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Organprotective and Antioxidant Activities of Amaranthus Retroflexus Leaves Against Paracetamol Induced Hepatic Damage in Albino Rats

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ABSTRACT

Amaranthus retroflexus is a well known medicinal plant. Our aim is to investigate its organ protection property. Aqueous extract of Amaranthus retroflexus leaves was taken and the parameters studied were serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, total bilirubin, direct bilirubin, total cholesterol, high density lipoproteins and alkaline phosphatase activities. Bio-chemical studies of blood samples of paracetamol treated animals showed significant increase in the levels of serum markers and decrease in high density lipoproteins reflecting the liver injury caused by paracetamol. Whereas, the animals treated with aqueous extract of Amaranthus retroflexus showed significant dose dependent decrease in the elevated levels of serum markers and increase in high density lipoproteins indicating the protection of hepatic cells. Results were analyzed by one-way analysis of variance by Dunnet's 't' test. Therefore aqueous extract afford significant protection against paracetamol induced hepatocellular injury and remarkable rejuvenation of these tissues found in histopathological studies which may be due to polyphenols and its antioxidant activities.

Keywords: Amaranthus retroflexus leaves, paracetamol, serum markers and polyphenols.

INTRODUCTION

Liver is one of the important organ in our body. It is vital 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, many of these hepatotoxic challenges overpower inbuilt protective mechanism and cause hepatotoxicity resulting in the hepatic necrosis and hepatitis.

Amaranthus retroflexus is a edible plant used as vegetable, it is also 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 phytoconstituents, antioxidant and hepatoprotective activities. This study was carried out by using aqueous extract of Amaranthus retroflexus (AR) as hepatoprotectant and paracetamol (PCM) as hepatotoxicant.

MATERIALS AND METHODS

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.

Extraction

150 gms of shade dried leaf powder of AR was extracted in Soxhlet's apparatus using petroleum ether for defatting and then it was extracted with

aqueous (ARAE). The solvent was 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), aqueouRAextract of AG, gallic acid (Rolex Chemical Industries, Mumbai). All chemicals and reagents used were of analytical grade.

Animals used

Wistar albino rats of either sex weighing between 160-180 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).

1. Evaluation of hepatoprotective activity in PCM-induced hepatotoxicity:

The method reported by R.R. Chattopadhyay was followed[2].

In the dose response experiment, albino wistar rats were randomly assigned into 5 groups of 6 animals in each.

Gp-I Animals (-ve control) were administered with 1ml/kg p.o. of saline for 7 days. Gp-II Animals (+ve control) were administered with 1ml/kg p.o. of saline for 7 days. Gp-III Animals were administered with silymarin 100mg/kg p.o., for 7 days.

Gp-IV Animals were administered with ARAE 200mg/kg p.o., for 7 days.

Gp-V Animals were administered with ARAE 400mg/kg p.o., for 7 days.

On 5th day, 30 minutes after the administration of normal saline, 100mg/kg silymarin, 200mg/kg and 400mg/kg of ARAE to Group-II, III, IV and V respectively, Paracetamol 2gm/kg was given orally. After 48 hours of paracetamol dosing, rats were sacrificed under mild ether anaesthesia and the blood samples were collected from the animals of each group through carotoid artery puncture and centrifuged immediately to get clear serum, for evaluating the serum biochemical parameters by using Aspen diagnostic kits. 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.

The results are shown in table-1.

2. Total Phenolic Content estimation[3]

Extraction of phenols from Amaranthus retroflexus leaves[4]

Method: In this process, 10 gms of shade dried, leaf powder of Amaranthus retroflexus (AR) is placed with the whole of the menstrum (250 ml) in a closed vessel for 2 days. During this period shaking is done occasionally. After 2 days, the liquid is strained and marc is pressed. The expressed liquid is mixed with strained liquid. It is then filtered to make a clear liquid. The final volume is not adjusted. It is evaporated on a water-bath at low temperature not exceeding (50°C) and preserved.

Plant extracts were prepared using two different extracting solvents :

AR_{CW} : Amaranthus retroflexus shade dried leaf powder macerated for 48 hrs with cold water (distilled water stored at room temperature).

AR_{HW} :AR shade dried leaf powder macerated for 48 hrs with hot water (50°C).

Total Phenolic Content

It was estimated by using Folin-Ciocalteau reagent according to the method reported by Singleton and Rossi[3] using standard curve generated with Gallic acid.

Procedure: For the preparation of Calibration curve, a series of calibrated 10 ml volumetric flasks were taken and appropriate aliquots of the working standard solution of gallic acid were pipetted out. To each flask, 5ml of Folin-Ciocalteu reagent (diluted ten fold) and 4ml of sodium carbonate solution (75g/l) was mixed with appropriate aliquots of gallic acid. The absorbance was measured after 30 min. at 765nm in Shimadzu 1700 UV-visible spectrophotometer.

Same procedure was applied for various extracts of Amaranthus retroflexus (1mg/ml) was mixed with the same reagent as described in the construction of calibration curve and after 30 min. the absorbance was measured for the determination of total phenolic compound. The total phenolic content was expressed as gallic actd equivalents (GAE) in milligram per gram of sample using a standard curve of gallic acid. Total amount of phenolic compounds in Amaranthus retroflexus were done in triplicates and concentrations of total phenolic content in various extracts were determined and expressed as gallic acid in mg equivalents per gm of sample using the standard curve generated with gallic acid.

The results obtained were compiled and represented as shown in table-2.

Statistical analysis

The data presented in table-1 (n=6) and 2 (n=3) were expressed as mean ± standard error of mean (SEM). Significant difference among the mean were calculated at the level of $p < 0.001$ and analyzed by one-way analysis of variance by Dunnet's 't' test. A value of $p < 0.05$ was defined as significant.

Table-1 Effect of aqueous extract of Amaranthus retroflexus leaves on hepatic enzymes in PCM induced hepatotoxicity

Treatment	Biochemical parameters Mean ± SEM						
	SGPT IU/L	SGOT IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl	Total Cholesterol mg/dl	HDL mg/dl	ALP IU/L
Negative control (1ml/ kg saline p.o.)	63.59 ±0.33	71.91 ±0.69	0.95 ±0.008	0.27 ±0.001	117.6 ±0.76	8.18 ±0.005	138 ±0.8

PCM treated (positive control) (1ml/kg saline p.o. + 2gms/kg p.o.)	206.5 ±0.57	270.55 ±0.58	4.12 ±0.006	0.90 ±0.005	195.6 ±0.71	4.35 ±0.007	339.5 ±0.75
Silymarin+ PCM (100mg/kg p.o.+ 2gms /kg p.o.)	75.92± 0.45***	105.1± 0.78***	1.16± 0.006***	0.32± 0.001***	115.8± 0.47***	7.09± 0.007***	148.5± 0.89***
ARAE + PCM (200mg/kg p.o. + 2gms /kg, p.o)	160.3± 0.76***	192.3± 0.57***	2.38± 0.005***	0.59± 0.001***	166.5± 0.42***	5.09± 0.004***	257.0± 1.06***
ARAE + PCM (400mg/kg p.o. + 2gms /kg, p.o)	88± 0.36***	130.0± 0.57***	1.44± 0.006***	0.45± 0.001***	143.5± 0.42***	6.73± 0.007***	179.8± 0.60***

Values are the mean ± S.E.M. of six rats/treatment.
P<0.001 Significance compared to PCM treatment.

Table-2 TOTAL PHENOL CONTENT IN AMARANTHUS RETROFLEXUS LEAF EXTRACT

Sl. No.	Name of the extract	Total phenol content mg/gms (in gallic acid equivalent)
1.	ARAE	225.484 ± 1.92
2.	AR_{CW}	108.097 ± 1.73
3.	AR_{HW}	160.070 ± 2.63

RESULTS

There is a marked increase in serum biomarkers which were observed in PCM treated group. But, these levels were reversed to near normal levels with the treatment of 200mg/kg and 400mg/kg of aqueous extract of AR. Whereas, the standard silymarin has restored the serum biomarker levels significantly which is depicted in table-1.

Results showed that, among the three different extracts of AR, AR_{CW} contained the lowest level of total phenol content, while ARAE contained the highest level. The observations are recorded in table-2.

DISCUSSIONS

The model selected to asses organ protection was the aqueous extract of AR which was tested against PCM induced hepatotoxicity in rats. The PCM treated group exhibited extensive fatty changes, congestion of sinsusoids, necrosis etc. upon histopathological observations. But treatment with AR, the 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 aqueous extract of AR for 7 days showed remarkable rejuvenation of hepatocellular architecture, the AR 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 AR respectively (compared to positive control w.r.t. biochemical parameters).

The hepatoprotection offered by AR may be attributed due to the presence of antioxidant phyto-constituents like flavonoids phytosterols and other polyphenolic constituents, by the virtue of which the extract nullified the powerful hepatotoxic radicals generated by PCM (i.e, hydroxyl radical and reactive oxygen species)[5-7] before they could initiate fatal consequences. These findings adds strength to our claim.

Total Phenolic Content of ARAE, AR_{CW} and AR_{HW} were determined. Total phenolic content was maximum in ARAE (225.484 mg/gms in terms of gallic acid equivalent).

CONCLUSIONS

Aqueous extract of AR has a powerful organ protection property and it also has a good in-vitro antioxidant properties which are attributed due to presence of antioxidant phyto-constituents. Therefore the above findings revels that the use of Amaranthus retroflexus leaves in our food protects our liver.

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