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Investigation of Hepatoprotective activity of Aerial Parts of *Canna indica* L. on carbon tetrachloride treated rats

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ABSTRACT

The hepatoprotective activity of methanol extract of aerial parts of *Canna indica* L. plant was evaluated against carbon tetrachloride induced hepatotoxicity. Extract at doses (100 and 200mg/kg) restored the levels of all serum parameters like SGPT, SGOT, TB which were elevated in CCl_4 administrated rats. A 10% liver homogenate was used for estimation of catalase, GSH content, LPO level for *in vivo* antioxidant status of liver. All LPO, Reduced GSH, Catalase levels were observed normal in extract treated rats. Histopathology demonstrated profound necrosis, lymphocytic infiltration was observed in hepatic architecture of carbon tetrachloride rats which were found to obtain near normalcy in extract plus carbon tetrachloride administrated rats. This clearly suggests that methanol extract of aerial parts of *Canna indica* L. has liver protective effect against carbon tetrachloride induced hepatotoxicity.

Keywords: Hepatoprotective, Serum parameters, Antioxidant, Histopathology, *Canna indica* L.

INTRODUCTION

Liver is an organ, which play an essential role in the metabolism of various foreign compounds entering in the body. Human beings are exposed these compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances. In addition, public consume lot of synthetic drugs, during diseased conditions which are alien to body organs. All these compounds produce variety of toxic metabolite if it is not eliminated properly from body it will directly damage liver. It will leads to various liver disease and disorders.(1) Conventional medicines used in liver treatments are often insufficient. Many chronic irreversible and acute hepatic diseases and disorders culminate in untimely death due to lack of adequate remedies in modern medicines. It is therefore necessary to search for alternative drugs for treatment of liver diseases to replace currently used drugs of controversial efficacy and safety. CCl_4 induced liver damage is the classic model for screening of hepatoprotective drugs. It has been stated that one of the principal causes CCl_4 induced hepatopathy is lipid peroxidation by CCl_3, a free radical derivative of toxin (2). The antioxidant activity or inhibition of generation of free radical is important in providing protection against such hepatic damage. An antioxidant effect has been reported to play a crucial role in hepatoprotective activity of many plants. (3) The herb *Canna indica* L. is commonly known as Devkali, (7) which is widely used in the indigenous system of medicine for the treatment of diuresis, fevers and dropsy. A seed's juice used to cure earaches. Leaves show significant analgesic activity. The flower are said to cure eye disease. (4)Flowers contain lutein, ß – carotene, violxanthin. Its leaves haves chemical constituents like lignin, furfural, hemicellulose. (5, 6) Plants having antioxidant chemical constituents showed good correlation with hepatoprotective activity. Previous study reported that this plant possess in vitro antioxidant activity. So, we proceeded to study its hepatoprotective potential.

MATERIALS AND METHODS

Sample Preparation:

Aerial parts of *Canna indica* L. was collected from Local gardens of National Nursery, Navi Mumbai. It is authenticated by Prof. D.R. Mahajan, Botanist, Head of Botany Department, KTHM College, Nasik, Pune University. A Voucher Specimen (no. 03) of sample has been deposited for future reference in Department of Botany, KTHM College, Nasik, Pune University. Aerial parts of plants collected and shade dried. This material then made into powder with the help of grinder. The aerial parts powder of *Canna indica* L. was extracted using methanol solvent in soxhlet extractor. After completion of extraction all the extract solvent removed using rotary vacuum evaporator. The extracts was then stored in desiccators for further use. (8)

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Table No.1 Effect of methanol extract of *Canna indica* L. on the levels of SGPT, SGOT, TP, TB, ALP in CCl_4 intoxicated rats.

Groups	SGPT(IU/L)	SGOT(IU/L)	Total Protein(mg/dl)	Total bilirubin(mg/dl)	ALP(IU/L)
I	78.75 ± 1.138	105.5 ± 0.7064	6.122 ± 0.0007	0.429 ± 0.0006	155.4 ± 0.6126
II	190.2 ± 2.327 [a]	207.6 ± 1.080 [a]	5.755 ± 0.0015 [a]	0.699 ± 0.0014 [a]	304.0 ± 0.7606 [a]
III	163.6 ± 1.266 [b]	174.2 ± 1.2600 [b]	5.663 ± 0.0387 [b]	0.648 ± 0.0046 [b]	284.9 ± 0.5267 [b]
IV	152.9 ± 1.335 [c]	153.0 ± 0.5041 [c]	5.593 ± 0.0007 [d]	0.613 ± 0.0008 [c]	264.3 ± 0.7360 [d]
V	135.7 ± 1.687 [c]	124.8 ± 1.053 [b]	5.990 ± 0.0142 [d]	0.483 ± 0.0024 [c]	250.1 ± 1.161 [c]

One way ANOVA followed by Dunnet's test performed, compared to CCl_4 control [b]p<0.05, [c]p<0.01, [d]p< 0.001, compared with normal control [a]p< 0.001.

Experimental Animals:

Male Swiss albino mice (20-25g), Male Sprague Dawley rats (160-180g) were obtained from animal house of Bharat Serums and Vaccines Ltd., Wagle Estate, Thane, Maharashtra. The polypropylene cages (32.5 x 21 x 14) and (24 x 14 x 12) lined with raw husk (renewed after every 48 hrs) were used to accommodate rat (6/ cage) and mice (4/cage) respectively. Animals received humane care and had free access to drinking water and feed with standard pellet diet manufactured by Amrut Laboratory, Mumbai. Experiment was carried out following the guidelines set by the Institute's Ethical Committee.

Acute Toxicity Study:

Acute oral toxicity study was performed on Male Albino mice as per OECD guidelines. The mice were observed for mortality as per OECD guidelines. Different groups of 2 mice in each group were given graded doses of plant extract (175-2000 mg/kg) orally and mortality was recorded 24 hours after drug administration.

Experimental design

In –Vivo evaluation of Hepatoprotective activity was performed on Male Sprague Dawley rats weighing 160-180 gm. Animals divided into 5 groups of 6 animals in each group. Carbon tetrachloride acts as Hepatotoxin in this study which had given intraperitoneally at a dose of 1ml /kg in liquid paraffin (1:2).Two doses of drug 100mg/kg body weight (test 1) and 200mg/kg (test 2) body weight were administrated orally for 10 days. Silymarin as standard drug given in 25 mg/kg for 10 days. Doses were given using 2% gum acacia as suspending medium. CCl_4 in liquid paraffin administrated intraperitoneally at every 72 hours .i.e. On Day 1, Day 4, Day 7, and Day 10 after administration of test drug doses and standard drug doses respectively. All Groups of animals get water as vehicle. While CCl_4 control also get same treatment of CCl_4 without administration of any drug. On 11th day animals were sacrificed by decapitation under ether anesthesia. Blood samples were collected and allowed to clot at room temperature for 1 hour. (9, 10, 11, 12) Serum separated was then processed for biochemical estimations viz. SGPT (13), SGOT (14), ALP (15), Total Protein (16), Total Bilirubin (17). A 10 % w/v liver homogenate was prepared using phosphate buffer pH 7.4 to carry out lipid peroxidation (18) GSH (19). While phosphate buffer pH 7 to carry out catalase activity.(20) The remaining liver tissue was fixed in 10 % formalin solution for Histopathological evaluation at Unique Bio-Diagnostic Lab., Mumbai.

Statistical analysis:

The data are given as means ±standard error of mean (S.E.M.) The comparison of the normal control, toxin control and treated groups were statistically analyzed by equality of all populations means collected were analyzed by one – way analysis of variance (ANOVA) and Dunnet's test . n = 6 animals in each group. Data found significant when One way ANOVA followed by Dunnet's test performed, compared to CCl_4 control [b]p<0.05, [c]p<0.01, [d]p< 0.001, compared with normal control [a]p< 0.001.

RESULTS

The extract did not produce any symptoms or mortality up to dose level of 2000mg/kg body weight in mice. And hence drug was considered to be safe for further pharmacological screening. So, selected doses of extract 100mg/kg and 200mg/kg used for study.

1.Serum Parameters Determination:

Grouping of animals used in hepatprotective study, Group I = Vehicle control, Group II= CCl_4 control, Group III = T 1(100mg/kg), Group IV=T 2 (200mg/kg), Group V= Silymarin (25mg/kg)

2. Antioxidant Status Evaluation:

Grouping of animals used in hepatprotective study, Group 1 = Vehicle control, Group 2= CCl_4 control, Group 3 = T 1(100mg/kg), Group 4 =T 2 (200mg/kg), Group 5 = Silymarin (25mg/kg)

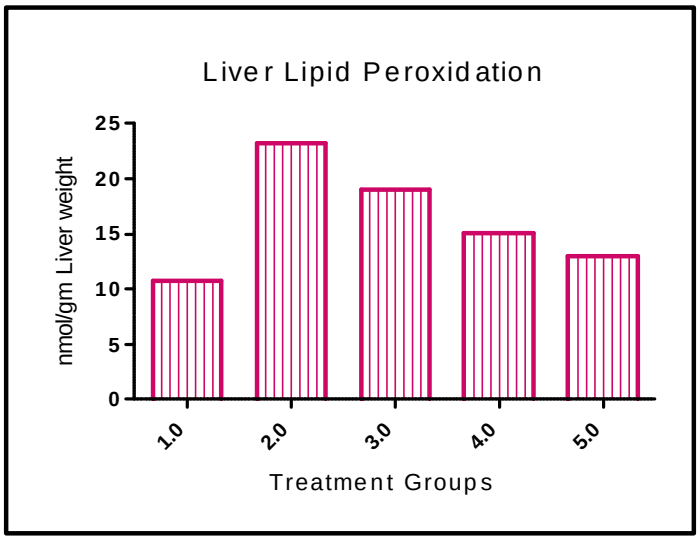

Fig.1 Effect of *Canna indica* L. extract on lipid Peroxidation level in CCl_4 treated rats

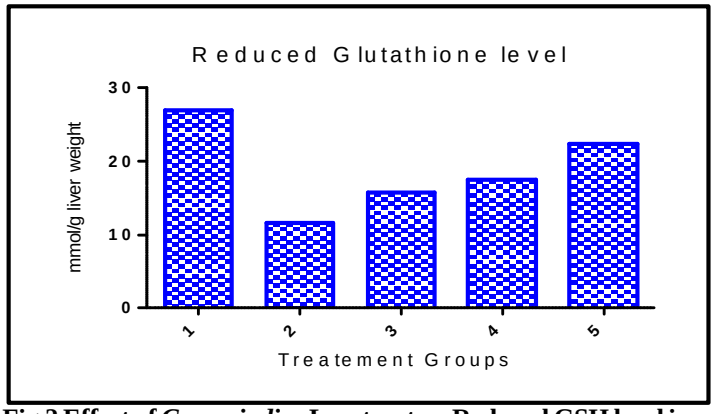

Fig.2 Effect of *Canna indica* L. extract on Reduced GSH level in CCl_4 treated rats

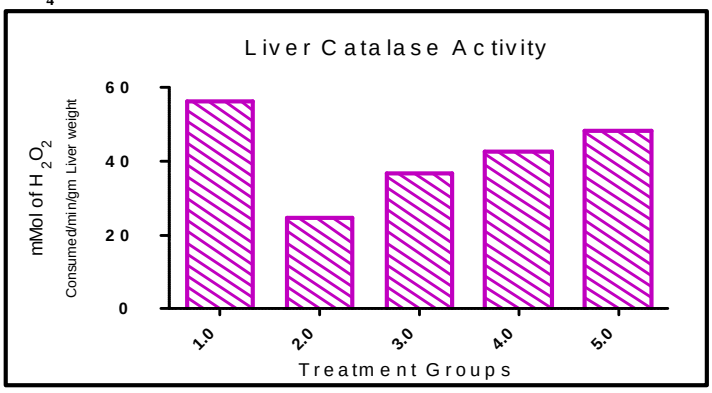

Fig.3 Effect of *Canna indica* L. extract on Catalase level in CCl_4 treated rats

3.Histopathological studies under light microscopy (H and E 100X):

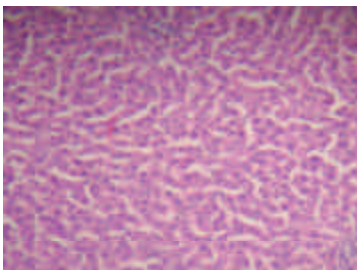

Fig. 4 Liver tissue: Normal Control Group

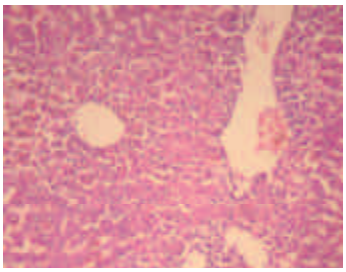

Fig. 5 Liver tissue: CCl_4 treated Group

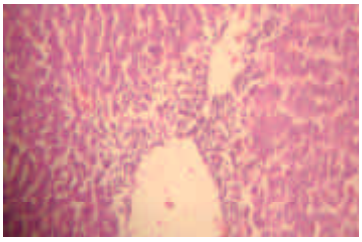

Fig. 6 Liver tissue: Silymarin treated Group

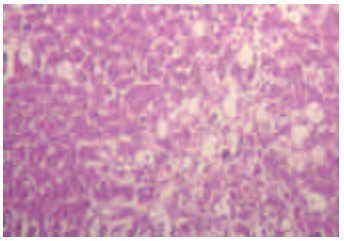

Fig. 7 Liver tissue: Test Drug (100mg/kg) Treated Group

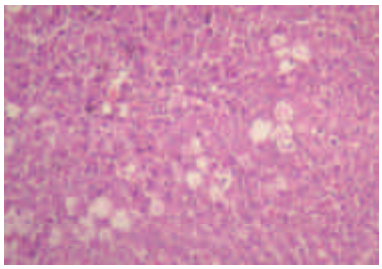

Fig. 8 Liver tissue: Test Drug (200mg/kg) Treated Group

DISCUSSION

The Hepatoprotective activity of *Canna indica* L. was measured by its protection against CCl_4 induced liver damage in rats. Liver damage induced by carbon tetrachloride is best characterized system of xenobiotic-induced hepatotoxicity and is a commonly used model for the screening of *in vivo* antioxidant and hepatoprotective activity of drugs. CCl_4 is first metabolized by cytochrome P450 2E1 in the liver endoplasmic reticulum to the highly reactive CCl_3 radical. One of the principal causes of CCl_4 induced liver injury is lipid peroxidation by free radical derivatives of CCl_4.Carbon tetrachloride is less harmful when compared to its metabolite. The hepatoprotection status was measured by lipidperoxidation level, enzymatic and non enzymatic antioxidant level. The lipid peroxidation was induced by CCl_3 radical and the level lipid peroxidation renowned by malondialdehyde, which forms a MDA-TBA adduct (LPO marker) with thiobarbituric acid. The lipid peroxidation reduced by the treatment of *Canna indica* L. which may act by reducing the metabolism of CCl_4 by inhibiting cytochrome P450 2E1 or scavenging the CCl_3 radical. Our results support LPO level in methanol extract of *Canna indica* treated groups were significantly reduced when it is compared to toxin control and inhibition was also dose dependent.The next important factor in the measurement of hepatoprotective status was non enzymatic antioxidant GSH. It plays the important role in the detoxification of the reactive toxic metabolites of CCl_4 and liver cells damages when the GSH depleted. GSH forms adduct with the toxic metabolites of CCl_4 and it is mediated through the activity of glutathione-S-transferase.Moreover GSH contributes to the detoxification of CCl_4, the experimental shows that GSH level was significantly reduced in the CCl_4 control groups when it is compared to normal animals and it was restored to normal by supplementation with *Canna indica* L. and silymarin respectively. The body has an effective defense mechanism to prevent and neutralize the free radical induced damage. This is accomplished by set of endogenous enzymatic antioxidant such Catalase. This enzyme has supportive team of defense against reactive oxygen species (ROS). In CCl_4 induced hepatotoxicity, the balance between ROS production and protection from ROS may be lost, 'oxidative stress' results, which through series of events deregulates the cellular functions leading to hepatic necrosis. The reduced activity of Catalase points out hepatic damage by administration of CCl_4.The methanol extract of aerial parts of *Canna indica* L.and silymarin treated groups showed significant increase in the level of this enzyme. Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by CCl_4. Treatment with different doses of methanolic extract of aerial parts of *Canna indica* produced only mild degenerative changes and absence of centrilobular necrosis, indicating its hepatoprotective efficiency. In addition hepatoprotective action of methanol extract of *Canna in-*

dica L. was assessed through measuring the level of biochemical enzymes. Hepatic damage caused by carbon tetrachloride intake was observed by recording SGOT, SGPT, SALP, total bilirubin and total protein level in drug treated, toxin control and normal groups because serum transaminases, serum alkaline phosphatase and serum bilirubin have been reported to be sensitive indicators of hepatic damage. Results shows that SGOT, SGPT, SALP and total bilirubin level in toxin treated animals were significantly elevated when compared with normal animals.

This disturbance in the transport function of the hepatocytes as a result of hepatic injury, cause the leakage of enzymes from cells due to altered permeability of membrane. This results in decrease in the biochemical enzymes in the liver and elevated in serum. This elevation significantly inhibited by the treatment of methanol extract of *Canna indica* L. and the level of SGOT, SGPT, SALP,TP and total bilirubin was came to normal level. (21, 22, 23)

CONCLUSION

An antioxidant effect has been reported to play a crucial role in the hepatoprotective ability of many plants. Therefore, search for a plant having antioxidant activity has become a central focus for study of hepatoprotection today. It is proved scientifically here that, it showed *In vivo* antioxidant activities by lowering lipid peroxidation and maintaining level of reduced GSH, catalase to normal level. *In vivo* study such as serum parameter estimations, histopathological study clearly suggest that this plant have comparable hepatoprotective activity that of silymarin. So this plant has shown good correlation not only with *in vivo* antioxidant activity but also with hepatoprotective activity. It leads to conclusion that this plant possesses hepatoprotective activity. Further studies are needed to isolate and characterize the active principles and their mechanism responsible for hepatoprotective activity of plant extract.

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REFERENCES

1. Rajesh MG, Latha MS, Protective effect of *Glycyrrhiza glabra* Linn. on carbon tetrachloride induced peroxidative damage, Indian Journal of Pharmacology, 36(5), 2004, 284-287.
2. Kuriakose GC, Kurup GM, Antioxidant activity of *Aulosira fertilisima* on CCl_4 induced hepatotoxicity in rats, Indian Journal of Experimental Biology, 46, 2008, 52-59.
3. Latha MS., Venukumar MS., Antioxidant activity of *Curculigo orchioides* in carbon tetrachloride induced hepatopathy in rats. Indian journal of Clinical Biochemistry, 17(2) 2002, 80-87.
4. Kolhe NM, Nirmal SA, Pal SC, Nonpolar compounds from *Canna indica* rhizomes, Physics, Chemistry and Technology, 6 (1), 2008, 141-146.
5. Deming RL, Tinoi J, Determination of major caratenoid constituents in Petal extract of Eight selected Flowering in the North of Thailand, Chiang J. Sciences,33(2),2006,327-334.
6. Anonymous, The wealth of India, raw materials vol. c, New Delhi, Council of Scientific and Industrial Research (CSIR), pp 194 -195.
7. Nadkarni AK, Indian Materia Medica, 3rd Edn, Vol.1, Bombay Popular Prakashan, Bombay, India, 1991, pp 255.
8. Mahibalan S., Jasemine S.,Jesupillai M.,Antiinflammatory effects of *Hedyotis umbellata* Linn., Internet journal of Alternative medicine, 7,(1),2005.
9. Mondal S., Chakraborty G., Gupta M., Hepatoprotective activity of *Disopyros malabarica* in Carbon tetrachloride intoxicated Rats., European Bulletein of Drug Research, 13,(1) 2005,25-29
10. Rao GM., Pushpagandhan P., ShirwaikarA., Hepatoprotective activity of *Nelumbo nucifera* Geartn.Flower : An ethanopharmacological study.,Acta Pharmaceutica Turcica. 47, 2005, 79-88.
11. Dhalwal K., Shinde V., Mahadik KR., Hepatoprotective activity of *Sida rhombifolia* ssp. against thioacetamide and Allyl alchol intoxication in Rats,Pharmacologyonline Pharmacologyonline, 3, 2006 , 259-266.
12. Blamurugan G., Muthusamy G., Observation of Hepatoprotective and antioxidant activity of *Trianthema decandra* Linn. on carbon tetrachloride trated rats, Bangladesh Journal of Pharmacology, 3, 2008, 83-89.
13. Wolf PL, Williams D., Coplon N., Transaminase Activities in Serum of Long-Term Hemodialysis Patients Clinc. Chem.18 (567) 1972.
14. Rej R., Fasce CF., Vanderlinde RE., A Discussion of Enzyme Reference Materials: Applications and Specifications Clin. Chem., 19(92) 1973.
15. Willkinson O.A., Winsten S., Clinical Chemistry 15 (487), 1969.
16. Flack CP., Woollen JW., Prevention of interference by dextran with biuret-type assay of serum proteins., Clinical Chem. 1984, 30, 559-561.
17. Pearlman PC., Lee RT., Detection and measurement of Total bilirubin in Serum, with use of surfactants as solubilising agents.Clin Chem., 20: 447 ,1974.
18. Buege JA, Aust SD., Microsomal lipid peroxidation. Methods in Enzymology, 52, 1978, 302-310.
19. Anderson ME., Determination of Glutathione and Glutathione disulfide in biological sample. Methods in Enzymology, Vol 113, 548-55
20. Aebi H., Catalase in vitro, Methods in Enzymology, 105, 1947, 121 – 125.
21. Ahmed S., Rahman A., Alam A., Saleem M., et al , Evaluation of efficacy of *Lawsonia alba* in alleviation of Carbon tetrachloride induced oxidative strees, Journal of Ethanopharmacology, 69,2000,157-164.
22. Lee K., Chul C., Jeong H., et al, Protective effects of Acetoside on Carbon tetrachloride induced Hepatotoxicity, Life sciences, 74, 2004, 1051-1064.
23. Kakoti B., Thamil S., Saha P., In vivo and In vitro antioxidant properties of methanol extract of *Sterblus asperlour*, Pharmacologyonline 2007, 3, 15-38.

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