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Hepatoprotective activity of some Indigeneous medicinal plants against paracetamol induced liver toxicity in albino rats

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Abstract
Hepatic disease is a united term for an entire group of trouble that afflicts the tissues, structures and cells of the human liver. Large number of important functions is performed by liver, so there are lots of opening for somewhat to go incorrect. One of the most common causes of liver disease is inflammation, which often results from abuse of alcohol, poor diet or even malnutrition. Drug induced liver damage or liver dysfunction is the most important health crisis that challenges not only medical personnel but also the pharmaceutical field and drug control board. 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 over dose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs. The present paper deals with the hepatoprotective activity of some indigenous medicinal plants containing aqueous extract of AI: Abutilon indicum (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) against paracetamol induced liver toxicity in albino rats. The results indicate that aqueous extract showed significant hepatoprotective activity as compared to the hepatotoxic control at the dose of 200 mg/kg.

Keywords: liver disorders, medicinal plants, paracetamol induced

Introduction
In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [1, 3].
Indian Indigenous medicinal plants are most widely used for the treatment of several diseases either in alone or in combination in raw as well as their extract. Synthetic hepatoprotective agents can produce several serious effects and also they are not suitable to use during pregnancy. In this light herbal are preferred in the treatment of liver disorders. Ancient ayurvedic literature reveals that the selected plants i.e., Abutilon indicum (Leaves), *Phyllanthus niruri* (Fruits), *Eclipta alba* (Leaves) and *Allium sativa* (Bulb) have been widely used in the treatment of liver disorders. These plants have been extensively used in ayurveda and traditional system of medicine for the treatment of liver disorders and found to be efficient and inexpensive as compared to synthetic drugs and not evaluated scientifically. Exhaustive literature review indicates that this kind of detailed scientific study has not been documented and carried out till now. Therefore, it was worthwhile to investigate the hepatoprotective activity of herbal extract containing *Abutilon indicum* (Leaves), *Phyllanthus niruri* (Fruits), *Eclipta alba* (Leaves) and *Allium sativa* (Bulb).

Material and Methods
Material(s)
Kanghi
Botanical Name: *Abutilon indicum* (Linn.) Sweet.
Family: Malvaceae
Habitat: Found in wild state in Central India.
Parts Used: Roots, Leaves, Fruits, Seeds
Phytochemistry: The plant contains saponins, flavonoids, alkaloids. The important constituents reported in the plant are β-sitosterol, vanillic acid, p-coumaric acid, caffeic acid, fumaric acid. The leaves of the plant contain steroids, sapogenins, carbohydrates and flavonoids.
Traditional Uses: Almost all the parts are of medicinal importance and used traditionally for the treatment of various ailments. The roots of the plant are considered as demulcent, diuretic, in chest infection and urethritis. The infusion of the root is prescribed in fevers as a cooling medicine and is considered useful in strangury, haematuria and in leprosy. The leaves are found to be good for ulcer and to treat liver disorders. The bark is used as febrifuge, anthelmintic, alexeteric, astringent and diuretic. The seeds are used in piles, laxative, expectorant, in chronic cystitis, gleet and gonorrhea [4].
Bhue amala
Botanical Name: Phyllanthus niruri *Linn.*
Family: Phyllanthaceae
Habitat: Found in India in rainy to winter season
Part Uses: Whole plant, Leaves, Fruit

Phytochemistry: The plant contains different classes of organic compounds. Major constituents are lignans, tannins, polyphenols, alkaloids, flavonoids, terpenoids and steroid.
Traditional Uses: It has extensive medicinal properties and has long history in the health care system of tropical countries. The plant is used as a folk medicine for treating kidney stones, gallbladder stones, liver related diseases such as liver cancer & jaundice, apart from these it is also administered for diuretic, hypoglycemic and hypertension cases and it also shows anti-inflammatory, anti-tumor, antinociceptive and anti-oxidant properties [5].

Bhringaraj
Botanical Name: *Eclipta alba* Linn.
Family: Asteraceae
Habitat: Found in wild state all over India
Parts Used: Whole plants, Leaves, Fruit
Phytochemistry: The plant contains wide range of active principles which includes coumestans, alkaloids, flavonoids, glycosides, polyacetylenes, triterpenoids. The leaves contain stigmasterol, β-terthienylmethanol, wedelolactone, demethyl wedelolactone and demethylwedelolactone-7-glucoside.
Traditional Uses: It has been as an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation. It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases. The plant is commonly used in hair oil all over India for healthy black and long hair. The fresh juice of leaves is used for increasing appetite, improving digestion and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma and popularly used to enhance memory and learning [6].

Garlic
Botanical Name: *Allium sativa* Linn.
Family: Lilliaceae
Habitat: Cultivated all over India for its bulb
Parts Used: Bulb, Leaves
Phytochemistry: It contains 33 sulphur compound, 17 amino acids, several enzymes and minerals such as selenium etc. Also, contains higher concentration of sulphur compounds than other Allium species. The sulphur compounds are responsible for its pungent odour and therapeutic action. Dried powdered garlic contain at least 1 % alliin (S-allyl cysteine sulfoxide).
Traditional Uses: Traditionally in Ayurveda, it has used for promotion of health as well as for the management of wide range of disorders including skin disorders, impaired digestive power, anorexia, indigestion, constipation, as analgesic, worm infestation, chronic cough, asthma and liver disorders [7].

Collection of herbs and their authentication
The plant parts viz., AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were collected from local sites of Malwa region of Madhya Pradesh, India during December-January 2018 and identified morphologically, microscopically and compared with standard pharmacopoeial monograph and authenticated by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory. Voucher specimen No. P/AI-L/113; P/PN-F/114; P/EA-L/115 and P/AS-B/116 were allotted to the selected plant parts.

Extraction of selected herbs
The shade dried coarsely powdered plant material (250 gms) of plant viz., AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were loaded in Soxhlet apparatus and was extracted with water for 48 hour. After completion of extraction, the solvent was removed by evaporation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined. [8-9]

Pharmacological screening

Acute Toxicity Studies of Extracts & Procurement of experimental animals
The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization. IEAC approval.

Hepatoprotective activity of extracts

Test Compounds
The aqueous extracts of plant viz., AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves), AS: *Allium sativa* (Bulb) and standard drug silymarin (50 mg/kg body weight) were used.

Chemicals and Reagents
Paracetamol, Silymarin.

Experimental Animal
Albino rats (200-250 g) used in the present studies was procured. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use.

Paracetamol Induced Model
The rats were divided into 11 groups of 6 animals in each [10, 11].

S/No.	Group	Treatments
1.	Group I (Normal)	Received vehicle gum acacia (5mg/kg p.o) for 7days
2.	Group I (Control)	Received vehicle gum acacia (5 mg/kg p.o) for 7 days once daily and paracetamol 500mg/kg once daily
3.	Group III (Standard)	Received silymarin as standard (50 mg/kg) for 7 days once daily and paracetamol 500mg/kg once daily
4.	Group IV	Received AEAIL (100 mg/kg) once daily and paracetamol 500mg/kg once daily
5.	Group V	Received AEAIL (200 mg/kg) once daily and paracetamol 500mg/kg once daily
6.	Group VI	Received AEPNF (100 mg/kg) once daily and paracetamol 500mg/kg once daily
7.	Group VII	Received AEPNF (200 mg/kg) once daily and paracetamol 500mg/kg once daily

8.	Group VIII	Received AEEAL (100 mg/kg) once daily and paracetamol 500mg/kg once daily
9.	Group IX	Received AEEAL (200 mg/kg) once daily and paracetamol 500mg/kg once daily
10.	Group X	Received AEASB (100 mg/kg) once daily and paracetamol 500mg/kg once daily
11.	Group XI	Received AEASB (200 mg/kg) once daily and paracetamol 500mg/kg once daily

On the seventh day, the blood samples were collected via orbital sinus puncture for the estimation of biochemical marker enzymes and allowed to clot and serum was separated by centrifuge at 2500 rpm for 15 min and analyzed for various biochemical parameters. Then the liver was carefully isolated and cleaned off extraneous tissue and preserved in 10% neutral formalin and then subjected to histopathological studies [13, 14].

Statistical Analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnette multiple Comparisons test. Statistically significance of * P<0.01, ** P<0.001, when compared with respective control. All values are expressed as mean ± SEM.

Assessment of Liver Function

The liver was removed and weighed**.** Biochemical parameters i.e., Serum glutamic Pyruvate transaminase (SGPT), serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP) were analyzed according to the reported methods [13, 14].

Histopathological Studies

Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro technique. 5µ section of the livers stained with alum haematoxylin and eosin, were observed microscopically for histopathological changes i.e., normal liver, damaged and recovered liver were studied and compared [13, 14].

Results and Discussion

The shade dried coarsely powdered plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) was extracted with water. The extracts obtained were evaluated for pH, color and % yield. The extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedure was adopted to perform the study. The results indicated the presence of flavonoids, phenols and alkaloids as a major constituent in all the extract.

The aqueous extracts of plant material i.e., AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were screened for acute toxicity study by OECD guideline no. 423 for determination of $LD_{50.}$ The results showed that the aqueous extracts i.e., AEAIL< AEPNF, AEEAL and AEASB were belonging to category-4. Hence, LD_{50} was 2000 mg/kg, therefore, ED_{50} was 200 mg/kg. Therefore, two doses of 100 and 200 mg were selected for present investigation. The results were presented in table 1.

Table 1: Determination of LD_{50} and ED_{50} of aqueous extract of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb)

No. of Animals	Extract Dose (mg/kg)	No. of death of animals			
		AEAIL	AEPNF	AEEAL	AEASB
3	5	0	0	0	0
3	50	0	0	0	0
3	300	0	0	0	0
3	2000	0	0	0	0

THepatoprotective Activity

Liver plays a key role in regulation of physiological processes. It is involved in several functions such as metabolism, secretion and storage. Furthermore detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are the most serious ailments. The results of biochemical parameters revealed that the elevation of enzyme level in paracetamol treated group, are almost restored to the normal level in the extract treated group.

Effect on SGPT

Aqueous extract of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) showed significant hepatoprotective activity as they reduced SGPT as compared to the hepatotoxic control at the dose of 200 mg/kg. The results of treatment with extract were tabulated in Table. SGPT is a cytosolic enzyme primarily present in the liver. The level of SGPT in serum increase due to leakage of this cellular enzyme into plasma by paracetamol induced hepatic injury. Serum level of SGPT can increase due to damage of the tissue producing acute hepatic necrosis. Since the extract significantly reduced the level of SGPT, this suggests that the extracts possess significant hepatoprotective activity.

Effect on SGOT

Aqueous extract of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) showed significant hepatoprotective activity as they reduced as compared to the hepatotoxic control at the dose of 200 mg/kg. The results of treatment with extracts were tabulated in Table. SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT level in serum due to the damage to the tissue producing acute necrosis such as several viral hepatitis and acute cholestasis. Since the extract significantly reduced the level of SGOT, this suggests that the extracts possess significant hepatoprotective activity.

Effect on ALP

Aqueous extract of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) showed significant hepatoprotective activity as they reduced as compared to the hepatotoxic control at the dose of 200 mg/kg. The results of treatment with extracts are tabulated in Table. In case of toxic liver, alkaline phosphate levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchyma or duct cells. Since the extract significantly reduced the level of ALP, this suggests that the extracts possess significant hepatoprotective activity.

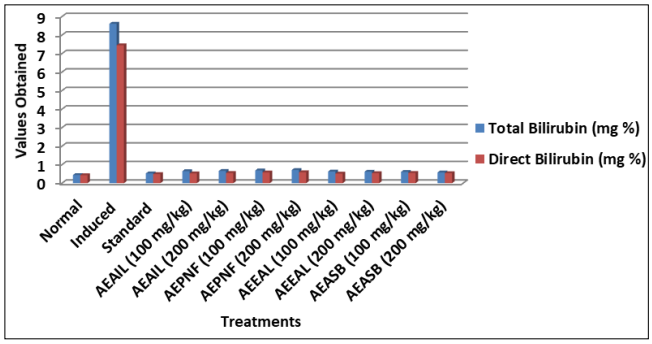
Effect on liver weight

The liver weight of animals treated with aqueous extract of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) was compared with that of the standard drug silymarin (50mg/kg) treated ones. Aqueous extract exhibited significant decrease in the weight of liver as that of the standard drug silymarin and thus suggest that the extracts possess significant hepatoprotective activity.

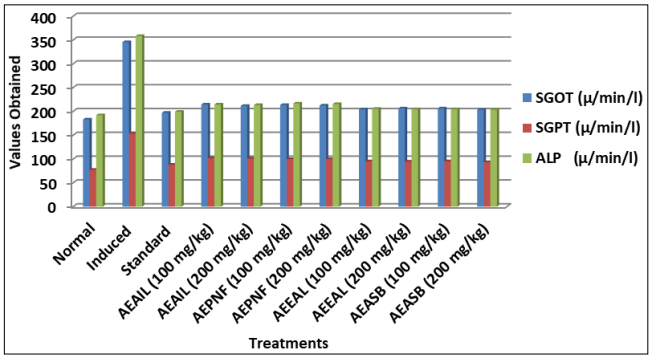
Table 2: Effect of aqueous of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) on paracetamol induced hepatotoxicity in rats

Treatment	Total Bilirubin (mg %)	Direct Bilirubin (mg %)	SGOT (µ/min/l)	SGPT (µ/min/l)	ALP (µ/min/l)
Normal	0.44 ± 0.21	0.43 ± 0.64	183.02 ± 2.1	77.40 ± 2.43	192.0 ± 6.2
Induced (PCM 2g/kg)	8.61 ± 2.46	7.45 ± 8.60	345.41± 10.42	153.7± 8.44	358.22±8.85
Standard (Silymarin 50mg/kg)	0.53 ±4.39**	0.49 ±0.19**	197.07±9.43**	88.07±8.79**	199.21 ±10.61**
AEAIL (100 mg/kg)	0.65 ± 4.59*	0.53 ± 0.19*	214.38± 8.58*	101.82± 4.58*	214.49± 9.59*
AEAIL (200 mg/kg)	0.66 ± 4.51*	0.55 ± 0.18*	211.38± 8.55*	100.82± 4.50*	213.31± 9.52*
AEPNF (100 mg/kg)	0.69 ± 4.62*	0.58 ± 0.28*	213.48± 8.64*	99.84± 4.62*	216.46± 9.64*
AEPNF (200 mg/kg)	0.71 ± 4.59*	0.59 ± 0.21*	212.41± 8.61*	99.82± 4.59*	215.41± 9.61*
AEEAL (100 mg/kg)	0.63 ±0 .64**	0.52 ±0.26**	204.24± 9.64**	95.24±8.24**	205.42± 8.48**
AEEAL (200 mg/kg)	0.62 ±0 .61**	0.54 ±0.22**	206.19± 9.66**	94.54±8.04**	204.43± 8.51**
AEASB (100 mg/kg)	0.61 ±0 .64**	0.55 ±0.26**	206.24± 9.64**	95.24±8.24**	204.52± 8.48**
AEASB (200 mg/kg)	0.58 ±0 .58**	0.54 ±0.31**	203.40± 9.14**	93.39±8.29**	203.47± 8.32**

Values are mean ±SEM, n= 6. (One-way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control



Graph 1: Estimation of total bilirubin and direct bilirubin of aqueous extracts on paracetamol induced hepatotoxicity in rats

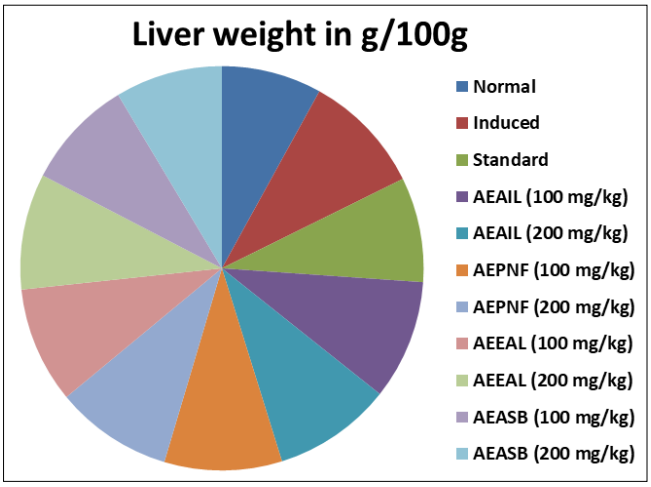


Graph 2: Estimation of SGOT, SGPT & ALP of aqueous extracts on paracetamol induced hepatotoxicity in rats

Table 3: Effect of of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) on liver weight variation of paracetamol induced hepatotoxicity in rats

Treatment	Liver weight in g/100g
Normal	6.82 ± 0.46
Induced (PCM 2g/kg)	8.22 ± 0.24
Standard (silymarin 50mg/kg)	7.12 ± 0.26**
AEAIL (100 mg/kg)	8.14 ± 0.62*
AEAIL (200 mg/kg)	8.07± 0.52*
AEPNF (100 mg/kg)	8.01 ± 0.62*
AEPNF (200 mg/kg)	7.98± 0.57*
AEEAL (100 mg/kg)	7.90 ± 0.44**
AEEAL (200 mg/kg)	7.88 ± 0.27**
AEASB (100 mg/kg)	7.47 ± 0.34**
AEASB (200 mg/kg)	7.31 ± 0.45**

Values are mean ±SEM, n= 6. (One-way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control.



Graph 3: Liver weight in g/100g of aqueous extracts on paracetamol induced hepatotoxicity in rats

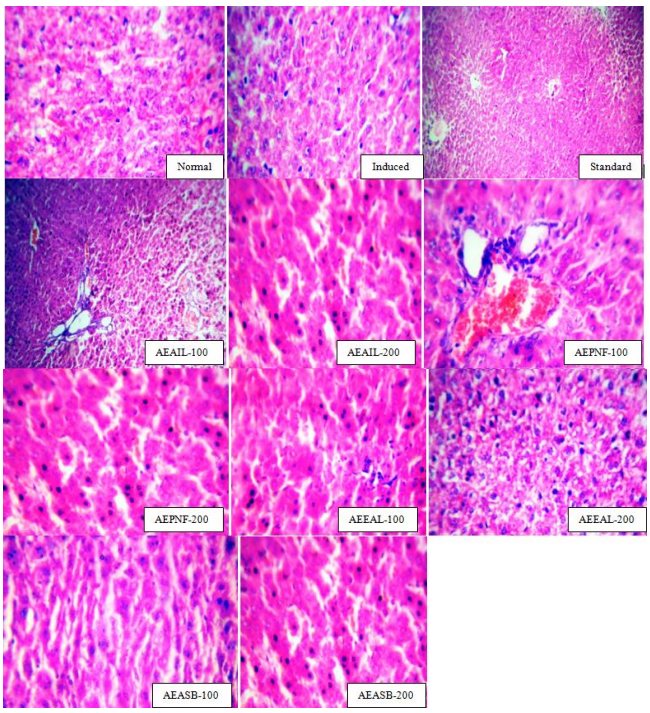


Fig 1: Histopathologic section of liver of rats in paracetamol induced hepatotoxicity

Normal: The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei. There is no periportal inflammation. Paracetamol induced (500mg/kg): The central veins show dilatation and congestion. The hepatocytes show feathery degeneration. The portal triads show mild peri-portal inflammation composed of lymphocytes. Silymarin (50mg/kg): The central veins appear normal. The hepatocytes show feathery degeneration. The portal triads show mild peri-portal inflammation composed of lymphocytes. AEAIL (100 mg/kg): The hepatocytes show moderate cytoplasm and moderately enlarged pleomorphic and hyperchromatic nuclei. The portal triads show mild peri-portal inflammation composed of lymphocytes. The central veins are normal. AEAIL (200 mg/kg): The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show show moderate cytoplasm and round to oval nuclei. AEPNF (100 mg/kg): The architecture is normal. The hepatocytes shows moderate cytoplasm and round nuclei. The portal triads appear normal. AEPNF (200 mg/kg): The central veins appear normal. The hepatocytes show moderate cytoplasm and enlarged pleomorphic nuclei. The portal triads show mild peri-portal inflammation composed of lymphocytes. AEEAL (100 mg/kg): The central veins show mild dilatation and congestion. The hepatocytes are normal. The portal triads appear normal. AEEAL (200 mg/kg): Section shows dilated and congested central veins. There is prominent periportal patchy necrosis and inflammation composed of mono-nuclear cells. AEASB (100 mg/kg): The hepatocytes show moderate cytoplasm and moderately enlarged pleomorphic and hyperchromatic nuclei. The portal triads show mild peri-portal inflammation composed of lymphocytes. The central veins are normal. AEASB (200 mg/kg): Section shows dilated and congested central veins. There is prominent periportal patchy necrosis and inflammation composed of mono-nuclear cells.

Conclusion

The liver disorders are very common and alarming. In the present work hepatoprotective activity of some indigenous medicinal plants containing aqueous extract of AI: Abutilon indicum(Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) against paracetamol induced liver toxicity in albino rats were investigated and the results indicates that aqueous extract showed significant hepatoprotective activity as compared to the hepatotoxic control at the dose of 200 mg/kg.

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