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Original Article

COMPARISON BETWEEN ETHANOLIC EXTRACTS OF THE PLANT MORINGA PTERYGOSPERMA, AGAINST CARBON TETRACHLORIDE INDUCED HEPATOPATHY

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ABSTRACT

Object: Comparison between protective effects of the ethanolic extracts of the plant Moringa pterigosperma against CCl_4 induced hepatic failure in male albino rats was investigated. **Materials and methods:** For acute and massive invasion of hepatopathy, CCl_4 (i.p injection of CCl_4+Olive Oil in 1:1 ratio; 2ml/kg) was used and the insidious intoxication was evidenced by evaluation of various biochemical parameters followed by significant weight loss in toxic control group. The administration of ethanolic extracts (250mg/kg and 150mg/kg of body weight) for 7 days, elicited protective action since the elevated levels of marker enzymes (AST, ALT, ALP) of liver functions were found to be decreasing progressively in a dose dependent manner with net weight gain. **Results:** In the ethanolic extract 250mg/kg treated rat group all the marker enzymes were analyzed to be decreasing significantly and the final body weight was also significantly increased when compared with the toxic control group. The serum total protein and the serum albumin were also approaching normal values. The results found in ethanolic extract 250mg/kg treated rat were quite promising and were comparable with a standard polyherbal drug Liv-52. **Conclusion:** The statistically processed results support the conclusion, that the ethanolic extracts of Moringa pterygosperma whole plant (250mg/kg and 150mg/kg) possesses dose dependent, significant protective activity against CCl_4 induced hepatotoxicity.

Keywords: Hepatotoxicity, marker enzymes, Asparate transaminase, Alanine aminotransferase, Alkaline Phosphatase.

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1.0 INTRODUCTION [1] [2][3] [4][5][6]

The aim of the present context is to evaluate the hepatoprotective potential of the ethanolic extract of Moringa pterygosperma whole plant in a conventional animal model of hepatopatotoxicity.

Moringa pterygosperma belongs to a onogeneric family of shrubs and tree, Moringaceae and is considered to have its origin in Agra and Oudh, in the northwest region of India, south of the Himalayan Mountains. They are an exceptionally good source of provitamin A, vitamins B, and C, minerals (in particular iron), and the sulphur-containing amino acids methionine and cystine. The seed oil of Moringa contains approximately 13 % saturated fatty acids and 82 % unsaturated fatty acids. It has a particularly high level of oleic acid (70 %). Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas, and that the protein quality of Moringa leaves rivals that of milk and eggs. The seeds contain 19 to 47 percent oil Known commercially as “ben oil”, it is similar to olive oil and is rich in palmetic, stearic, behmic, and oleic acids, and are used for human consumption, and in cosmetics and soaps. In traditional Indian medicine various parts of the tree are used therapeutically, including for treatment of ascites, rheumatism, venomous bites, and as cardiac, anti diabetic and circulatory stimulants. The root of young trees and also the root bark are considered rubefacient, vescicant carminative, stomachic, and abortifacient; among other uses, they are commonly applied externally to cure inflammatory swellings. The flowers and roots contain pterogospermin, an antibiotic that is highly effective in the treatment of cholera. The flowers are also used as a tonic and diuretic. The leaves, rich in vitamin A and C, are considered useful in scurvy and respiratory ailments; they are also used as an emetic. The juice extracted from the leaves has strong antibacterial and antimalarial properties a paste of the leaves is used as an external application to promote healing of wounds. The seed oil is applied externally to treat rheumatism and gout.

CCl_4 is a widely used industrial chemical and a potent hepatotoxin. It induces hepatotoxicity by producing free radical, putting oxidative stress hence causing lipid peroxidation in liver tissues, consequently necrotic liver damage [7].

2.0 MATERIALS AND METHODS

2.1 Plant material

The whole part of plant Moringa pterygosperma plant was collected from young matured plant from the rural belt of Balasore, Orissa during the month of Jan-Feb and identified by the botanist of Department of Botany, Utkal University, Bhubaneswar by comparing with the voucher specimen present in the herbarium. After

authentification fresh plant materials were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The coarse powder was used for further studies.

Animals

Healthy albino male rats of Wistar strain weighing between 150-200 g were selected for the investigation (Brought from Ghosh Enterprises, Kolkata, India). The animals were kept under maintained uniform laboratory conditions (12 ± 1 hr, day and night Schedule; temperature maintained between 11-20^0 ± 2^{0}C; housed in large spacious hygienic cages) before a week of the experiment for acclimatization. The approval of the protocol by I.A.E.C/U.D.P.S/990/2005 -Vanivihar, Bhubaneswar. Following C.P.C.S.E.A guideline.

2.2 Chemicals

Chemicals used in the study were of analytical grade and were procured from Merck specialties private limited, Mumbai, India. All biochemical assay kits were purchased from Ecoline: Merck specialties private limited, Mumbai, India.

2.3 Extraction of plant material and preparation of test dose

About 200 gm of coarse dried powder of whole plant of the Moringa pterygosperma was taken in the soxhlet apparatus and extracted successively [8] using the selected solvents in order (i.e. Pet. ether→Chloroform→Ethanol). The extraction for each solvent was carried out for 18 to 20 hours. The extract was collected by evaporating the solvents by slow heat treatment. Total 1.4kg of pulverized stem was subjected under solvent extraction to produce the required amount of test extract.

Calculated amount of dried ethanolic extract was suspended in 0.5% w/v of sodium-CMC in normal saline solution to get the test doses (200mg/kg and 400mg/kg per ml.). The dose limits were selected on the basis of previously performed oral acute toxicity studies in mice, in accordance with the OECD guidelines. [9]

2.4 Acute toxicity studies:

In the acute toxicity test carried out in mice we take six doses and 10 mice in each dose of both aqueous and ethanolic extract i.e. 500, 1000, 1500, 2000, 2500, 3000 mg/kg body weight. All groups of test drug showed neither any toxic effect nor any lethal effect in the dose range of 500 to 3000 mg/kg body weight. In 3000 mg/kg of ethanolic extract showed altered behavior of some mice had been observed. So we had taken a minimum and maximum dose 150mg/kg and 250 mg/kg of body weight for ethanolic extract for further screenings.

2.5 Hepatoprotective activity study:

30 rats were divided randomly into 5 groups, each comprising 6 animals. Group I (Normal Control) received oral dose of 0.5% Sodium CMC (1 ml each) for 7 days. Group II (Toxic Control) received single dose of CCl_4 (CCl_4 + Olive oil in 1:1 ratio; 2ml/kg of body wt; i.p.) on day 1 and day 7 of the experiment [10] . Group III, IV and V received standard polyherbal

drug 'Liv-52' (5 ml/kg; p.o.) [11](Liv-52 Syrup, Himalaya Drug Company., Bangalore, India), ethanolic extract 250 mg/kg of body wt. and 150mg/kg of body wt. once in a day for 7 days respectively, along with the i.p dose of CCl_4 on day 1 and day 7 as mentioned above. The treatment duration was of 7 days. On the 8th day of the study the animals were sacrificed under anesthesia and blood samples were collected from each animal to produce the serum for biochemical assay. The biochemical investigations were performed by using a Biochemical semi auto analyzer (EBRA-Chem-5 Plus. V2., West-Germany). The biochemical parameters considered were: Serum AST (SGOT) i.e. Asparate transaminase, ALT (SGPT) i.e. Alanine aminotransferase, ALP (alkaline phosphatase), Serum albumin and total protein.[12][13][14]

2.6 Statistical Analysis.

Results of all the estimations done were indicated in terms of Mean ± SEM. Statistical significance of data were assessed by analysis of variance (One Way- ANOVA), followed by comparison between different groups using 'Tukey-Kramer' multiple comparison test. The significance was set at the level of $p<0.05$. The toxic control group was compared with the normal control group and all other treatment groups were compared with the toxic control group.

3.0 RESULT AND DISCUSSION

The biochemical assays of hepatoprotective study were performed on 8th day. The results were presented in Table 1. (Change in body weight; Serum albumin and total protein) and Table 2. (Assay of marker enzymes).

Table 1: Change in body weight, Serum albumin, total protein and assay of Marker Enzymes on 8th day.

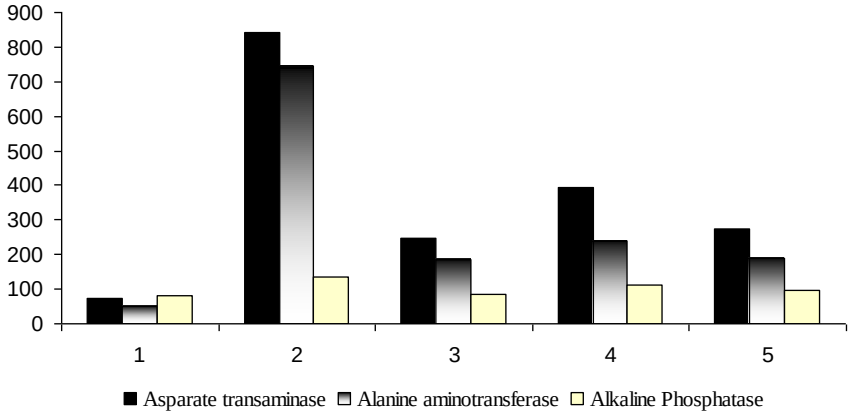
Group.	Change in Body wt. (gm)	Albumin. (gm/dl)	Total protein. (gm/dl)	AST. (IU/l)	ALT. (IU/l)	ALP. (IU/l)
Normal Control.	10.66±1.28	2.83±0.17	5.70±0.35	75.04±7.23	49.21±4.90	80.30±3.58
Toxic Control.	-12.83±1.13 ^{c}	1.40±0.10 ^{c}	3.11±0.16 ^{c}	840.37±28.26 ^{c}	746.75±18.94 ^{c}	133.65±3.47 ^{c}
Standard.	6.83±0.70^{c}	2.71±0.27 ^{c}	5.09±0.20 ^{c}	246.23±16.93 ^{c}	186.38±14.60 ^{c}	86.49±7.17 ^{c}
$EEMP_{150}$ Group.	3.33±1.90 ^{c}	2.55±0.16 ^{b}	4.70±0.36 ^{b}	393.08±12.29 ^{c}	241.07±11.73 ^{c}	112.58±6.82
$EEMP_{250}$ Group.	6.16±1.01 ^{c}	2.61±0.18 ^{c}	4.96±0.31 ^{b}	272.77±24.08 ^{c}	189.15±7.16 ^{c}	97.15±6.54 ^{b}

a - $p<0.05$, b - $p<0.01$, c - $p<0.001$. For n=6. Toxic control group was compared with Normal control group and all the other groups were compared with toxic control group.

- EEMP= Ethanolic extract of the plant Moringa pterygosperma
- AST = Asparate transaminase.
- ALT = Alanine aminotransferase.
- ALP = Alkaline Phosphatase.

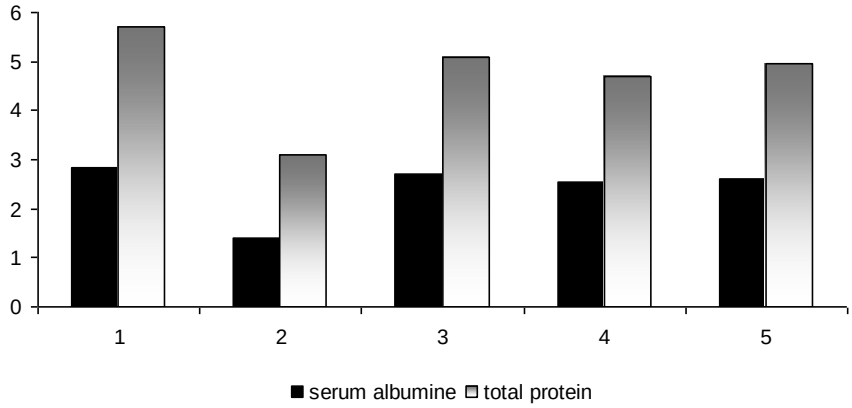
Histogram no.1:

Graphical represnation of the Assay of Marker Enzymes on 8th day



Histogram no.2:

comparison of serum albumine and total protein of different group in CCl4 induced hepatopathy on 8th day



- 1 = Normal control group
- 2 = Toxic control group
- 3 = standard group
- 4 = Ethanolic extract of the plant Moringa pterygosperma 150 mg/kg treated rats
- 5 = Ethanolic extract of the plant Moringa pterygosperma 250 mg/kg treated rats

A significant elevation in serum AST, serum ALT, serum ALP levels and significant decrease in serum Total Protein and serum albumin level followed by significant weight loss were found in Toxic control group, when compared with the Normal control group.

In the $EEMP_{150}$ group (ethanolic extract of the plant 150mg/kg treated rat group), the final body wt. was observed to be elevated as the change in body wt. which was found to be significant. Both the serum AST and the serum ALT were found to be significantly low when compared with the

Toxic control group. But the serum ALP level was not significantly decreased. The serum albumin and serum total protein were likely to be approaching normal values and found significant 1, when compared with that of Toxic control group.

The EEMP $_{250}$ group (ethanolic extract of the plant 250mg/kg treated rat group), the final body wt. was significantly elevated, when compared with the Toxic control group. The serum total protein and the serum albumin were almost approaching normal values and comparable with the results observed in Standard group. Serum AST and serum ALT were found to be significantly lower and serum ALP level was also significantly decreased (when compared with the Toxic control group), approaching almost normal values as that of observed in case of Standard group, supports the restoration of normal hepatic functions.

The above results suggest that ethanolic extract treated rats (at a dose level of 250mg/kg and 150mg/kg treated rats) had gained normalcy against the hepatocellular injury caused by CCl_4 during the 7 day treatment period and both dose levels were found almost equipotent. The result of these investigations was comparable and matches the previously reported protective effects of other plants. [15][16]

4.0 CONCLUSION

In the present pharmacological evaluation the whole plant extracts (ethanolic) of Moringa pterygosperma plant was extensively investigated for its Hepatoprotective potential against substance (CCl_4) induced hepatopathy. At the end of our study, a strong conclusion can be drawn that, the ethanolic extract of Moringa pterygosperma possess Hepatoprotective activities more or less depending on the dose levels. The ethanolic extract of the plant at a dose level of 250mg/kg exhibited competent, potent and comparable results where as ethanolic extract at a dose level of 150mg/kg, observed to have moderate level of efficacy, promoting Moringa pterygosperma plant as a promising Hepatoprotective plant species, seeking vast multidimensional future research work up to the molecular level to establish new up-to-date scientific data about this plant species and to elucidate its exact mechanism of protective effect. Future studies may be aimed at hepatoprotective study in other chronic models of Hepatopathies, antioxidant and free radical scavenging potentials, toxicological studies and other pharmacological activities as well.

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