WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1921-1929. Research Article ISSN 2277– 7105

EFFECT OF COLEUS AROMATICUS LINN., ON CCL4 INDUCED HEPATOTOXICITY IN SWISS ALBINO RATS

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Article Received on
17 May 2015,

Revised on 08 June 2015,
Accepted on 29 June 2015

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ABSTRACT

The present study has been carried out to evaluate the effect of *coleus aromaticus* leaves on CCl4 induced hepatotoxicity in swiss albino rats. Elevation of SGOT, SGPT, LDH, GGT and decline in NADH-dehydrogenase, Glucose-6-phosphatase, Na+/K+ dependent ATPase, Total ATPase and ca^{2+} ATPase was noted in CCl4 administred rats. Total reversal of all the above said parameters was noted in both ethanolic extract treated and Silymarin treated rats. The efficacy of the plant drug has been found to be more than the standard drug Silymarin.

KEYWORDS: Biochemical analysis, *Coleus aromaticus.*

INTRODUCTION

The body depends on the liver to perform a number of vital functions such as synthesis, storage, secretion, transformation breakdown and detoxification. Many chemical agents, certain drugs and toxics produce cell injury.[1]

The phase one pathway is the cytochrome p-450 mixed function oxidase system. These enzymes convert toxic chemicals into less harmful substances. This is called the conjugation pathway, where the liver cells add another substances (glutathione sulfate, glycine and glucuronide) to a toxic chemical (or) drug, to render it less harmful.[2]

Cabon tetrachloride (CCl_4) has been used to study the pathogenesis of liver injury. Liver is the main target organ for CCl_4 toxicity. CCl_4 reaches maximum concentrations in liver parenchymal cells which lead to alterations in the structure of the endoplasmic reticulum, plasma membrane, mitochondria and Golgi apparatus. The attack of CCl_4 and /or it metabolite son covalent binding to proteins and lipids leads to change in cell structure and function.[3]

Karpuravalli (*Coleus aromaticus* L.) with its distinctive smelling leaves is a common home remedy for infantile cough, cold and fever. They are useful in cephalagia, anorexia, dyspepsia, colic, diarrhea and cholera especially in children, halitosis, convulsions, epilepsy, chronic asthma, bronchitis, renal vesical calculi stroangury, hepatopathy and malarial fever. Juice is mixed with sugar is give to children in colic. Its also useful for gonorrhea, piles. Crushed leaves are used as a local application of the head in headache and relieve the pain and irritation caused by sting centipedes.[4]

MATERIALS AND METHODS

Plant material

The leaves of *Coleus aromaticus* were collected from S.T.E.T Medical plant garden, Mannargudi, Thiruvarur District and authenticated by Botany Department of A.V.V.M. Sri Pushpam College, Poondi. After anthentification the plant material were washed under running tap water.

Preparation of Plant Extract

Coleus aromaticus leaves were dried (without direct sunlight) and converted to powder form. The powder obtained was successively extracted in methanol and distilled water by using soxhlet apparatus. It was stored at 4°C until used when needed the residual extract was suspended in distilled water and used in the study.

Animals

A healthy swiss albino rats were housed in well ventilated hygienic atomosphere. Animals with 100 – 150g were used our study. Animals were fed with commericial rat feed (Saidurga feeds & foods, Bangalore) and tap water adlibitum. After randamization into various groups, the rats were acclimatized for a period of 2-3 days in the new environment before initiation of experiment.

Chemicals

All of the chemicals were of analytical grades and were obtained from Central Drug House Pvt. Ltd (New Delhi, India).

Experiment design

In the experiment, a total of 24 rats were used. The rats were divided in to following 4 groups of 6 each.

Group I : Control

Group II : CCl_4 treated (Intraperitoneal administration of CCl_4 at a dosage of 1.5ml/kg/body weight for 14 days).

Group III : CCl_4 and silymarin (Intraperitoneal administration of CCl_4 as the above mentioned dose along with oral administration of 25mg of silymarin/ml of paraffin/kg/body weight for 14 days).

GroupIV : CCl_4 and *coleus aromaticus* treated (Intraperitoneal administration of CCl_4 as the above of 300mg of *coleus aromaticus* 1 ml of paraffin/kg/bodyweight for 14 days).

Sample Collection

After 14 days of herbal treatment, the blood sample were collected from the anaesthetized rats by puncturing the orbital sinus. After the collection of blood, it was allowed to stand for 10 mts.

Biochemical measurements

Tissue and plasma SGOT[5], SGPT[6], LDH[7], GGT[8], Glucose-6-phosphatase[7], Na+/K+ dependent ATPase[9], Total ATPase[10] and ca^{2+} ATPase[11] were determined.

Statistical analysis

Results are expressed as mean ± SE from six observations.

RESULT

Table1 represent the mean values of SGOT and SGPT in experimental animals and that of normal control. Administration of CCl_4 for 14 days at a does of 300mg/kg caused significant hepato toxicity recognized by increased in the serum SGPT activity, a vital control (188.91 IU/L). Increased level of SGPT are 156.70 IU/L, 193.94 IU/L. After 14 days increased values

of above parameters were found to be decreased. A remarkable recovery was seen in Group IV animals after herbal treatment the values were found to be 82.92 IU/L, 80.02 IU/L.

The observed values in the Group IV animals were found nearly normal with that of Group I animals. In Group III animals which received the standard drug silymarin, which also showed the same result compared between Group III and Group IV animals,herbal received groups showed result when was similar to silymarin received groups.

Table 2 depicts the membrane bound enzyme (Ca^{2+} ATPase, Na^{+} K^{+} ATPase and total ATPase) values in mean ± S.E form experimental animals. In Group I the values were found to be normal but it was reduced in Group II animals. The reduced values in Group II animals get increased in other groups after *Coleus aromatics* treatment. This was seen increased value of Group IV animals. The value are 0.73, 1.09 and 2.845mg. The significant effect produced by herbal treatment is similar to that of the effect produced by silymarin treatment (0.79, 1.150 and 3.32mg).

Table 3 shows the levels of glucose-6-Phosphatase, LDH and GGT in tissue. Activity of principal glucose-6-Phosphatase are significantly lower in Group II rats when compared with Group I normal rats.

After the treatment of *Coleus aromaticus* extract resulted in elevation of glucose-6-Phosphatase activities towards near normally as compared to Group II rats, reflecting its ability to increase the level of glucose-6-Phosphate. The activity of LDH & GGT was significantly higher in Group II rats as compared with Group I control rats. After the herbal treatment of *Coleus aromatius* resulted in near normal level of LDH and GGT activity.

Table 1 showing the level of SGOT and SGPT in serum of normal and experimental groups.

S.No	Groups	SGOT (IU/L)	SGPT (IU/L)
1.	GP-I	70.16 ± 3.58	71.82 ± 3.70
2.	GP-II	188.91 ± 51.9	156.70 ± 3.74
3.	GP-III	72.64 ± 3.61	75.16 ± 3.63
4.	GP-IV	82.92 ± 3.77	80.02 ± 3.78

(Values are mean ± S.E from 6 rats in each group)

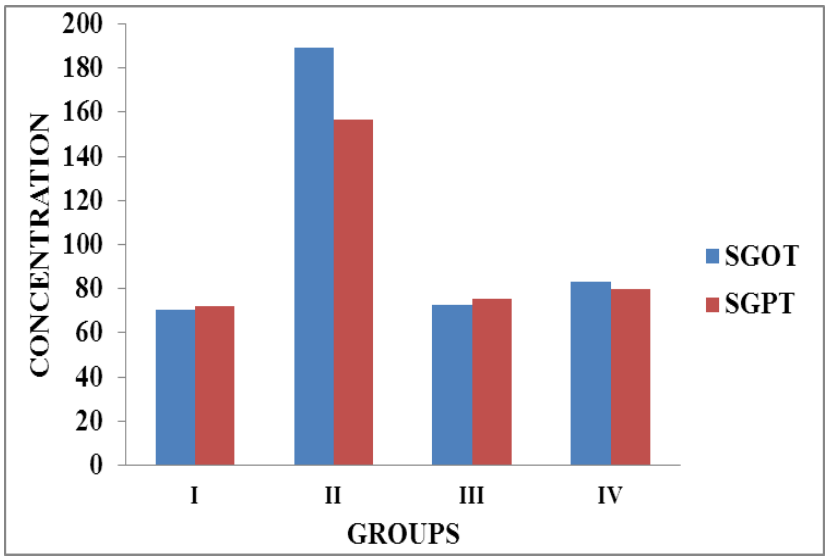

Figure 1

Table 2 showing the level of membrane bound phosphatase in liver homogenate of normal and experimental groups of rat in liver.

S.No	Groups	Ca^{2+}ATPase (X/mg Protein)	$Na^{+}K^{+}$ATPase (Y/mg Protein)	Total ATPase (Z/mg Protein)
1.	GP-I	0.82 ± 0.358	1.250 ± 0.445	3.52 ± 0.699
2.	GP-II	0.61± 0.0376	0.980 ± 0.035	2.792 ± 0.906
3.	GP-III	0.79 ± 0.039	1.150 ± 0.280	3.32 ± 1.19
4.	GP-IV	0.730 ± 0.039	1.090 ± 0.288	2.845 ± 0.785

(Values are mean ± S.E from 6 rats in each group)

X = µ moles of inorganic phosphorous liberated minutes.

Y= µ moles of Pi liberated.

Z= µ moles of Pi liberated.

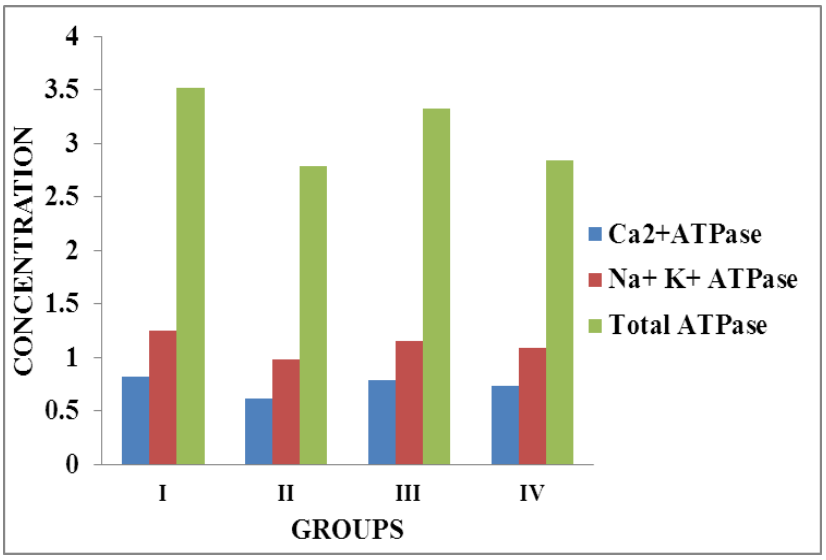

Figure 2

Table 3 showing the level of glucose-6-Phosphatase, lactate dehydrogenase in liver homogenate of normal and experimental group.

S.No	Groups	Glucose-6-Phosphate (X/mg Protein)	LDH (Y/mg Protein)	GGT (IU/L)
1.	GP-I	2.190 ± 0.792	12.40 ± 3.42	0.54
2.	GP-II	1.440 ± 0.464	25.70 ± 3.60	1.63
3.	GP-III	2.000 ± 0.755	13.30± 3.76	0.88
4.	GP-IV	1.980 ± 0.840	14.50 ± 3.58	0.64

(Values are mean ± S.E from 6 rats in each group)

X = µ moles of Pi liberated per minute.

Y= µ moles of Pyruvate liberate.

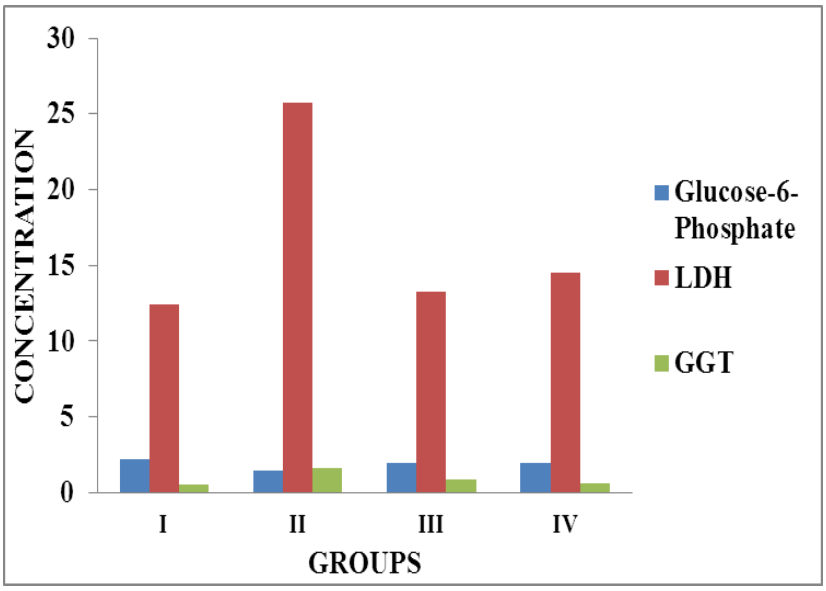

Figure 3

DISCUSSION

Hepatic necrosis by CCl_4 is usually associated with elevated level of serum enzymes that are indicated of cellular leakage and loss of functional integrity of the cell membrane in liver.[12] Amino transaminases such as SGOT and SGPT are liver specific enzyme and are considered to be very sensitive and reliable indices for measuring hepatotoxic as well as hepatoprotective (or) curative effect of various compounds. SGOT is ubiquitously distributed in the body tissue including the heart, liver and muscle where as, SGPT is found primarily in the liver. In hepatocyte, SGPT is located in mitochondria, where as SGPT is located in cytosol.[13] SGOT and SGPT increases nearly in all types of liver disorders like toxin induced liver injury and prolonged circulatory collapse.[14]

The present data indicates that SGOT and SGPT levels are increased in serum following CCl_4 toxicity. The increase in SGOT and SGPT are increase in serum following CCl_4 toxicity. However, both SGOT and SGPT levels are known to go up due to CCl_4 toxicity affecting liver cell integrity, which the results from hepatic plasma membrane damage. It may involve other cellular organelles including the mitochondria. The tendency of enzymes such as SGOT and SGPT, to return towards a near normally in drug treated rats is a clear manifestation of antihepatotoxic effect of *Coleus aromaticus*.

Membrane bound enzymes

i. Na^+, K^+ ATPase
ii. Total ATPase
iii. Ca^{2+} ATPase

Activities of the membrane – bound ATPases (Na^+, K^+ ATPase, Total ATPase, Ca^{2+} ATPase) were significantly lowered in Group II as compared with group I and normal rats. This concurred with previous reports of Sakaguchi et al., (1995).[15] ATPases are lipid dependent as well as SH dependent membrabe – bound enzyme and alterations in membrane fluids. Further enhance susceptibility to lipid peroxidation of membrane can lead to of protein thiol, there by change in membrane function.

The plasma membrane Na^+, K^+ ATPase is concerned with the maintaince of a low concentration of Na^+ and consequently of cellular water content. Decrease activity as Na^+, K^+ ATPase can lead to to decrease in sodium efflux and there by alter the membrane permeability.[16] Ca^{2+} ATPase regulates the calcium pump activity. Decrease Ca^{2+} ATPase activity has been reported during oxidative stress due to hydroperoxides and drugs in hepatocytes.[17] The intracellular concentration of calcium regulates the acitivity of the Na^+, K^+ ATPase. Therefore Ca^{2+} may play a role in the regulation of sodium reabsorption.

The steady- state Ca^{2+} concentration is postulated to be regulated only be calcium uptake by this Ca^{2+} activated ATPase.[18] *Coleus aromaticus* extract pretreatment protects the membrane bound enzymes from inactivation probably by the restoration of antioxidants[19] and there by arresting free radical – induced damage.

CCl^4 is known to induced lipid peroxidation in liver damaging cytochrome p-450 and the membranes of smooth endoplasmic reticulam.[20] Similarly loss in glucose – 6- phosphatease

is also an indication of the damage of smooth endoplasmic retriculum.[21] Glucose – 6 – phosphatase is unique among the key gluconeogenic enzymes. Since it is multifunctional and belongs to more than one metabolic pathway (Gluconeogenisis and glycogenolysis). Activities of the principal gluconeogenic enzyme (glucose – 6 – phosphatase) are significantly lower in group II CCl_4 intoxicated rats when compared with group I normal rats. Increased activities of glycolytic enzyme (Lactate dehydrogenase) in group II CCl_4 toxic rats, Deoliveria, et al., 1992[22] indicated that CCl_4 – induced reduction of ATPsynthesis in the impaired mitochondria may induced an activation glycolysis, disappearences of glycogen granules and accumulation of hepatocellular fat. A significant rise in LDH activity in CCl_4 – induced hepatic damage has already been reported.[23] The rats treated with *Coleus aromaticus* extract showed near normal levels of glycolytic enzymes is compared with that of group II CCl_4 intoxicated rats.

Gamma – glutamyl tranferase (GGT) is enzyme embedded in the hepatocytoplasma membrane, the liberation of this enzyme into plasma indicates damage to the cell membrane. Gamma – glutamyl tranferase (GGT) activity indicates to be one of the best indicators liver damage.[24] Elevated activity of Gamma – glutamyl tranferase (GGT) in our study indicate server hepatic damage by CCl_4.

CONCLUSION

In the present bring out the hepato protection activity on *Coleus aromaticus* against CCl_4 induced hepatotoxicity in rats. The use of *Coleus aromaticus* as chronic cough syrup seems to be effective. To rationalise use of the plant however, more work needs to be carried out at molecular level.

ACKNOWLEDGEMENT

The authors are grateful thank to the Correspondent and principal of S.T.E.T womens college, Mannargudi -614001, Tamilnadu, India for providing necessary laboratory facilities to complete this manuscript.

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