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Hepatoprotective Activity of *Atylosia rugosa* Against Carbontetrachloride and Paracetamol Induced Hepatotoxicity in Rats

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Abstract

Liver is a vital organ that plays a major role in the elimination of xenobiotics from the body. Diseases that affecting the liver become major health problems and challenge to the health-care professionals as well as to the pharmaceutical industry.The conventional treatment for liver diseases is associated with a wide range of adverse effects hence herbal formulations are considered as safer than conventional drugs. The plant Atylosia rugosa was collected from tirumala hills, dried around 1000gms of whole plant was powdered.Thewhole plant of Atylosia rugosa was successively extracted using soxhlet apparatus with solvents as petroleum ether (60^0C-80^0C), chloroform, acetone, ethyl acetate and ethanol. The phytochemical screening of various extracts showed the presence of various phytoconstituents like flavonoids, terpenoids and phenolic compounds etc. The present study was aimed to study theinvivoacute toxicity and hepatoprotective activity of ethanolic extract of whole plant of Atylosia rugosa in albino wistar rats. The studies were conducted byusing the two popular inducing agents paracetamol (2mg/kg.p.o)in 1% CMC and carbontetrachloride (2mg/kg).N-acetyl- cysteine(100mg/kg .b.w) and Silymarin(50mg/kg.p.o) were used as reference drugs in the respective models.

The degree of protection was measured by estimating biochemical parameters such as serum glutamate Oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT),total protein (TP), alkaline phosphatase (ALP) and the level of total serum bilirubin. Evaluation of the change in body weight and liver weight, histopathologic examination against CCl4-induced hepatotoxicity were also carried, the ethanolic extract (100mg/kg, 200mg/kg, 400mg/kg) exhibited significant hepatoprotection against carbontetrachloride and paracetamol in toxicated rats in a dose dependant manner. It also suppressed the plasma levels of AST, ALT and ALP ($p<0.05$) in the aforementioned doses. A positive, significant linear relationship was observedbetween hepatoprotective activity and TPC and TFC content, showed that phenolic compounds and flavonoids were the dominant. The present study revealed there is no toxicity in animals upto 3200mg/kg of th extract, and showed a significant hepatoprotective activity of the plant is because of its active metabolites in the plant in the *Atylosia rugosa.*

KeyWords: *Atylosiarugosa*, Hepatoprotective activity, Carbontetrachloride, Paracetmol, Silymarin

Introduction

In ancient Indian literature, it is mentioned that every plant on this earth is useful for

human beings, animals and other plants.Therefore, plantshas been considerable interest in complementary and alternative medicines for the treatment of various disorders. Naturalproductsmayreducetheriskofdevelopingtoxicities with the drugs(1).

The use of herbal medicine stotre atliver diseases has increased worldwide,and this is due to the belief that herbal medicines are harmless and free from serious adverse reactions, in addition, they are available and easily obtained from nature. Moreover, the limited therapeutic choices and sometimes unsatisfactory therapeutic failure of modern medicine have increased the usage of alternative medicine including herbal preparations (2,3) .The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision ,reproduction (4) and a frequent target for a number of toxicants(5) . In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (6) .Now only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders (7). The disorders associated with the liver are also numerous and varied (8).

Morethan900drugshavebeenimplicatedincausingliverinjury(9) and it is the most common reason for a drug to be withdrawn from the market. Drug- induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (10 ,11) .In spite of tremendous scientific advancement in the field of hepatology during recent years, liver problems are on the rise. Regrettably there are only a few drugs with serious side effects available for the treatment of liver ailments (12).

In view of the undesirable side effects of synthetic agents, there is growing focus towards the therapeutic evaluation of medicinal plants using systemic research methodology.*Atylosia rugosa* known as *Cajanus rugosus*belonging to the family Fabaceae commonly known as peddaadavikandi, Adaviualva. It is a slender, twining herb with densely grey-dowry stems (13). The plant was reported to contain flavonoids, alkaloids, glycosides, terpenoids, tannins and phenolic compounds. The whole plant or the roots are being crushed and prescribed for vitality to the mother after child birth, used in bronchitis and tooth paste. Nothing is reported on biological work of *Atylosia rugosa.* Legumes also provide essential minerals required by humans (14) and produce health-promoting secondary compounds that can protect against human cancers (15,16)and protect the plant against the onslaught of pathogens and pests (17,18) . In addition to their blood cholesterol-reducing effect(19), grain legumes generally also have a hypoglycemic effect, reducing the increase in blood Glucose after a meal and, hence are acting as insulin. Legumes are, therefore, included in the diet of insulin-dependent diabetics (20). Certain legumes, however, produce antinutritional factors, such as trypsin inhibitors and phytohemagglutinins (21)and allergens, the later being a severe problem in peanut(22). Genomics approaches, including metabolomics and proteomics, are essential to understanding the metabolic pathways that produce these antinutritional compounds and to eliminating these factors from the plant. Silymarin,aflavonolignanfrommilkthistle(Silybummarianum)plantisused almostexclusivelyforhepatoprotection(23).This study was aimed at investigation of chemical constituents of the leaves of *Atylosia rugosa* grown in India, in addition to pharmacologicalevaluation.This study was aimed at investigation of chemical constituents of the leaves of *Atylosiarugosa* grown in India, in addition to pharmacologicalevaluation.

Materials and Methods

Plant Material

The fresh plant of *Atylosiarugosa* was collected in the month of August from Tirupathi, Andhra

Pradesh, India. The plant was authenticated by Dr. Madhava Chetty, Taxonomist, S.V.University, Tirupathi, India. The plant was washed immediately after collecting and shade dried at 40°C for a week, powdered mechanically, sieved with #40 mesh and stored in air-tight containers.

Chemicals

Petroleum ether, Chloroform, Acetone, Ethyl acetate, Ethanolwere purchased from Sigma Aldrich.And all theotherchemicalreagentswereusedof are analyticalgrade.

Preparation of extracts

The shade dried powered of *Atylosia rugosa*was reduced to fine powder, around 1000g of powder was subjected to successive hot continuous soxhlet extraction with petroleum ether, chloroform, acetone, ethyl acetate and ethanol. Each time before extracting with the next solvent the powdered material was air dried. After the effective extraction the solvents were distilled off and the extract was then concentrated by distillation and solvent recovery. The solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland). The semisolid mass obtained was dried in an oven at 40°C, powdered, labelled as EEAR stored in desiccators. The obtained extracts were subjected to phytochemical investigation and pharmacological evaluation.Preliminary phytochemical analysis of secondary metabolites

Table 1: Extraction yield (%) of Atylosia rugosa with various solvents by hot soxhlation method

S.No	Ex tract(200gm)	Color in day light and consistency	% Yield
1	Petroleum Ether	Solid Greenish	6.305
2	Chloroform	Solid Dark Brown	5.1
3	Acetone	Solid Dark Brownish Black	9.245
4	Ethyl Acetate	Solid Dark Brown	19.12
5	Ethanol	Semi-Solid Reddish Brown	24.08

All the extracts were subjected to preliminary phytochemical screening (24) for the presence or absence of various secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, saponins, tannins, glycosides using analytical grade solvents and reagents. The yields and preliminary phytochemical investigation results were given in the **Table 1.0** and 1.1.

Experimental animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of MNR College of Pharmcy, Sangareddy, Hyderabad. The animals were housed in standard cages by maintaining a temperature of 22±20°C at 12:12 hours light dark cycle. The animals were provided with pellet diet and water ad libitum(25) .

The animals were allowedfreeaccesstotapwaterandlaboratorypelletandacclimatizedtolaboratoryconditionsforoneweek before theexperiment, during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups andbeforeinitiationofexperiment,theratswereacclimatizedfora period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum.The experimental procedure were carried out in strict compliance with the ethical guidelines for investigations of experimental pain in conscious animal framed by the Animal Ethical Committee rules and regulations in this institute.

Phytochemical investigation

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in ethanol extracts(26-28).

Assessment of hepatoprotective activity

A toxic dose or repeated doses of a known hepatotoxins such as carbon tetrachloride, paracetamol, thioacetamide, rifampicin, alcohol, D-galactosamine, allyl-alcohol etc; are administered to induce liver damage in experimental animals(29,30). Ifthe hepatotoxicity produced by the toxin is prevented or reduced, then the test substance is considered as an effective hepatoprotective agent(31,32). In the present investigation, rats (n=6) were randomized into following groups and the pharmacological investigation was carried using carbon tetrachloride and paracetamol as inducing agents and the test EEAI at dose levels of 100,200,400 mg/kg as hepatoprotectiveagent.

1)Group I – 1% w/v CMC perorally for 21days.

2)Group II – CC14 (2 ml/kg) administered by i.p + 1% w/v CMC per orally for 21 days.

3)Group III – Paracetamol (2gm/kg) in 1% CMC per orally for 21days.

Group IV – CC14 (2 ml/kg) administered by i.p + EEAR (100mg/kg) in 1% w/v CMC per orally for 21days.

Group V – CC14 (2 ml/kg) administered by i.p + EEAR (200mg/kg) in 1% w/v CMC per orally for 21days.

Group VI – CC14 (2 ml/kg) administered by i.p + EEAR (400mg/kg) in 1% w/v CMC per orally for 21days.

Group VII –Paracetamol (2 gm/kg) and EEAR (100mg/kg) in 1% w/v CMC per orally for 21days.

Group VIII –Paracetamol (2 gm/kg) and EEAR (200mg/kg) in 1% w/v CMC per orally for 21days Group IX –Paracetamol (2 gm/kg) and EEAR (400mg/kg) in 1% w/v CMC per orally for 21days.

Group X – CC14 (2 ml /kg) administered by i.p + silymarin (50mg/kg) in 1% w/v CMC per orally for 7days.

Group XI – Paracetamol (2gm/kg) and N-acetyl l-cystine (100mg/kg) in 1% w/v CMC per orally for 21days.

.Animals were divided into eleven different groups, each having 6 rats and treated accordingly Treatment with plant extract was started after 24 hrs of administration of inducing agents. After 21 days of such treatment, rats serum analysis was done.

Blood biochemistry

Blood samples were collected in glass tube from retro- orbital puncture to coagulate for 30 min at 37^0C followed by centrifugation (3000 rpm for 15 min) and subject obtain haemolysis-free clear serum used for the analysis of SGOT and SGPT (33), ALP(34) and bilirubin (35) by standard method.Serumtotalproteinwasmeasuredaccordingtothemethod of (36).

Estimation ofoxidative stressmarkers

On the 31stday, all the animals were euthanized after blood collection with the spinal dislocation method under light ether anesthesia and the liver was removed for study of oxidative stress markers like Superoxide dismutase (SOD)Catalase(CAT)(37), Liver was dissected out, washed with ice cold Phosphate Buffer Saline (PBS) (0.1 M, pH 7.4) and 10% tissue homogenate used for different biochemical analysis. A part of the liver was used for histopathological studies.

Histopathology

Histopathology of liver was carried out by a modified Luna (38). In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 h. Then the livers were paraffin embedded and 5 µ thickness microtone sections were made (39).The sections were processed in alcohol-xylene series and stainedwithhaematoxy linandeosin.The slides were studiedunder a light micro-scope for any histological damage/protection.

Statistical analysis

The results are expressed as Mean±SEM of six animals from each group. The data were evaluated by Dunnets comparision tests. *p values <0.05 was considered statistically significant. for liver enzymes.

Results and Discussion

Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials which are rich in phenolics and flavonoids, are increasingly being used in the food industry for their health benefits. The powder of *Atylosia rugosa* was successively extacted by soxhalationwith petroleum ether, chloroform, acetone, ethyl acetate, ethanol. The results of yields were described in the **Table no.1.0**. The petroleum ether of *Atylosia rugosa* extract was solid greenish colour and the yield was 6.305%w/w, chloroform extract was solid dark brown in colour and the yield was 5.1%w/w, acetone extract was solid dark brownish black in colour and the yield was 9.245%w/w, ethyl acetate was solid dark brown in colour and the yield was 19.12%w/w, ethanolic extract was semisolid reddish brown in colour and the was 24.08%w/w. In the present research the total phenolic and flavonoid content in *Atylosia rugosa* has shown high phenolic and flavonoid contents as described in **Table 2.0**. The presence or absence of phytoconstituents in all the solvents are described in the **Table 3.0**. Flavonoids and phenolics are major classes of compounds in ethanolic extracts of *Atylosia rugosa,* the results of the present study showed that the ethanolic extract of *Atylosia rugosa* which has highest amount of flavonoids, phenolic and triterpenes.

Acute Toxicity studies of ethanolic extract of *Atylosia rugosa (*EEAR) did not show any sign and symptoms of toxicity or mortality up to 3200mg/kg body weight on oral administration. Thus, the extracts could be considered as safe as per OECD guidelines 423. Body weight before and after administration were noted and any changes in skin, fur, eyes, mucous membranes, breathing, vascular, automatic and central nervous system were observed, sign of salivation, diarrhoea, tremors, convulsions, lethargy, sleep and coma were comprehended. The onset of toxicity and signs of toxicity were not seen in the rats upto two weeks of observations period. This indicates the safety of extract. Hence, the 100mg/kg, 200mg/kg, and

Table2:Total phenolic and flavonoid contents of *Atylosia rugosa*

S.No	Extract	a Total Phenolic content	b Total Flavonoid content
1	Ethyl acetate	31.63±1.06	23.36±1.65
2	Ethanolic Extract	34.12 ±1.4 6	20.6±1.2

Table 3: Qualitative phytochemical investigation of *Atylosia rugosa*

Nature	Pet Ether	Chloroform	Acetone	Ethyl Acetate	Ethanol
Alkaloids	-	+	-	+	+
Amino acids	-	-	-	-	+
Flavonoids	-	+	++	++	++
AnthraquinoneGlycosides	-	-	-	+	++
Phytosterol & Triterpenoids	+	+	-	-	+
Reducing Sugar	+	+	++	++	++
Gums	-	-	+	+	+
Tannins and Phenolics	-	+	++	++	++
Saponins	+	+	++	+	+
Fixed oils	+	+	+	+	+

+ = present, - = absent

Table 4.0 Acute toxicity studies of Ethanolic Extract of *Atylosia rugosa*

Route of administration	Treatment	Dose mg/kg	No. of animals	No. of Survival	No. of Death	LD_{50}
Peroral	Control	10 ml/kg	20	20	0	>3.2 gms kg.p.o
	SPE	100	20	20	0	
		200	20	20	0	
		400	20	20	0	
		800	20	20	0	
		1600	20	20	0	
		3200	20	20	0	
Intraperitonial	Control	10 mg/kg	20	20	0	>3.2 gms/kg.p.o.
	SPE	100	20	20	0	
		200	20	20	0	
		400	20	20	0	
		800	20	20	0	
		1600	20	20	0	
		3200	20	18	2	

400mg/kg doses were selected for the further pharmacological studies. **Table 4.0** Results suggested that the extracts administered doses have not exhibited signs toxicity.

Carbon tetrachloride and paracetamol are thewell known hepato-destructive agents that are widely used to induce acute-toxic liver injury in laboratory animals(40).Rats treated with carbon tetrachloride and paracetamol showed a significant hepatic damage as observed from elevated levels of hepato-specific enzymes as well as severe alteration in different liver parameters asdiscussed in **Table 5.0** and **Table 6.0**. The CCl_4 and paracetamol administration resulted in elevated activities of AST, ALT and ALP in serum against their respective control values. Similarly, serum bilirubin level was also found to be increased significantly as a result of CCl_4 and paracetamol toxicity. On the other hand, total serum protein level was lowered in response to CCl_4 and paracetamol administration when compared with control. Abnormally higher activities of serum ALT, AST and ALP after CCl_4 and paracetamol administration are an indication of the development of hepatic injury, which is responsible for leakage of cellular enzymes into the blood. When liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation(41).The changes associated with CCl_4-induced hepatic damage are similar to that of acute viral hepatitis(42).The hepatotoxicity of CCl_4 has been reported to be due to its biotransformation by cytochrome P-450 system to produce trichloroethylene free radicals. These free radicals may again react with oxygen to form trichloroethylene peroxy radicals, which exert their action on lipids membrane of endoplasmic reticulum to evoke lipid peroxidation(43). Overdose of paracetamol causes a potentially fatal, hepatic centrilobular necrosis.The hepatotoxicity of paracetamol has been attributed to the formation of a toxic metabolite, *N-acetyl-p-benzoquinoneimine*(NAPQI) by the action of cytochrome P4502E1 (44).Oral administration of various doses of EEAR to CCl_4 and paracetamol intoxicated rats resulted in gradual normalization of the activities of AST, ALT and ALP. This evidently suggests the protective effect of the extract in improving the functional integrity of liver cells.Serum bilirubin is considered as an index for the assessment of hepatic function and any abnormal increase indicates hepatobiliary disease and severe disturbance of hepatocellular architecture(45). CCl_4 and Paracetamol administration resulted

Table 5.0: Ethanolic extract of ***Atylosia rugosa*** (EEAR) on AST, ALT &ALP ,TP &TB serum enzymatic activity in CCL4 and Paracetamol induced Liver damage in rats (n=6)

	AST—IU/L	ALT—IU/L	ALP (KA Units)	Total Proteins(mg/dl)	Bilirubin(mg%) (Total)
Group I	33.84± 0.347*	36.06 ± 0.33*	292.56 ± 0.420*	191.23 ± 0.359*	0.281 ± 0.013*
Group II	71.40± 0.535*	135.20±0.507*	839.05 ± 1.581*	68.28 ± 0.377*	4.340 ± 0.111*
Group III	71.10± 0.316*	113.50± 0.513	891.58 ± 0.221	72.55 ± 0.534	4.027 ± 0.087
Group IV	54.10±0.647*(↓46.05)	80.67±0.276*(↓55.00)	647.82±0.685*(↓34.99)	98.10±0.324 *(↑24.25)	1.640±0.104*(↓66.50)
Group V	42.70±0.327*(↓76.41)	63.80±0.719*(↓72.01)	475.87±1.156* (↓66.45)	128.90±0.100 *(↑49.30)	1.043±0.056*(↓81.28)
Group VI	39.60±0.375**(↓84.60)	45.72±0.414*(↓90.25)	402.80±1.23*(↓79.82)	157.60±0.870*(↑72.64)	0.696±0.072*(↓89.90)
Group VII	49.00±0.176**(↓59.31)	73.61±0.301**(↓51.51)	622.44±0.768**(↓44.93)	94.75±0.261**(↑18.70)	1.617±0.034*(↓64.43)
Group VIII	41.90±0.373*(↓78.36)	57.39±0.490*(↓72.45)	483.11±0.981*(↓75.70)	125.40±0.394*(↑44.53)	1.107±0.783**(↓78.27)
Group IX	38.50±0.983**(↓87.49)	42.93±0.495**(↓91.12)	403.23±0.802**(↓81.52)	161.30±0.537*(↑74.78)	0.665±0.076*(↓89.83)
Group X	39.92±0.462**(↓83.81)	42.42±0.545**(↓93.58)	358.82±0.266*(↓87.87)	178.50±0.439**(↑89.64)	0.590±0.076**(↓92.36)
Group XI	36.40±0.732**(↓93.12)	38.40±0.470**(↓96.97)	381.63±0.647**(↓85.13)	182.01±0.508**(↑92.23)	0.568±0.024**(↓92.51)

The results are expressed as Mean ± SEM of six animals from each group. The data were evaluated by Dunnetscomparision-tests.Where, * represents significant at <0.05, ** represents highly significant at p<0.01,. All values are compared with toxicant were considered as statistically significant. EEAR = Ethanolic extract of *Atylosia rugosa*.

in increased serum bilirubin level, thereby suggesting severe hepatic injury and confirming the hepatotoxic nature of CCl_4andparacetamol.

.Treatment with EEAR significantly decreased the elevated level of total bilirubin in serum towards normalcy indicating its hepatoprotective efficacy. Hepatotoxins impair the capacity of liver to synthesize albumin. Decreased total serum protein level in CCl_4 and paracetamol treated rats may be attributed to impaired protein synthesis by damaging liver tissue. Subsequent treatment of CCl_4 and paracetamol intoxicated rats with EEAR increased the total serum protein (TSP) level. This further signifies the curative nature of extract against CCl_4 and paracetamol toxicity.Hepatic lipid peroxidation (LP), expressed as TBARS (thiobarbituric acid reacting substances), increased significantly in CCl_4 and paracetamol toxicity. While, the activities of protective enzymes such as Superoxide dismutase (SOD) and catalase (CAT) and glutathione and glycogen content in liver tissue were lowered after paracetamol administration. Enhanced LP and reduced activities of SOD and CAT is an indication of generation of free radical stress as a mark of hepatic damage due to CCl_4 and paracetamol toxicity. Marked reductions in the activities of these free radical scavenging enzymes, SOD and CAT, associated with CCl4 and paracetamol toxicity were significantly reversed to normal on oral feeding of EEAR in a dose dependent manner conferring the antilipid peroxidative ability to the extract. Paracetamol gets metabolically activated to a reactive metabolite NAPQI by cytochrome P4502E NAPQI, in turn, is detoxified by conjugating with glutathione (GSH). Thus, GSH constitute the first line of defence against paracetamol induced generation of free radicals. In paracetamol toxicity, total hepatic GSH was found to be depleted due to the damage caused to hepatic cells. As a result, formation of NAPQI

Table 6.0: Ethanolic extract of *Atylosia rugosa* (EEAR) on LP,SOD,CAT,GSH and Glycogen content in Liver in rats (n=6)

Group	LP(n molesofMDA formed /mg Protein)	SOD (Units of Activity / mg Protein)	CAT(µg/mg Protein)	GSH(µg/mgProtein)	Glycogen(mg/gm of wet tissue)
Group I	27.16±0.701*	15.4 ± 0.169*	116.00± 0.146*	4.57±0.100*	7.53 ± 0.130*
Group II	592.26±0.812*	8.250 ± 0.283*	50.64 ± 0.176*	1.80 ± 0.049*	4.43 ± 0.135*
Group III	612.93±1.416*	7.890 ± 0.203*	62.00 ± 0.440*	2.33 ± 0.114*	5.13 ± 0.191*
Group IV	369.14±0.962*(↓39.49)	10.80±0.049*(↑35.66)	65.10±0.282*(↑22.17)	2.96±0.077* (↑41.84)	5.72±0.214*(↑41.61)
Group V	210.12±0.422*(↓67.62)	12.70±0.067*(↑62.23)	83.40±0.203*(↑50.15)	2.72 ± 0.070* (↑33.21)	6.35±0.088*(↑61.93)
Group VI	129.16±0.628*(↓81.95)	13.50±0.140**(↑73.42)	94.90±0.506**(↑67.73)	3.58±0.107**(↑64.25)	6.37±0.151**(↑62.58)
Group VII	396.18±0.824*(↓37.00)	10.30± 0.150**(↑32.09)	78.00± 0.270**(↑29.62)	2.78±0.145**(↑20.08)	5.97±0.211**(↑35.00)
Group VIII	206.14±0.112*(↓69.44)	12.40±0.151*(↑60.05)	85.90±0.234**(↑44.25)	3.00±0.40**(↑29.91)	6.32±0.065**(↑49.58)
Group IX	129.19±0.428*(↓82.58)	12.50±0.194*(↑61.38)	98.80±0.179**(↑68.14)	3.73±0.148*(↑62.50)	6.51±0.204**(↑57.50)
Group X	98.12±0.842*(↓87.44)	13.70 ±0.094**(↑76.22)	106.00 ±0.354*(↑84.70)	4.05 ± 0.057*(↑81.22)	7.21±0.133(↑68.67)
Group XI	60.19±0.624*(↓94.36)	12.90 ± 0.195*(↑66.71)	107.06±0.163**(↑83.44)	4.16±0.039*(↑81.69)	7.02±0.122*(↑78.75)

The results are expressed as Mean ± SEM of six animals from each group. The data were evaluated by Dunnetscomparision-tests.Where, * represents significant at <0.05, ** represents highly significant at p< 0.01,. All values are compared with toxicant were considered as statistically significant. EEAR = Ethanolic extract of *Atylosia rugosa*

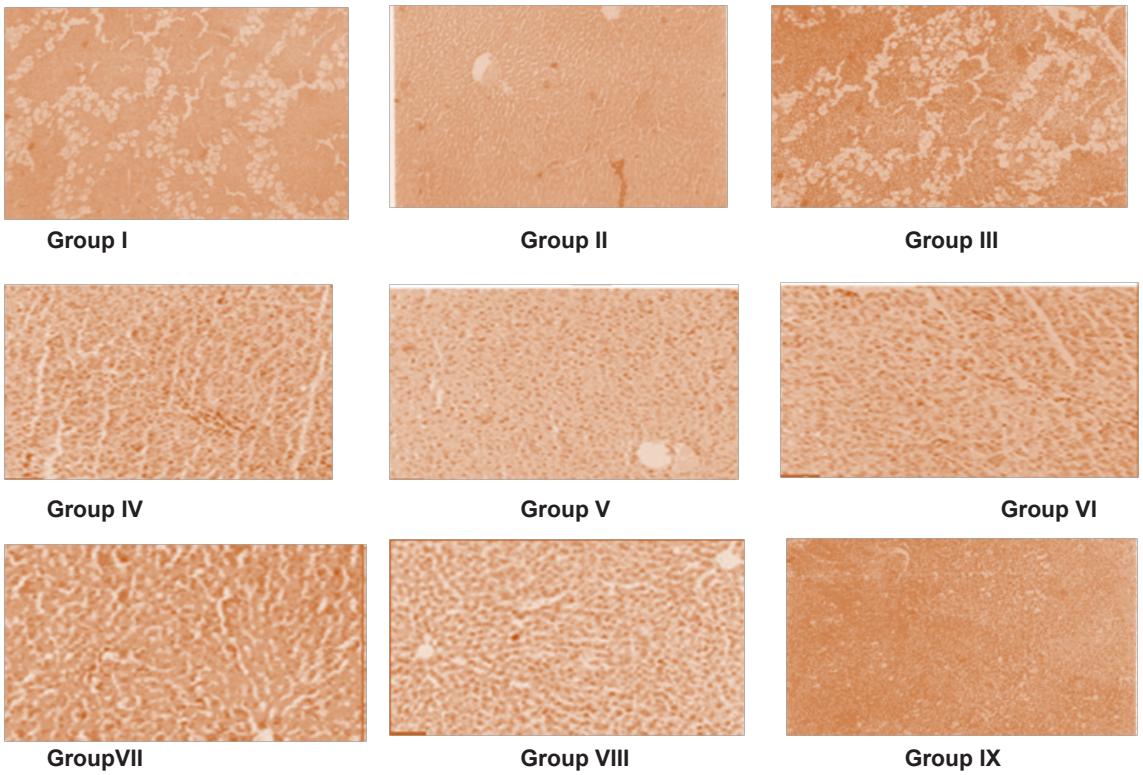

Fig.1.0: Histopathological changes showing effect of EEAR on the rats intoxicated with PCT & CCl_4

glutathione conjugate is diminished. Administration of EEAR effectively replenished the paracetamol induced depletion of hepatic GSH presumably due to diminished production of toxic metabolite, NAPQI through the inhibition of cytoP450 enzymesystem. Histopathological examination of liver sections of the normal control group showed normal cellular architecture with distinct hepatic cells as in **Fig 1.0**. However, distinct hepatic necrosis was noted after $CCl_4$and paracetamol administration with destruction of hepatic cells. EEAR treatment to such and paracetamol intoxicated rats showed recovery of the hepatocytes from necrosis. This also CCl_4 suggests that the plant extract has a tremendous potential to reverse the changes induced by paracetamol toxicity back tonormal. The curative efficacy of EEAR was dose dependent as evidenced by gradual reversal of the altered values of various biochemical markers back to normal following oral administration. This may, probably be through promotional activation of antioxidative enzymes and regeneration of hepatocytes that restore the structural and functional integrity of liver. The protective effects due to treatment with *Atylosia rugosa* extract strongly indicated the possibility of the extract being able to prevent and/or mitigate any leakages of marker enzymes into circulation, condition the hepatocytes to accelerate regeneration of parenchymal cells, and preserve the integrity of the plasma membranes and hence restore these enzymes levels(45).

Thus, the present investigation confirms that the ethanolic extract of *Atylosia rugosa* (EEAR) posses alkaloids, flavonoids, tannins, terpenes, proteins etc. These phytosterols can enhance adaptive immunity through the stimulation of innate immune system termed as the “adptogen”which promotes overall health without side effects(46). Thus Ethanolic extract of

Atylosia rugosa (EEAR) is safe *i*n possessing significant hepatoprotective effect in tested animals, it is may be due to the presence of bioactive substances or a mixture of compounds which has proven biological activity.

Conclusion

The results of the present study suggested that the ethanolic extract of *Atylosia rugosa* in doses of 100mg/kg, 200mg/kg, 400mg/kg are safe and significantly reduced the toxicity of liver in rats in a dose dependent manner.The underlying mechanism may be that ethanolic extract of *Atylosia rugosa* has potent hepatoprotective activity against carbon tetrachloride and paracetmol induced liver damage in rats, the presence active constituents such as alkaloids , flavonoids and sterols in alchololic extract of *Atylosia rugosa* revealed the hepatoprotective nature of the plant.

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