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Hepatoprotective activities of *Amaranthus gangeticus* leaves against paracetamol induced hepatic damage in albino rats

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Abstract: ***Amaranthus Gangeticus*** **is a medicinal plant. Our aim is to investigate its organ protection property. Hence ethanolic extract of** ***Amaranthus Gangeticus*** **leaves (AG) was taken and the parameters studied were SGPT, SGOT, total bilirubin, direct bilirubin, total cholesterol, HDL and ALP activities. Results of bio-chemical studies of blood samples of Paracetamol treated animals showed significant increase in the levels of serum markers and decrease in HDL level reflecting the liver injury caused by Paracetamol. Whereas, the animals treated with ethanolic extract of AG showed significant dose dependent decrease in the elevated levels of serum markers and increase in HDL levels indicating the protection of hepatic cells. Therefore these results concluded that, the ethanolic extract afford significant protection against Paracetamol induced hepatocellular injury and remarkable rejuvenation of these tissues found in histopathological studies which may be attributed due to polyphenols and antioxidant in it.**

Keywords: **Ethanolic extract of** ***Amaranthus Gangeticus*** **leaves and serum markers.**

1. INTRODUCTION

Liver is an important organ in the body. It is the key organ of metabolism and excretion. During its normal physiological functioning it metabolizes various endogenous and exogenously administered chemicals so as to terminate or inactivate these agents. Hence due to this function, it protects the whole body from the various environmental and chemical challenges. In addition to this liver has got an inbuilt mechanism to protect itself and to regenerate on several occasions, but many of these hepatotoxic challenges overpower inbuilt protective mechanism and cause hepatotoxicity resulting in the hepatic necrosis and hepatitis.

Amaranthus gangeticus is one such edible plant used as vegetable which is being used by native practitioner as hepatoprotective in treating various types of jaundice. The leaves of this plant contain polyphenolic compounds like tannins and flavonoids. These polyphenolic compounds have antioxidant property and anti-oxidants have known to possess hepatoprotective activity. Keeping the native knowledge and the above mentioned literature information[1], this plant was selected for present study to screen the leaves of this edible plant for the presence of category of phytoconstituents, antioxidant and hepatoprotective activities. This study was carried out by using 70% ethanolic extract of AG (AGEE) as hepatoproectant and Paracetamol as hepatotoxicant.

2. MATERIALS AND METHOD

Collection and identification of plant: The plant was collected from Kusnoor village (Gulbarga district), Karnataka in the month of March and was authenticated by Dr. Srinath Rao, chairman, P.G. Department of Studies and Research in Botany, Gulbarga University, Gulbarga, Karnataka. The plant was thoroughly cleaned to remove adherent soil and other impurities, the leaves were shade dried and made into a coarse powder by rubbing in the palms.

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Extraction

150 gms of shade dried leaf powder of AG was extracted in Soxhlet's apparatus using petroleum ether for defatting and then it was extracted with 70% ethanol. The solvent evaporated on a water bath at a low temperature (50°C) and finally the residue was obtained.

Materials used

Paracetamol (Esteem Pharmaceutical Pvt. Ltd. Agra), Silymarin (SD fine chemicals, Mumbai), Ready to use diagnostic kits (Aspen Labs Pvt. Ltd., Delhi-India) 70% ethanolic extract of AG. All chemicals and reagents used were of analytical grade.

Animals used

Wistar albino rats of either sex weighing between 150-200 gms were housed in polypropylene cages and were maintained at 27° ± 2°C with 12:12 hr, light/dark cycle. They were fed with commercial diet (VRK Nutritional Laboratory, Sangli) and water at libitum during the experiment. The study was permitted by Institutional Animal Ethical Committee (Reg. No. 342).

Evaluation of AGEE against Paracetamol induced hepatotoxicity:

Hepatoprotection offered by AGEE, the method reported by R.R. Chattopadhyay was followed[2].

In this biological screening technique, albino wistar rats were randomly assigned into 5 groups of 6 animals each as follows:

Gp-I: Animals (-ve control) were administered 1ml/kg p.o. of saline for 7 days.

Gp-II: Animals (+ve control) were administered 1ml/kg p.o. of saline for 7 days.

Gp-III: Animals were administered with silymarin 100 mg/kg p.o. for 7 days.

Gp-IV: Animals administered with AGEE 200 mg/kg p.o. for 7 days.

Gp-V: Animals administered with AGEE 400 mg/kg p.o. for 7 days.

On 5th day, 30 minutes after the administration of normal saline, 100 mg/kg silymarin, 200 mg/kg and 400 mg/kg of AGEE to Group- III, IV and V respectively. Paracetamol 2 gm/kg was given orally to Group- II, III, IV and V . After 48 hours of Paracetamol dosing, rats were sacrificed under mild ether anaesthesia and blood samples were withdrawn through carotid artery puncture and centrifuged immediately to get clear serum and they were subjected to various biochemical studies for evaluating the serum biochemical parameters by using Aspen diagnostic kits and liver was dissected out, the blood was blotted off, washed with saline and stored in 10% formalin and proceeded for histopathology to evaluate the details of hepatic architecture in each group microscopically.

Finally, the results were compiled, tabled and graphically represented.

Table. No.1 Effect of 70 % ethanolic extract of Amaranthus Gangeticus leaves on hepatic enzymes in Paracetamol induced hepatotoxicity

Treatment	Biochemical parameters Mean ± SEM						
	SGPT U/L	SGOT U/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl	Total Cholesterol mg/dl	HDL mg/dl	ALP IU/L
Negative control (1ml dist. Water p.o. + 1ml/ kg liquid paraffin s.c.)	63.0± 0.57	69.82±0.70	0.95± 0.006	0.26±0.001	114.7±0.76	8.25± 0.004	133.3±0.76
Paracetamol treated (positive control) (1ml dist. Water p.o 2gm/kg p.o.	208± 0.57	274.3±0.55	4.15± 0.006	0.99±0.001	187.5±0.61	4.40± 0.007	332.7±0.55

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Paracetamol + Silymarin (2gm/kg p.o. + 100mg/kg, p.o.)	78.6± 0.09***	102.2±0. 60***	1.15± 0.003***	0.31± 0.001***	114.8± 0.40***	7.13± 0.004***	147.0± 0.85***
Paracetamol + AGEE (2gm/kg p.o. + 200 mg/kg, p.o)	153.3± 0.76***	187.3±0. 57***	2.34± 0.005***	0.53± 0.001***	163.5± 0.42***	5.16± 0.004***	243.0± 1.06***
Paracetamol + AGEE (2gm/kg p.o. + 400 mg/kg, p.o)	79± 0.36***	112.0±0. 57***	1.37± 0.006***	0.34± 0.001***	126.5± 0.42***	6.76± 0.007***	160.8± 0.60***

Values are the mean ± S.E.M. of 6 rats/treatment. *** P<0.001 Significance compared to Paracetamol treatment

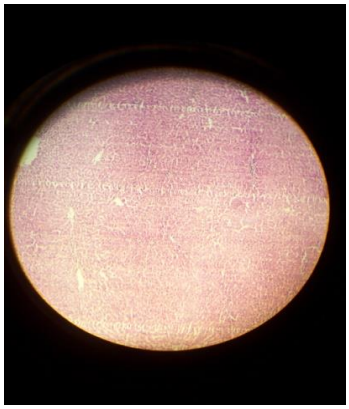
Table. No. 2 Percentage recovery of biochemical parameters in Paracetamol induced hepatotoxicity

Sl. No.	Groups	SGPT IU/L	SGOT IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl	Total Cholesterol mg/dl	HDL mg/dl	ALP IU/L	ALP IU/L
1.	PCM+ Silymarin (100 mg/kg.p.o)	62.21	62.74	72.23	68.69	38.77	86.42	55.82	56.26
2.	PCM + AGEE (200 mg/kg.p.o)	26.30	31.72	43.61	46.46	12.80	60.56	26.96	28.42
3.	PCM + AGEE (400 mg/kg.p.o)	62.02	59.17	66.99	65.66	32.53	81.94	51.67	52.64

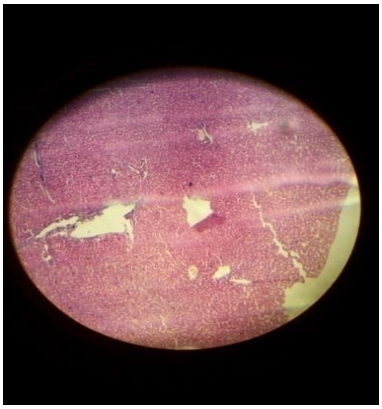
Table No. 3 Aggregate protection in Paracetamol induced hepatotoxicity

Sl. No.	Treatment	Percentage protection
1.	Paracetamol + Silymarin (100 mg/kg.p.o)	63.84
2.	Paracetamol + AGEE (200 mg/kg.p.o)	35.49
3.	Paracetamol + AGEE (400 mg/kg.p.o)	60.00

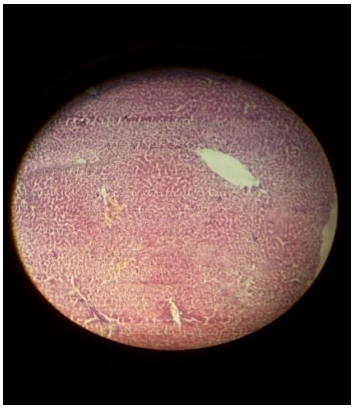
Fig. No. 1 Histopathological slides of Paracetamol induced hepatotoxicity model



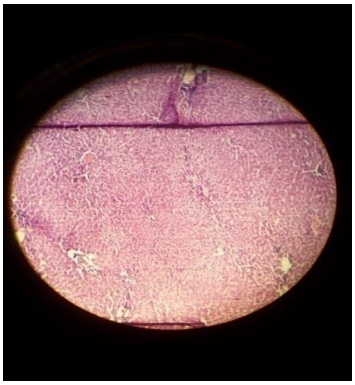
Normal Group



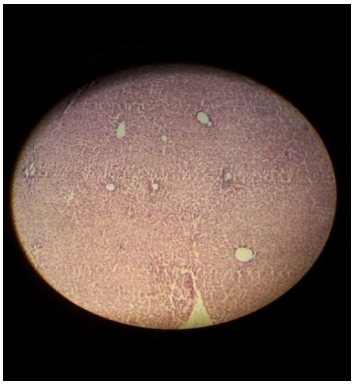
Paracetamol Treated (2gms/kg)



Silymarin Treated + PCM



200 mg/kg of AGEE + PCM



400 mg/kg of AGEE + PCM

3. RESULTS

Histopathological Studies in Paracetamol induced hepatotoxicity:

- **Group A:** In the case of normal control (-ve control) hepatic globular structure, central veins, portal tract and kuffer cells look normal.

Suggestive: **Normal liver**.

- **Group B:** In the case of paracetamol treated group (+ve control), hepatic globular architecture was normal, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes and proliferation of kuffer cells. Liver sinusoids were congested. Centri-lobular necrosis was observed.

Suggestive: **Fatty liver**.

- **Group C:** In the case of 100 mg/kg silymarin treated group the hepatic globular architecture was normal. There were occasional fatty cells and few cells have shown hyaline and cytoplasm. There were occasional areas of lymphocytic infiltration and kuffer cell proliferation.

Suggestive: **Regeneration liver**.

- **Group D:** In the case of 200 mg/kg 70% ethanolic extract of leaves of A***G*** treated group the hepatic globular architecture was normal. A few areas show lymphocytic infiltration. Majority of hepatocytes are normal.

Suggestive: **Light regeneration of hepatocytes.**

- **Group E:** In the case of 400 mg/kg 70% ethanolic extract of leaves of A***G*** treated group the hepatic
- architecture was maintained. Areas of kuffer cells proliferation and sinusoids appear to be normal.

Suggestive: **Regeneration of hepatocytes.**

Paracetamol has markedly increase in SGPT levels due to hepatocellular injury in unprotected group (208 IU/L). However, the SGPT levels were reversed to near normal levels (79 IU/L) with the treatment of 400 mg/kg of AGEE. Whereas, the standard Silymarin 100 mg/kg has restored the SGPT levels significantly i.e. 78.6 IU/L.

Serum SGOT levels were also elevated in the Paracetamol treated group (274.3 IU/L). Treatment with standard Silymarin 100 mg/kg has brought back the SGOT levels to the near normal levels i.e. 102.2 IU/L. Meanwhile treatment with the AGEE restored the SGOT levels in a dose dependent manner at both the doses (200 mg/kg and 400 mg/kg) to 187.3 and 112 IU/L respectively, which are statistically significant.

On treatment with Paracetamol, there was an increase in the total and direct bilirubin (4.15 and 0.99mg/dl respectively). Treatment with 400 mg/kg of AGEE could reverse the total and direct bilirubin serum levels to 1.37 mg/dl and 0.34 mg/dl respectively, which are statistically significant, when compared with Paracetamol treated group. The reversal by standard Silymarin 100 mg/kg was obviously significant i.e. 1.15 mg/dl (total bilirubin) and 0.31 mg/dl (direct bilirubin).

Serum total cholesterol levels were also elevated in the Paracetamol treated group (187.5 mg/dl). Treatment with standard Silymarin 100 mg/kg has brought back the total cholesterol levels to the near normal levels i.e.114.8 mg/dl. But, treatment with the AGEE restored the levels in a dose dependent manner at both the doses (200 mg/kg and 400 mg/kg) to 163.5 and 126.5 mg/dl respectively, which are statistically significant.

Serum HDL levels were reduced in the Paracetamol treated group (4.40 mg/dl). Treatment with standard Silymarin 100 mg/kg has brought back the HDL levels to the near normal levels i.e.7.13 mg/dl. However treatment with the AGEE restored the levels in a dose dependent manner at both the doses (200 mg/kg and 400 mg/kg) to 5.16 and 6.76 mg/dl respectively,which are statistically significant.

Similarly, rise in ALP serum levels due to Paracetamol challenge was remarkable (332.7 IU/L) and the same was brought back significantly by both doses of AGEE to near normal level i.e.243 IU/L and 160.8 IU/L respectively, which are statistically significant. As expected standard Silymarin 100 mg/kg responded well and restored the ALP levels to 147 IU/L. Various biochemical markers were significantly lowered by both extracts & Silymarin treatment. These results are summarized in Table no. 1 and shown in Fig. No. 1.

On the basis of the results presented in Table no. 1, an attempt was made to translate results into percentage protection (Table no. 2 & 3) in comparison with positive control. Later, these parameters of test extracts & standard were averaged to estimate aggregate protection offered by them. Wherein, Silymarin has shown 63.84% protection, followed by 60 % & 35.49% protection exhibited by 400mg/kg & 200mg/kg of AGEE respectively.

4. DISCUSSIONS

The model selected to asses organ protection for ethanol extract of AG was tested against Paracetamol induced hepatotoxicity in rats. The PCM treated group exhibited extensive fatty changes, congestion of sinsusoids, necrosis etc. upon histopathological observations. Treatment with PCM, showed serum levels of biochemical markers of hepatocellular damage like SGPT, SGOT, bilirubin (total and direct), ALP, cholesterol were increased as a mark of fatty change, congestion, inflammation etc.

The animals treated with AGEE for 7 days showed remarkable rejuvenation of hepatocellular architecture, the AGEE reversed the elevated serum markers of liver damage proportionate to the doses employed. Protection was offered by silymarin, (100mg/kg), 200mg/kg and 400mg/kg of AGEE respectively (compared to positive control w.r.t. biochemical parameters).

Paracetamol is usually safe at therapeutic doses but, produces centri-lobular or massive hepatic necrosis along with damage to subcellular organelle like mitochondria followed by congestion and failure at the dose of 2gm/kg (p.o). This happens due to exhaustion and non-availability of safer ways of eliminating Paracetamol such as sulphation and glucuronidation. As a result, a number of isoenzymes of CYP-450 namely CYP $2E_1$, CYP $1A_2$, CYP $2A_6$, CYP $3A_4$, CYP $2D_6$ are released and produce N-acetyl p-benzoquinine amine (NAPQI). This toxic electrophile donates an electron to surroundings, generating hydroxyl radical and ROS, meanwhile it gets itself converted to highly reactive semi quinone radical[3-5].

Paracetamol treatment (2gm/kg) causes centrilobular or massive hepatic necrosis along with damage to subcellular organelle resulting in the leakage of biochemical markers into the blood stream thereby the levels of biochemical markers in the serum was raised in the present study.[6]

The animals treated with AGEE dose showed praise-worthy reversal of all the elevated parameters indicating the prevention of liver damage. The animals pretreated with Silymarin and AGEE showed regeneration of hepatocytes. It is clear that the free radicals mediated assault made by NAPQI forms the central dogma of Paracetamol induced pathogenesis.[7]

5. CONCLUSION

AGEE has a powerful organ protection property and significantly it has a good *in-vitro* antioxidant properties which are attributed due to presence of antioxidant phyto-constituents like flavonoids, phytosterols and other polyphenolic constituents Therefore the above findings revels that the use of *Amaranthus gangeticus* leaves in our food protects our liver.

These findings adds strength to our claim.

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