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Phytochemical screening and hepatoprotective activity of *Celosia argentea* Linn.

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ABSTRACT

Ethanol extract of *Celosia argentea* linn. (Amaranthacea) was evaluated for hepatoprotective activity against paracetamol induced liver injury in rat. The plant extract (ECA) at the dose of 250 & 500 mg/kg, p.o Showed a remarkable hepatoprotective activity against paracetamol induced hepatotoxicity as judged from the serum marker enzyme. Paracetamol induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase, bilirubin and decrease in total protein. Treatment of rat with ethanol extract (250&500mg) significantly (P<0.001) Altered Serum Marker Enzyme levels to near normal against paracetamol treated rats. The activity of the extract was comparable to the standard drug, silymarin (50mg/kg, p.o). Histopathological changes of liver sample were compared with respective control. Result indicates that celosia aregentea possesses hepatoprotective effect on paracetamol –induced hepatotoxic in rats.

KEY WORDS: *Celosia argentea*, paracetamol, ethanol, silymarin.

INTRODUCTION

Botanical name: *Celosia argentea* linn, Family: Amaranthacea. The plant has healing properties, the seed are useful in diarrhea, the seeds are bitter, aphrodisiac, reduce the inflammations, strength the liver, useful in gonorrhea, burnt leaves are stypice (Unani). The dried plant is considered antiscorbutic and cooling in chin. The seed have a reputation of clearing the vision and healing diseases of the eye .The scope of work is to carry out the preliminary phytochemical studies of the plant extract, to carry out the acute toxicity studies, to study the hepatoprotective activity of the plant extract against paracetamol induced hepatotoxicity.

MATERIALS METHODS

Method of extraction is Continuous hot percolation process by using Soxhlet apparatus.

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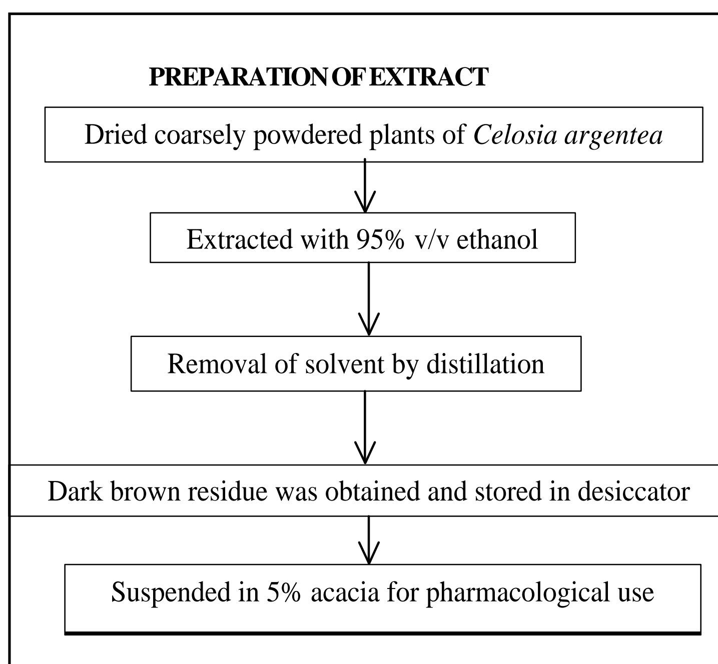
MATERIALS

Soxhlet apparatus, alcohol 95 % V/V, Shade dried coarse powder of *Celosia argentea*.



IDENTIFICATION OF PHYTOCHEMICAL CONSTITUENTS

TEST FOR CARBOHYDRATES AND GLYCOSIDES		
TEST	**OBSERVATION**	**INFERENCE**
Molisch test	The filtrate was treated with 2-3 drops of 1% alcoholic alpha napthol and 2ml of conc sulphuric acid was added along the sides of the test tube.	Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.
Fehling's test	The filtrate was treated with Fehling's A and B and heated on a water bath.	a)A reddish precipitate indicate the presence of carbohydrates b)Another portion of the extract were hydrolysed with dilute HCL for few hours on a water bath and the hydrolysate was subjected to the below tests.
Legal test	To the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside was added and then it was made alkaline with sodium hydroxide solution.	Appearance of pink to red colour indicate the presence of glycosides
Borntragers test	Hydrolysate was treated with chloroform and the chloroform layers were separated. To this equal volume of dilute ammonia solution was added.	Ammonium layer acquires pink colour indicate the presence of glycosides.
DETECTION OF FIXED OILS AND FATS		
Filter paper test	Small quantities of various extract were pressed separately between the filter papers	Appearance of oil stain on the paper indicate the presence of fixed oil
Saponification test	Few drops of 0.5 N alcoholic potassium hydroxide was added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours.	Formation of soap indicate the presence of fixed oil and fat
DETECTION OF PROTEINS AND FREE AMINOACIDS		
Millons test	The above prepared extract was treated with millons reagent	Red colour was formed shows the presence of proteins and free amino acids
Biuret test	To the above prepared extract equal volume of 5% sodium hydroxide and 1%copper sulphate solution were added.	Violet colour was produced shows the presence of proteins and free amino acids
Ninhydrin test	The extracts were treated with ninhydrin reagent	Purple colour was produced shows the presence of proteins and free amino acids

DETECTION OF SAPONINS:

The extract was diluted with 20ml of distilled water and it was agitated in a measuring cylinder for 15minutes. The formation of 1 cm layer of foam shows the presence of saponins.

DETECTION OF TANNINS AND PHENOLIC COMPOUNDS

Small quantity of various extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagent

1) 5% Ferric chloride solution - violet colour
2) 1% solution of gelatin containing 10% sodium chloride – white precipitate
3) 10% lead acetate solution – white precipitate

Above findings show the presence of phenolic compounds and tannins

DETECTION OF ALKALOIDS

Small quantity of various extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests

1) Mayer's reagent - cream precipitate
2) Dragendroffs reagent - orange brown precipitate
3) Hagers reagent - yellow precipitate
4) Wagner's reagent - reddish brown precipitate

DETECTION OF PHYTOSTEROLS AND TERPENOIDS		
Salkowski test	To 1ml of prepared chloroform solution ,a few drops of conc sulphuric acid was added	Brown colour was produced shows the presence of phytosterols
Libermann Burchard test	The above prepared chloroform solution was treated with few drops of concentrated sulphuric acid followed by few drops of dilute acetic acid,3ml of acetic anhydride	A bluish green colour was appeared indicate the presence of phytosterols

DETECTION OF GUMS AND MUCILAGES

A small quantity of various extracts were added separately to 25ml of obsolete alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicate the absence of mucilage and gum.

DETECTION OF FLAVONOIDS:

1. Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow indicate the presence of flavonoids.appearence of yellow colour indicate the presence of flavonoids.
2. To the small portion of each extract, concentrated sulphuric acid was added. Yellow orange colour was obtained shows the presence of flavonoids .

3. **Shinoda test:**

Small quantities of the extract were dissolved in alcohol. To those pieces of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

PHARMACOLOGICAL STUIDES

ANIMALS:

Albino male Wister rats weighing 120-160g were purchased from the animal house of St. John's animal house, St. John's College of pharmacy, Bangalore, India. They were kept in departmental animal house in a well-ventilated room at 27±2 degree and 44-56% relative humidity, light and dark cycles of 10 and 14 hours respectively for 1 week before and during the experiment. Animals were provided with the standard rodent pellet diet and the food was withdrawn 18-24hours before the experiment though water was allowed ad libitum .All the experiments were performed in the morning according to the current guidelines for the care of laboratory animals and the study was conducted after obtaining institutional animal ethical committee clearance.

GENERAL BEHAVIOUR AND ACUTE TOXICITY STUDY:

Acute oral toxicity (AOT) of ECA was determined using Swiss albino mice. The animals were fasted for 3 hours. Prior to the experiment and were administered with single dose of extracts dissolved in 5% gum acacia (dose ranges from 500-2000 mg kg^{-1} at various dose levels) and observed for mortality up to 48hours (short term toxicity).based on the short term toxicity the dose of next animal was determined as per OECD guidelines 425. All the animals were observed for long term toxicity (14 days). The LD50 of the test extract was calculated using 425 software provided by the environmental protection agency, USA.

HEPATOPROTECTIVE STUDIES

PARACETMOL INDUCED HEPATOTOXICITY:

Paracetmol is a common anti-pyretic agent, with those safe in therapeutic doses causes fatal hepatic necrosis in man, rat and mice. Rats were divided in to four groups, each group consists of six animals. Group I served as control receives normal saline (2mg/kg).group II receives paracetamol (1000mg/kg p.o) at every 72hours for 10 days. Group III receives ECA 250 mg/kg p.o for 10 days and simultaneously administered paracetmol 1000 mg/kg body wt every 72 hours. Group IV received ECA 500 mg/kg p.o for 10 days and simultaneously administered paracetmol 1000 mg/kg body weight every 72 hours and group V receives silymarin 50 mg/kg p.o and simultaneously administered paracetamol 1000mg/kg body wt. every 72hours.

At the end of experimental period the animals were starved overnight and then sacrificed by cervical decapitation. Blood samples were collected. Serum was separated by centrifuging at 2500 rpm for 15 min and analysed for various biochemical parameters.

ASSESSMENT OF LIVER FUNCTIONS:

Biochemical parameters i.e aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total bilirubin and total protein were analysed according to the reported methods. The liver was removed, weighed and morphological changes were observed. A portion of liver was fixed in 10% formalin for histopathological studies.

HISTOPATHOLOGICAL STUDIES:

After draining the blood liver samples were excised, washed with normal saline and processed separately for histopathological observations. Initially the materials were fixed in 10% buffered neutral formalin solution for 48 hours and then in bovine solution for 6 hours. Paraffin sections were taken at 5mm thickness processed in alcohol-xylene series and was stained with alum haematoxylin and eosin. The sections were examined microscopically for histopathological changes.

STASTICAL ANALYSIS:

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Turkey multiple comparison test and data on liver weight variations were analysed using one way ANOVA followed by Dennett test P values less than 0.05 were considered as significant

RESULTS AND DISCUSSION

Based on ethno pharmacological literatures, the plant celosia argentea was selected and the project work was carried out. The phytoconstituents was extracted by using alcohol by continuous hot percolation method. The extractive value was found to be 18.7g, 7.48% w/w. The phytoconstituents were identified by chemical test which shows the presence of various phytoconstituents such viz.alkaloids, glycosides, polyphenols, tannins, flavonoids, phytosterols saponins and terpenoids (Table 1).

Table 1. Preliminary Phytochemical Screening of Ethanol & Extract of *Celosia Argentea*

Sl.No	Main Active constituent	Present or Absent
1	Carbohydrates	present
2	proteins	absent
3	Alkaloids	present
4	Glycosides	present
5	Phytosterols	present
6	Terpenoids	present
7	Flavonoids	present
8	Saponins	present
9	Tannins and phenolic compounds	present
10	Fixed oils and Fats	present

The extract treated animals were observed for mortality up to 48hours (short term toxicity) and for long term toxicity (14days) based on the result the extract did not produce toxicity up to 2000mg/kg, to optimize the dose levels 250,500mg/kg of ECA were selected for determining the hepatoprotective activity. The effects of the ethanol extract of celosia argentea on paracetmol intoxicated rats are shown in table 2.

Table.2. Effect of ethanol extract of *Celosia argentea* on biochemical parameters in paracetmol induced hepatotoxicity in rats

Design of treatment	Dose (mg/kg)	AST U/L	ALT U/L	ALP U/L	Total bilirubin mg %	Total protein mg%
Normal saline	2ml/kg	62.5±1.34	48.25±1.78	196.5±2.45	0.8±0.05	8.13±1.4
Paracetmol	750	142.2± 2.67[a]	85.8±1.63[a]	378.10±5.48[a]	1.1±0.08[b]	6.35±0.35[b]
ECA	250	113.6±2.85[a,c]	65.8±1.83[a,c]	267.50±3.78[a,c]	0.95±0.06[d]	7.43±0.28
ECA	500	98.5±3.27[a,c]	52.6±1.34 [c]	218.10±2.85[b,c]	0.86±0.04	7.85±0.15[d]
Silymarin	50	88.75±1.14[a,d]	58.35±1.62[a,d]	192±2.93[b]	0.91±0.02[a,d]	8.1±0.36[b]

N=6, [a]p<0.001;[b]p<0.01 vs Normal, [c]p<0.001;[d]p<0.05 vs acetaminophen, Data was analysed by one way ANOVA followed by Turkey multiple comparison test

Intoxication of rats treated with paracetmol significantly (P<0.001 &0.01) increased the levels of AST, ALT, ALP, total bilirubin and total protein serum. The treatment with the ECA at 250 and500 mg/kg body weight reduced the level of AST,ALT, ALP, total bilirubin and total protein serum. The results produced by the tested doses were comparable with the standard drug silymarin (P<0.001 &0.0). The effect of ethanol extract of celosia argentea on liver weight variation of paracetmol induced hepatotoxicity in rats were shown in table 3.

Table.3. Effect of ethanol extract of *Celosia argentea* on liver weight variation of paracetmol induced hepatotoxicity in rats.

Design of treatment	Dose (mg/kg)	Liver wt. per 100g body weight (g)
Normal(normal saline)	2ml/kg	4.5±0.04
paracetmol	750	6.27±0.26*
ECA	250	5.65±0.12*
ECA	500	5.04±0.10
Silymarin	50	4.73±0.23

N=6, *p<0.01 vs Normal, Data was analysed by one way ANOVA followed by Dunnett test.

The liver weight of paracetamol intoxicated rats was significantly high when compared to normal. But the treatments with ECA at the dose of 500mg/kg level reduces the weight of liver and bring back to almost normal. The result produced by the extract is comparable with the group treated with standard silymarin. Both silymarin and plant extract decreased paracetmol induced elevated enzyme levels in the tested groups, indicating the protection of structural integrity of hepatocytic cell membrane.

Possible mechanisms that may be responsible for protection against paracetmol induced hepatocellular damage by celosia argentea extract include the following mechanisms.

1. *Celosia argentea* extract by itself could act as a free radical scavenger intercepting those radicals involved in the paracetmol metabolism by microsomal enzymes. Thus by trapping oxygen related radicals argentea could hinder their interaction with poly unsaturated fatty acids and abolish the enhancement of lipid per oxidative processes.
2. Treatment with ethanol extract of argentea exhibited a novel effect on the glutathione of the liver cells. These results suggest that higher levels of glutathione levels in the liver would afford the tissues a better protection against oxidative stress thus contributing to the abolishment of hepatotoxicity induced by different hepatotoxins.

The preliminary chemical examination of ethanol extract of *Celosia argentea* shows the presence of terpenoids and flavonoids. The terpenes may offer hepatocellular protection at least in part, through its diminution of oxidative stress inhibition of cytochrome p-450 and by bolstering the levels of antioxidants and antioxidant enzyme system. The mode of action shown by celosia argentea also predicts that hepatocellular protection may be due to the cell membrane stabilization, hepatic cell regeneration and activation of anti-oxidative enzyme systems.

The histological observations also basically support the results obtained from serum enzyme assays mechanisms by which paracetmol produces liver injuries must rely on the cytochrome P-450 system to produce reactive metabolites-acetyl-P-benzo-quinoneimine.Therefore the possible hepatoprotective mechanisms of ECA may be due to preventing the process of liquid peroxidation, inhibiting the cytochrome P-450 activity and stabilizing the hepatocellular membrane. There are reports on the hepatoprotective activity of triterpenes and flavonoids though it can't be concluded with the certainty, it seems possible that the better activity of ECA may be due to triterpenes and flavonoids present therein.

All these data point to possibly developing the ethanol extract of Celosia argentea as a novel and potential in the management of liver disorders.

SUMMARY AND CONCLUSION

The phytochemical and pharmacological study on *Celosia argentea* was done. The phytochemical constituents were extracted by ethanol by continuous hot percolation process. The phytochemical constituents were identified by chemical tests and tests shows the presence of various phytochemical constituents like carbohydrates, glycosides, alkaloids, tannins, saponins, phenolic compounds, phytosterols and flavonoids.

The results of the pharmacological studies showed that ethanol extract of *Celosia argentea* has protective activity on paracetamol induced hepatotoxicity in dose dependent manner the results obtained were supported by the histological findings, the results were also comparable with the standard silymarin.

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