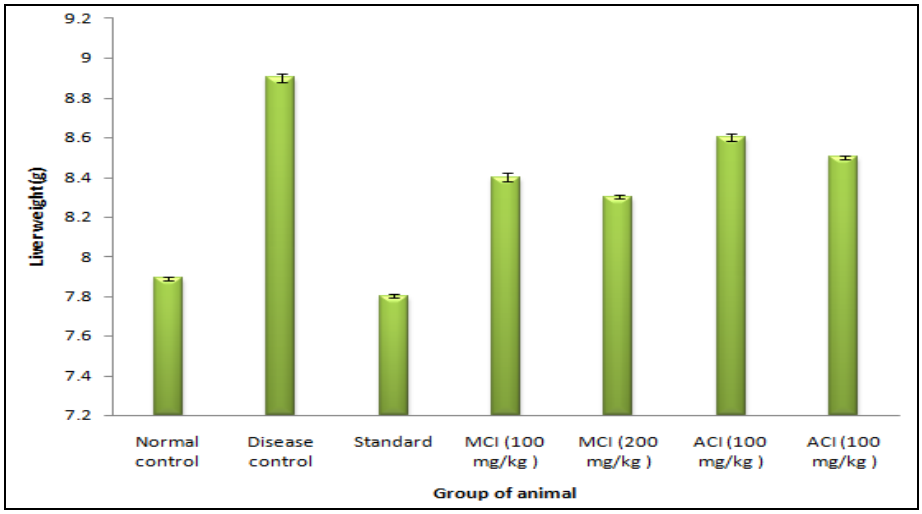

FIG. 5: EFFECT OF *CANNA INDICA* EXTRACT ON ABSOLUTE WEIGHT OF LIVER

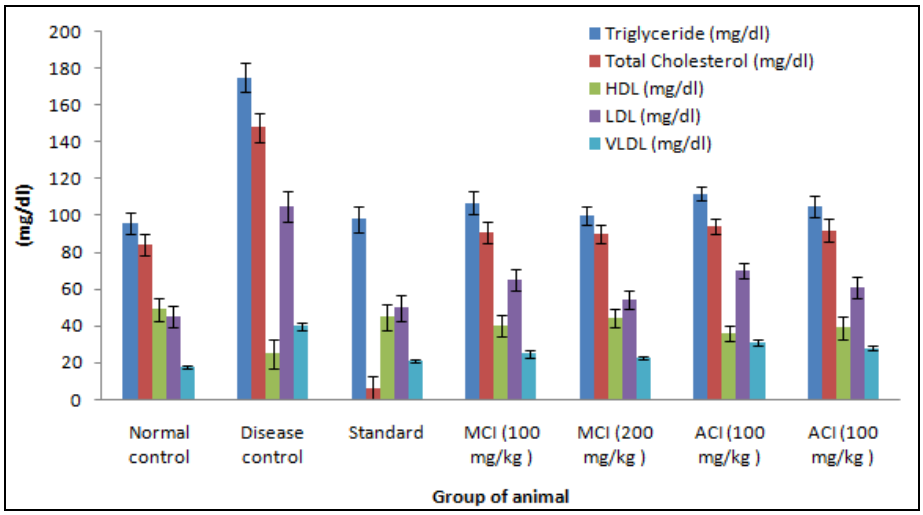

FIG. 6: EFFECT OF *CANNA INDICA* EXTRACT ON LIPID PROFILES

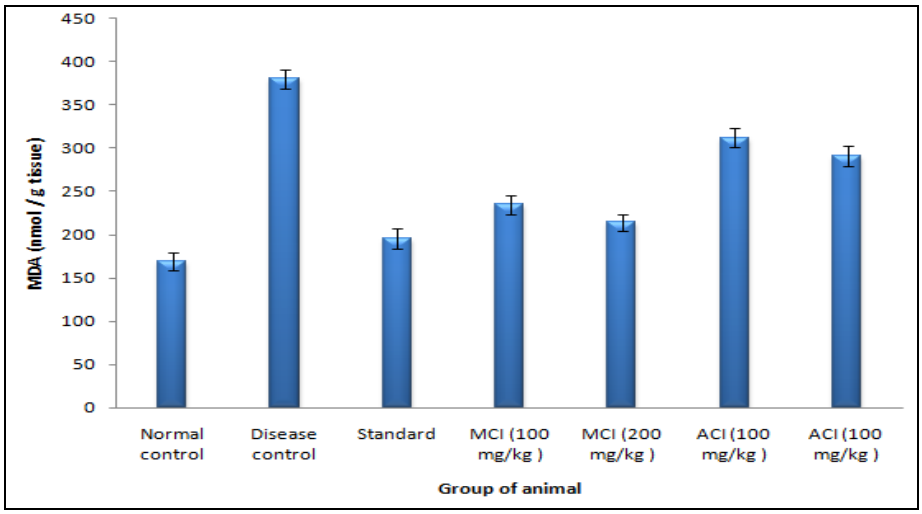

FIG. 7: EFFECT ON MDA CONTENTS IN LIVER OF ANIMALS TREATED WITH *CANNA INDICA* EXTRAC

The levels of AST activity in the blood plasma of animals in different experimental groups were performed. Treatment with *Canna indica* control AST level in dose dependent manner. Methanol extract of *Canna indica* control the AST level more effective than aqueous extract. 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. ALP, a hydrolysable enzyme excreted through the bile, is present in biliary cells as well as other organs such as bone, placenta, intestine, and kidney. Hepatotoxicity causes the biliary congestion leading to the inability of excretion of the ALP from the body that leads to elevation of the ALP level as seen in the vehicle group. The methanol extracts of *Canna indica* extracts showed reduced Bilirubin level in blood plasma of animals. In group received methanol extract of *Canna indica* (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 *Canna indica* (200mg/kg) was significantly restored the liver weight of treated group than aqueous extract.

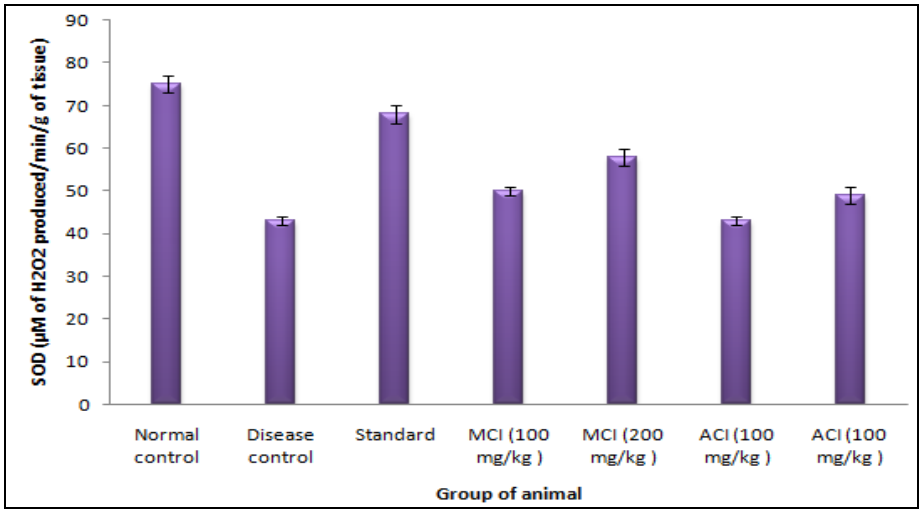

FIG. 8: EFFECT OF *CANNA INDICA* EXTRACT ON SOD LEVEL IN LIVER

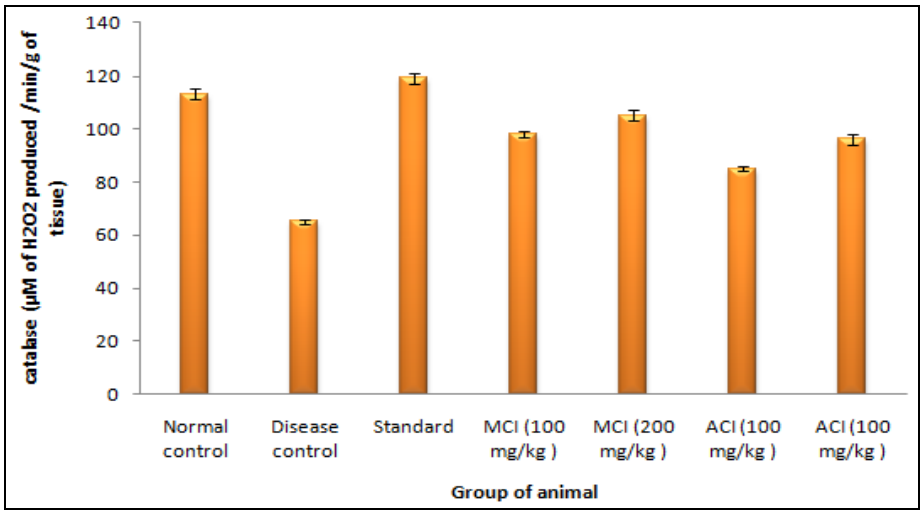

FIG. 9: EFFECT OF *CANNA INDICA* EXTRACT ON CATALASE ACTIVITY

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 *Canna indica* (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 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 *Canna indica*. The result of superoxide dismutase (SOD) activity level in the liver of the animals of various experimental groups. The paracetamol treated animal group significantly reduce SOD level compared with the normal

control group. Standard and methanol extract (200mg/kg) treated group reversed the situation and significantly increases the SOD level. The SOD level of treated group with extract was restored with treatment of plant extract. Methanol extract of *Canna indica* (200mg/kg) was significantly control the SOD level of treated group. The diseased animals have almost 52.8 % reduction in catalase activity. In a dose dependant manner, various extracts of *Canna indica* caused an increase in catalse activity in comparison to the disease control, to take the animals towards normalcy.

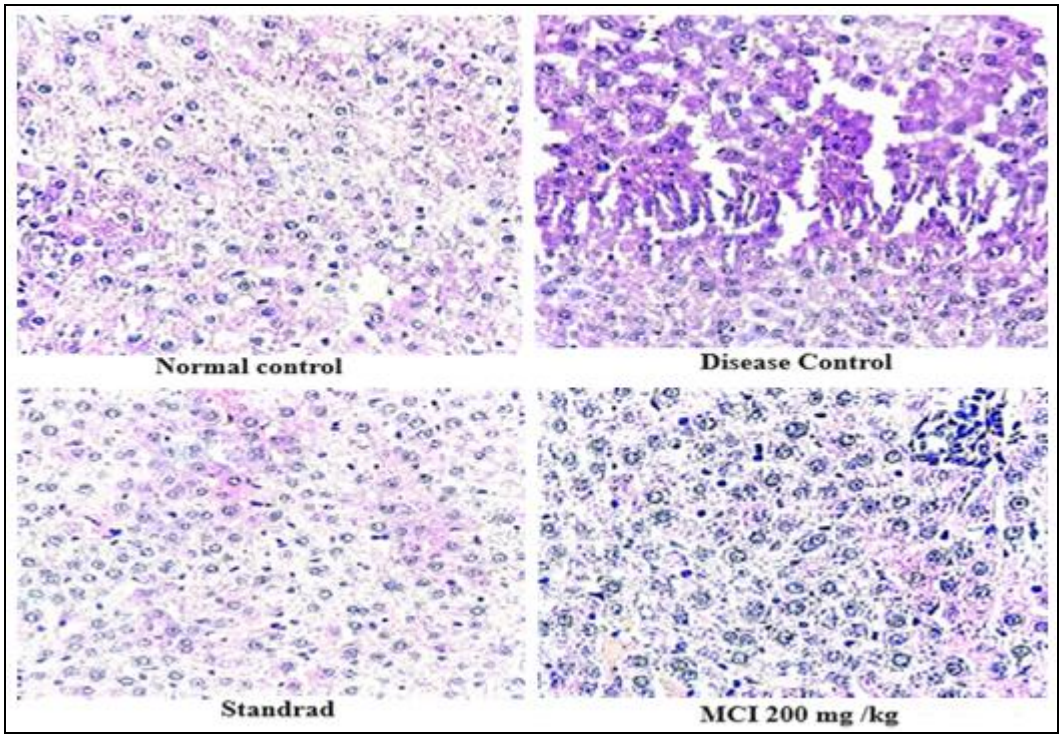

FIG. 10: HISTOPATHOLOGY OF LIVER OF THE ANIMALS TREATED WITH EXTRACTS

Histological sections of liver of normal control Group, revealed normal histology. The central vein and hepatocytes surrounding it were observed. The hepatocytes are polyhedral cells with one or rarely two spherical nuclei and contains abundant cytoplasm. The cytoplasm of such cells is granular and strongly eosinophilic. The nuclei of the hepatocytes are large with peripherally dispersed chromatin and prominent nucleoli. 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 aqueous extracts of *Canna indica* showed multifocal mild cytoplasmic vacuolation.

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. The over-usage of analgesic, antipyretic and certain medicines that is easily available across the counter in pharmaceutical outlets, overdrinking of alcohol has producing toxicity. Though all drugs have been found to be safe when used within limit of therapeutic dose, it may produce a fatal condition *i.e.* centrilobular hepatic necrosis when consumed in larger doses and/or over prolonged periods.

In the present study, hepatoprotective activity of the extracts of *Canna indica* was carried out using model of paracetamol induced hepatotoxicity in experimental animals (Wistar rats). Administration of paracetamol and different extracts of *Canna indica* showed no mortality or morbidity in the animals during the period of study. The values of AST, ALT, ALP, and total bilirubin were all substantially raised by paracetamol. Significant hepatoprotective effects were obtained by pretreatment with *Canna indica* methanol extract.

Histopathological examination of the liver tissues of paracetamolcontrol group represented the presence of marked foci of mononuclear infiltration in the hepatic parenchyma tissue, sinusoid, and around central vein, as well as disorganization of hepatic plates, necrosis, and fatty changes of hepatocytes.

Pretreatment with *Canna indica* extract reversed these alterations Methanol extract was found most effective among other extract and protect the liver towards normal, similar to standard treated group. This is suggestive of the fact that methanol extract of the *Canna indica* protect the liver from paracetamol induced hepatotoxicity.

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CONFLICT OF INTEREST: Authors have no Conflict of interest

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