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IRON OVER LOAD INDUCED LIVER TOXICITY OF AQUEOUS LEAF EXTRACT OF *CARICA PAPAYA* IN RATS

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ABSTRACT

The leaf extract of *Carica papaya* have been widely used in Ayurveda to treat a variety of common disorders like neurodegenerative diseases, cardio vascular diseases, cancer. In the present investigation, the aqueous leaf extract of *C.papaya* was evaluated for antioxidant activity in iron over load induced liver toxicity. For the evaluation of iron over load liver toxicity method was daily treatment of *C.papaya* extract on the 5th day iron load was induced. The animals were sacrificed by light ether anesthesia and it was dissected .Tissue homogenate and blood samples were assess the SGOT, ALP, and LPO, Reduced glutathione, Protein & Catalase in normal and iron over load groups. The daily administration of aqueous leaf extract of *C.papaya* at doses of 50 mg/kg, 100 mg/kg and 200 mg/kg body weight to iron over load liver toxicity methods were significantly reduced in a dose dependent manner. However, no change observed in the normal animals at all the doses studied. The extract also produced significant reduction of hydroxyl radicals in comparison to ascorbic acid in a dose dependent manner. The result have shown that the aqueous extract of the Leaf of *Carica papaya* possesses antioxidant properties, supported by the decrease in lipid peroxidation and increase in the reduced glutathione and catalase activity in *in-vivo* method. Such antioxidant activity of *Carica papaya* might be responsible for its importance in traditional medicine for the treatment of various disorders.

Keywords: *Carica papaya*, Free radical scavenging activity, Hepatoprotective activity, Ferrous sulphate.

INTRODUCTION

In general up on entry of any foreign particle enters in to our body. Free radicals can be generated, which is based on the size of the foreign particle or physico chemical characteristics of that foreign particle [(1)].

The free radicals are not inactivated; their chemical reactivity can damage all cellular macromolecules including proteins, carbohydrates, lipids and nucleic acids. Their destructive effect on protein may play a role in the causation of diseases, like cataracts. Free radical damage to DNA is also implicated in the causation of cancer and its effect on LDL cholesterol is very likely responsible for heart disease. Free radicals are also responsible for ageing [(2)].

Iron over load causes liver toxicity by producing hydroxyl radicals by promoting Fenton reaction[(3)]. Free radicals also can be able to cause various diseases like neurodegenerative diseases [(4)], cardio vascular diseases [(5)], cancer etc. So the work started because of these serious effects up on the free radical generation, expecting that Herbal medicines have been used able to generate antioxidants, which can be provide balance between free radical generation and anti-oxidant levels or those anti-oxidants prevents the liver damage and gastric ulceration caused by free radicals.

METHODS

Collection of extract and drug preparation

The leaves of *C. papaya* were collected during December and January 2010 from Thimmapur, Karimnagar district, Andhra pradesh. The leaves were cut in to small pieces and dried at room temperature for 15 days, finely fine powdered and

used for extraction. The powdered material was extracted with distilled water using soxhlet apparatus. The extract was concentrated *in vacuo* and kept in vacuum dessicator for complete removal of solvent. The yield was 14% w/w with respect to dried powder. The qualitative analysis of the aqueous leaf extract showed the presence of alkaloids, citric acid, maleic acid and Vit-C. The dried extract thus obtained was used directly for the assessment of antioxidant activity through various methods [(6)].

Chemicals used

5, 5' dithiobis (2-Nitrobenzoic acid) and 1, 1, 3, 3-Tetra ethaoxy propane were purchased from Sigma Aldrich, Thiobarbituric acid was purchased from Central Drug House, Newdelhi. Acetic acid was purchased from Molechem, Mumbai. Ascorbic acid was purchased from Finar chemicals Ltd, Ahmedabad, While Ferrous sulphate was purchased from Central Drug House, Newdelhi.

Animals

Albino rats of either sex weighing between 150-220 g were obtained from Mahaveer Enterprises Ltd, HYD and kept in polypropylene cages. Standard condition of humidity, temperature and light (12 h-light/dark cycles) are maintained. They were given a week time to get acclimatized with the laboratory conditions. Rats were fed with standard pellet diet and given water *ad libitium*.

Iron over load liver toxicity model [(3)]

Animals were divided in to eight groups of five rats each. Group one was served as normal control and was given in distilled water. Group two was served as iron over load group given only ferrous sulphate (30mg/kg) [(7)]. Group three, four and five was treated with 200mg/kg 100mg/kg and 50mg/kg of *Carica papaya* with iron over load that is 30mg/kg of ferrous sulphate. Group six was given 50 mg/kg of Ascorbic acid and Iron over load of 30 mg/kg of ferrous sulphate. Iron over load causes liver toxicity by producing hydroxyl radicals by promoting Fenton reaction [(3)]. The aqueous leaf extract of *Carica papaya* was given for five days and on the fifth day ferrous sulphate (30 mg/kg) was given one hour prior to scarification. On the 5^{th} day blood samples were collected and to separate the plasma was used for the estimation of SGOT, ALP and Total protein. The animals were sacrificed by light ether anesthesia and it was dissected. The liver was removed, weighed and kept in normal saline. Then it was homogenized in 8 ml. of ice-cold 0.9 % saline for 5 minutes using glass homogenizer. The homogenate was then centrifuge at 8000rpm for 10 minutes at 4^0. The homogenate was used for the estimation of protein [(8)], LPO [(9)], Catalase [(10)] and reduced glutathione.

Statistical Analysis [(11)]

The observed data were statistically analyzed for the significance using student *t*-test for unpaired data. For stress induced gastric ulcer model all the data were compared with the stress induced group except the normal control group. In iron induced liver toxicity model also all the group except normal control and the group without iron overload were compare with the iron overload group. The P-value of $P<0.05$ was considered as the level of significance.

RESULTS

The different drug treated groups were statistically analyzed for their significance using student *t*-test for unpaired data with respect to the stress induced group in ulcer model and iron over load group in liver toxicity model. The results were given in below. Effect of different treatment groups on SGOT, ALP values all P values were considered as highly significant ($P < 0.01$) and also Total protein values also same except *C.papaya* 100mg/kg group were Non-significant because the P values >0.05.

.As shown in figure there was a considerable increase in lipid peroxidation in iron over load group in liver toxicity model as compared to normal control.

And there is a considerable decrease in lipid peroxidation in a dose dependant manner with the aqueous extract of leaf of *Carica papaya*, which was significant at $p<0.05$. At dose 200mg/kg of aqueous extract of leaf of *Carica papaya* there was very little change in the lipid peroxidation. The effect were also comparable to ascorbic acid which was taken as standard. Glutathione levels in the stress induced group in iron over load group in liver toxicity model was significantly decreased in rats ($P<0.001$) compared with normal control. Treatment with aqueous extract of leaf of *Carica papaya* at a dose of 50mg/kg, 100mg/kg & 200mg/kg had notable effect on increased glutathione levels, the P values ($P < 0.001$) were considered highly significant dose dependent manner.

Total protein levels in the iron over load group in liver toxicity model was significantly decreased in rats ($P<0.001$) compared with normal control. Treatment with aqueous extract of leaf of *Carica papaya* at a dose of 50mg/kg, 100mg/kg & 200mg/kg had notable effect on increased Total protein levels, the P values ($P < 0.001$) were considered highly significant dose dependent manner.

CAT levels in the stress induced group in ulcer model and iron over load group in liver toxicity model was significantly decreased in rats (P<0.001) compared with normal control. Treatment with aqueous extract of leaf of *Carica papaya* at a dose of 50mg/kg, 100mg/kg & 200mg/kg had notable effect on increased CAT levels, the P values (P < 0.001) were considered highly significant dose dependent manner.

Histopathology Reports

The liver tissues of treated animals from different groups were evaluated for histopathological examination and the results were shown in following figures. Liver Section of this group shows Revealed hepatocytes are in cards, trabeculae and sinusoidal pattern. Hepatocytes are normal in cell morphology with moderate eosinophilic cytoplasm with round nucleus contains fine chromatin. Hepatic vein, central vein, and portal triads are in normal in position and arrangement in **normal control** group (A). Section B group shows Revealed there are ballooning degeneration of hepatocytes with patchy and perivascular inflammatory component comprising of lymphomononuclear cell aggregates. There is mild central vein dilation and focal fibrosis seen in toxicant **ferrous sulphate** group (B). Section C show still persisting of less degenerated hepatocytes with sparse inflammatory component. Bile duct, central vein, portal triad are normal in ***C.papaya* 200mg/kg (C).** Liver section of this group shows disappearance of fibrous septae and hepatocytes appeared to be normal. Very less mononuclear inflammatory infiltration is found in ***C.papaya* 100mg/kg** (D).The liver section show still persisting of degenerated hepatocytes with sparse inflammatory component. Bile duct, central vein, portal triad are normal in ***C.papaya* 50mg/kg** (E).And also liver tissue of this group show hepatocytes are normal in cell pattern and arrangement. No inflammatory component. No thrombosis. Portal triad, central vein and bile duct are more or less normal in **Ascorbic acid** (F).

DISCUSSION

Metal induced liver toxicity is one of the major causes of liver disease. Iron induced liver damage is mainly due to Fenton's reaction which result formation of hydroxyl radical from hydrogen peroxide.This hydroxyl radical increases lipid peroxidation and also decreases the level of glutathione and catalase. The result of the present study has shown that iron over load there was significant increase in lipid peroxidation; decrease in catalase, protein and glutathione level in liver was found [12]. According to various studies, chemical induced damage may result from disturbance of pro-oxidant and antioxidant balance that are found in cells. In this study, the measurement of oxidant-antioxidant parameters was done in blood and blood because it is a better indicator of changes in metabolite and energy metabolism related enzyme activity [13]. Malondialdehyde, an end product of lipid peroxidation, is widely used as a marker of lipid peroxidation. Glutathione peroxidase is an important enzyme which plays a key role in the elimination of hydrogen peroxide and lipid hydroperoxide in hepatic cells. Currently, there is a consensus that former deleterious effects of iron on hepatocytes are the consequence of enhanced lipid peroxidation and decreased GPx level or *vice versa* [14].

In present study the antioxidant activity of aqueous extract of leaf of *Carica papaya* was found. From the study it was also found to decrease lipid peroxidation and significant increase in catalase, glutathione and protein with the aqueous extract of leaf of *Carica papaya* in dose dependant manner. Literature regarding phytoconstituents shows that aqueous extract of leaf of *Carica papaya* contains Alkaloids Carpain, Pseudo carpain, and dehydro carpaine I & II, Choline, Caproside, Vitamin C & E. Since all these constituents are found in the leaf of this plant it is possible that these constituents may be implicated in antioxidant activity of the species. Therefore, it was concluded that the aqueous extract of the leaf of *Carica papaya* possess antioxidant principles and has free radical scavenging activity.

CONCLUSION

The result have shown that the aqueous extract of the leaf of *Carica papaya* possesses antioxidant properties, supported by the decrease in lipid peroxidation and increase in the reduced glutathione and catalase activity in in vivo method. The in vivo antioxidant activities have been supported by its free radical scavenging activities in in-vitro method. Such antioxidant activity of *Carica papaya* might be responsible for its importance in traditional medicine for the treatment of various disorders. The present study opens many new areas of research work. This work can be continued in the future to study and confirm liver protective activity in different experimental models and also to isolate, identify, characterize and standardize the active principle(s) that are responsible for this activity.

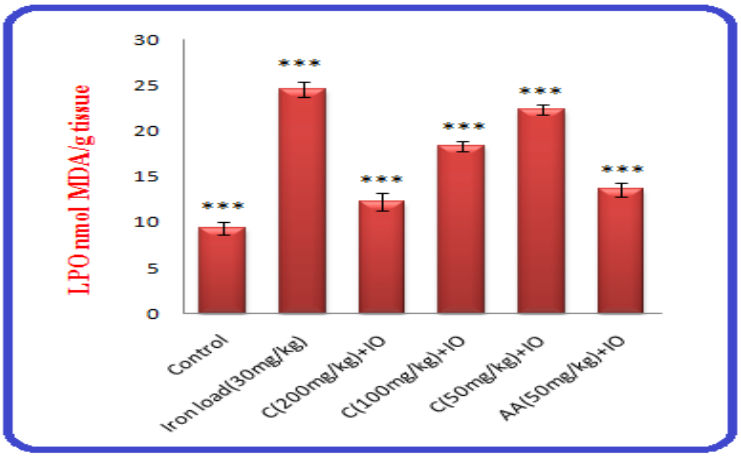

Fig .No-1 Effect of different treatment group on LPO in iron over load liver toxicity

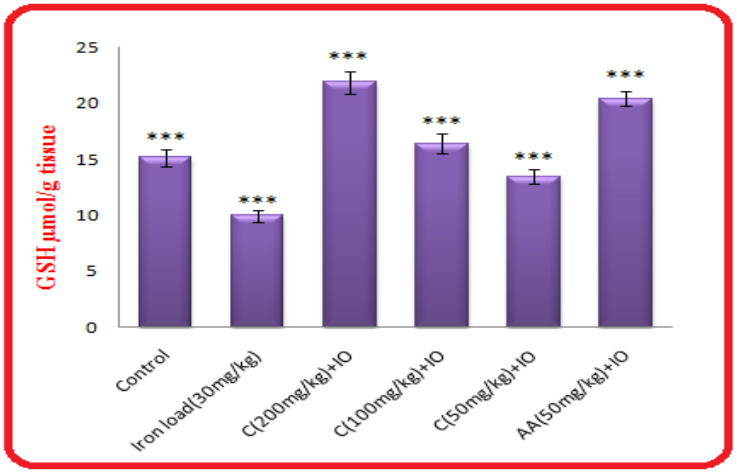

Fig .No-2 Effect of different treatment group on GSH in iron over load liver toxicity

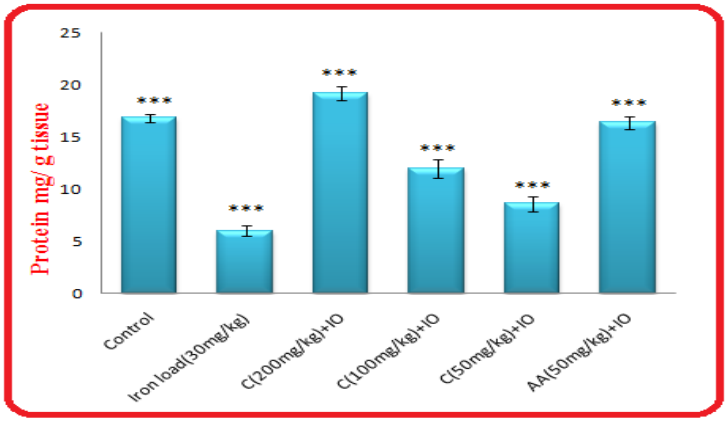

Fig. No-3 Effect of different treatment group on PROTEIN in iron over load liver toxicity

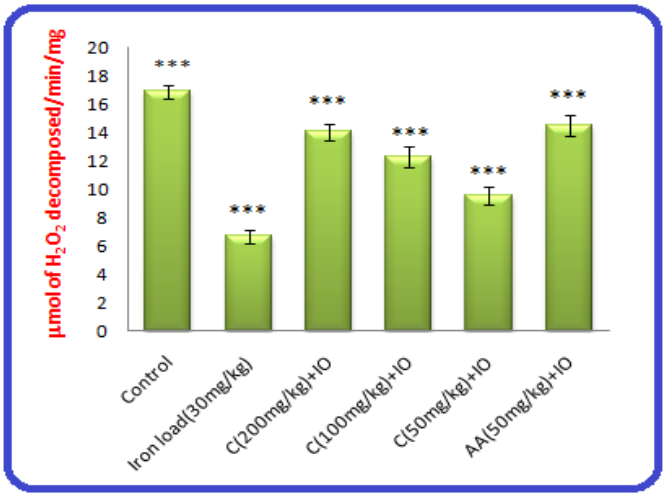

Fig .No-4 Effect of different treatment group on CATALASE in iron over load liver toxicity

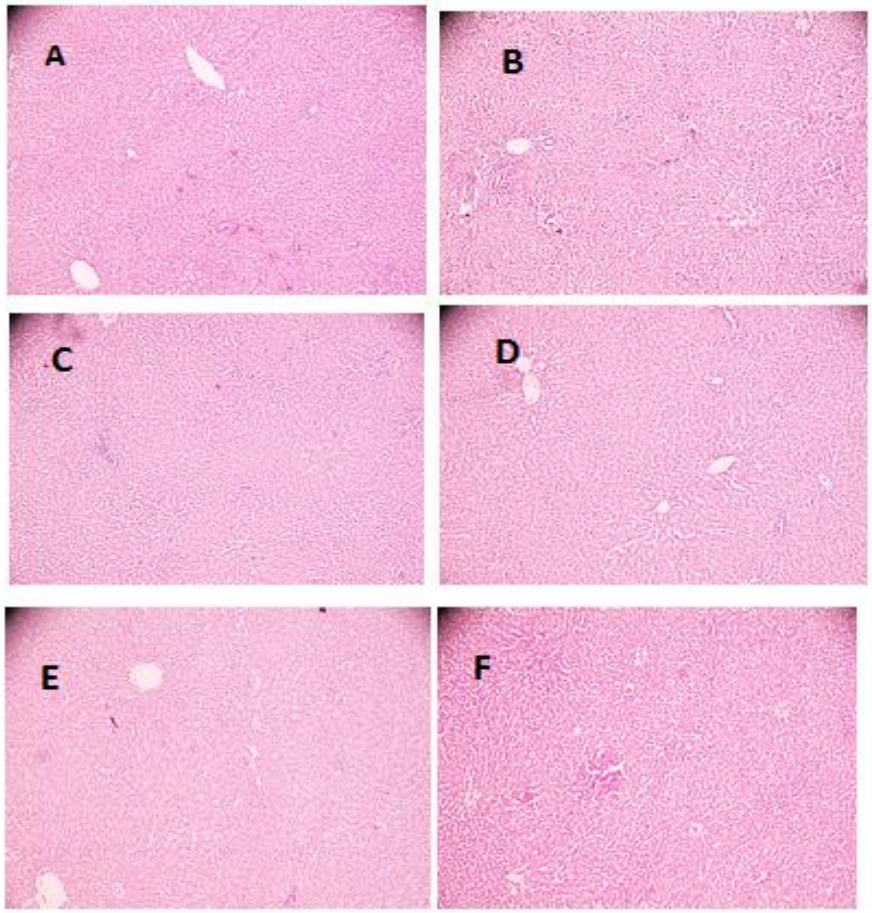

Figure: 5 Histopathological changes in rat liver apexes. 5A:- Normal control group, 5B:-.Ferrous sulphate (30 mg/kg), .5C,D&.E:-*Carica papaya* (200 mg/kg, 100mg/kg and 50 mg/kg) respectively and .5F:- Ascarbic acid group(stanadard).

Table 1: Effect of different control, and treatment group on SGOT, ALP and PROTEIN, in Iron over load induced Liver toxicity model

Groups	SGOT(U/L)	ALP (U/L)	TOTAL PROTEINS(mg/dL)
Normal control	31.34±0.61	110±0.63	14.92±0.58
Toxicant	82.51±0.61***	212±0.66***	4.81±0.74***
C.papaya(200mg/kg)	63.62±0.35***	150±0.94***	19.02±0.68***
C.papaya(100mg/kg)	68.55±1.05***	175±0.51***	15.73±0.38^{ns}
C.papaya(50mg/kg)	71.76±0.71***	204±0.73***	10.65±0.58***
Ascorbic acid (50mg/kg)	61.6±0.85***	162±0.67***	13±0.63***

******* indicates (*P*< 0.001), ****** (*P*< 0.01),***** (P<0.05).

Effect of different treatment groups on SGOT, ALP values all P values were considered

Table 2. Effect of different treatment group on LPO, GSH, and TOTAL PROTEIN & CAT in iron over load induced liver toxicity model

Groups	Treatment	LPO (nmol MDA/g tissue)	GSH (μmol/g tissue)	Protein (mg/ g tissue)	CAT (μmol of H_2O_2 decomposed/min/mg protein)
1.	Normal control	9.57±0.375	24.53±0.707	15.25±0.148	14.17±0.210
2.	Toxicant	24.59±0.353	12.33±0.226	5.97±0.222	6.67±0.214
3.	C.papaya (200mg/kg)	12.21±0.424	21.84±0.347	19.18±0.285	14.01±0.206
4.	C.papaya (100mg/kg)	18.30±0.249	16.40±0.415	17.96±0.403	12.26±0.176
5.	C.papaya (50mg/kg)	22.30±0.249	12.66±0.253	8.58±0.324	6.44±0.276
6.	Ascorbic acid (50mg/kg)	13.53±0.362	20.42±0.294	13.57±0.276	14.48±0.344

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