WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1901-1908. Research Article ISSN 2277– 7105

PROTECTIVE EFFECT OF *COLEUS AROMATICUS* LINN., ON RESPIRATORY MARKER ENZYMES DURING CCL_4 -INDUCED HEPATOTOXICITY

Dr. Jayachitra J.*[1] and Dr. Chitra M.[2]

[1]Guest Lecturer, Post Graduate Department of Biochemistry, Dharamapuram Gnanambigai Govt. Arts College, Mayiladuthurai – 609 001, Tamilnadu, India.

[2]Assistant Professor Biochemistry, Agricultural college and Research institute. Echangkottai, Thanjavur– 613 501, Tamilnadu, India.

Article Received on
17 May 2015,

Revised on 08 June 2015,
Accepted on 29 June 2015

***Correspondence for Author**
Dr. Jayachitra J.
Guest Lecturer, Post Graduate Department of Biochemistry, Dharamapuram Gnanambigai Govt. Arts College, Mayiladuthurai – 609 001, Tamilnadu, India.

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 ALP and ACP, and decrease in succinate dehydrogenase, NADH dehydrogenase and cytochrome-c oxidase was noted in CCl_4 administred rats. Total reversal of all the above said parameters was noted in both ethanolic extract of *coleus aromaticus* 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, Respiratory enzymes, *Coleus aromaticus* and CCl_4.

INTRODUCTION

Cabon tetrachloride (CCl_4) is of considerable industrial and environmental importance and is a natural product. The toxic effect of CCl_4 on the liver has been studies extensively by a large number of investigators.[1] CCl_4 is a lipid –solution compound and is thus well distributed through out the CCl_4 can be metabolished by cytochrome p-450, specially in the centrilobular region. The cytochrome p-450 functions in a reductive mode and catalyses the additionof a electron to CCl_4 which then allows hemolytic cleavage and the loss of a chloride ion with the formation of trichloromethyl radical. The resulting trichloromethyl radical may then undergo one of the several reactions. It may abstract a hydrogen atom from a suitable donor such as the

methylene bridge on poly unsaturated fatty acids thiol group producing chloroform which which is a known metabolite of carbon tetrachloride. The trichloro methyl radical, can covalently bind to microsomal lipids, protein and reacts with membrane phospholipids and cholesterol. The production of a reactive radical metabolite is the start of a cascade of events leading to lipid peroxidation.[2]

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.[3]

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

Serum ALP[4], ACP[5], succinate dehydrogenase[6], NADH dehydrogenase[7] and cytochrome-c oxidase[8] were determined.

Statistical analysis

Results are expressed as mean ± SE from six observations.

RESULTS

Table 1 represent the mean values of ALP and ACP in experimental animals and that of normal control. Administration of CCl_4 for 14 days at a dose of 300mg/kg caused significant hepato toxicity recognized by increased in the serum level of ALP and ACP was observed after CCl_4 administration. The level are 193.94 IU/L and 6.230 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 74.02 IU/L and 5.95 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 the changes in the activities of succinate dehydrogenase, NADH dehydrogenase and cytochrome - c - oxidase are given in Table 2. The activity of the enzymes succinate dehydrogenase, NADH dehydrogenase and cytochrome – c – oxidase were lower in Group II CCl_4 treated rats, as compound with Group I normal rats. After the herbal treatment of *Coleus aromaticus* extract resulted in elevation enzyme activities towards near normal. The significant effect produced by herbal treatment is similar too that of the effect produced by silymarin treatment.

Table 1 showing the level of ALP, ACP in serum of normal and experimental group.

S.No	Groups	ALP (IU/L)	ACP (KA Units/100ml)
1.	GP-I	69.94 ± 3.39	3.01 ± 0.738
2.	GP-II	193.94 ± 3.41	6.230 ± 0.979
3.	GP-III	70.02 ± 3.22	4.24 ± 1.38
4.	GP-IV	74.02 ± 3.76	5.95 ± 1.25

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

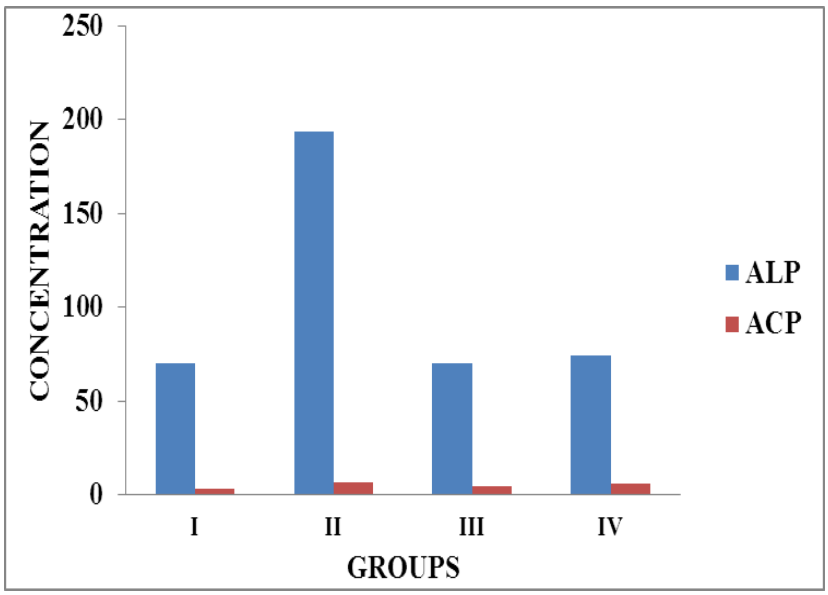

Figure 1

Table 2 showing the level of Succinate dehydrogenase, NADH dehydrogenase and Cytochrome- c- oxidase in the liver mitochondria of normal and experimental groups.

S.No	Groups	Succinate dehydrogenase (X/mg protein)	NADH dehydrogenase (Y/mg protein)	Cytochrome - c - oxidase (Z/mg protein)
1.	GP-I	28.72 ± 3.37	30.02 ± 4.15	4.80 ±1.59
2.	GP-II	10.06± 2.98	15.08 ± 3.68	2.0007 ± 0.730
3.	GP-III	26.74 ± 2.38	28.01 ± 3.74	3.200 ± 0.596
4.	GP-IV	25.02 ± 3.74	24.02 ± 3.66	3.100 ± 0.339

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

X = µ moles of succinate dehydrogenase per minute.

Y= µ moles of oxygen per minute.

Z= µ moles of NADH oxidized per minute.

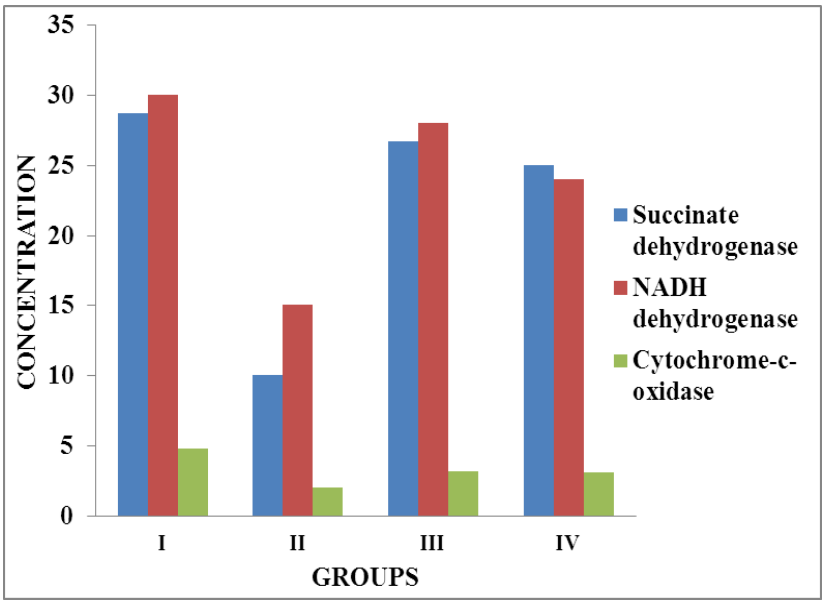

Figure 2

DISCUSSION

Alkaline phosphatase

Alkaline phosphatase is a membrane bound enzyme and is released unequally depending on the pathological phenomenon. Elevation of serum alpha-glutamyl transferase concentration is generally regarded as one of the most sensitive indices of hepatic damage.[9] Serum alkaline phosphatase concentration are known to be markedly increased elevated in cholestasis and to be minimally increased in chronic hepatocellular disease.[10]

Toxicants also showed similar type of elevation, which attributed the primarily to hepatic necrosis.[11] Various reports stated that increase in ALP occur in parenchymal liver disorder such as hepatitis induced by drugs.

Increased level of ALP may represent increased production as well as release from necrotic cell. Study of ALP level has yielded an interesting data about provide inclusive evidence for liver toxicity by CCl_4 treated animal when compared to the normal control.

Effective control of ALP activity towards an early improvement in secretary mechanism of hepatic cells is achieved by treatment with extract of *Coleus aromaticus*.

Acid phosphatase

Acid phosphatase is frequently employed as a maker enzyme to assess the iysosomal changes invivo because it is localized almost exclusively in the particular and its release parallels that of lysosomal hydrolyses.[12] A significant increase in the acid phosphatase as a result of *Coleus aromatics* treatment against CCl_4 induced liver damage indicates protection against the rupture of lysosomes and the leakage of the enzyme.

Succinate dehydrogenase

Activities of the enzyme involed in the aerobic oxidation of pyruvate in mitochondria (succinate dehydrogenase) are lower in CCl_4 treated animals as compared with those in group I controls significant decreases in the activities of these mitochondrial oxidative enzyme after intraperitoneal administration of CCl_4 have already been reported Anandan et al., (1998).[13]

Jikk et al., (1984)[14] stated that the level of NADH increased in mitochondria only when the metabolite overload on hepatic cell was prolonged. A significant rise in NADH/NAD ratio has been reported in CCl_4 treated animals (Miyahra et al., 1982).[15] This could be attributes to a reduction in the activities of tricarboxylic acid cycle enzyme by the mechanism of mass action. Prior oral administration of the *Coleus aromaticus* resulted in elevation of TCA cycle. Enzyme towards near – normally, reflecting its ability to increase the level of NAD^+ and to decrease the lipid peroxidation.

Respiratory marker enzymes

i. **NADH dehydrogenase**

ii. **Cytochrome – c – oxidase**

CCl_4 drug decreases NADPH and NADH oxidation accelerates the inactivation of cyt – p450 to cyt – p420 and cause destruction of nucleus, mitochondria and endoplasmic reticulum.[15] Change in the concentration of respiratory components, phosphorylative activity, cytochorome – c – oxidase activity and hepatic dalmatic change level have also been reported in CCl_4 induced hepatocellular damage Padama et al., (1997).[16] The effect of CCl_4 induced liver damaged on energy metabolism may be due to reduction in gluconeogenesis and involves a shift an aerobic metabolism to anaerobic glycolysis.

CONCLUSION

Thus, our study shows that *coleus aromaticus* at a dose of 300mg/kg body weight effectively protects the tissues against CCl_4-induced liver damage. *Coleus aromaticus* can be used for effective protection of hepatic disorders, their potential under field conditions needs to be evaluated. Further investigation regarding the hepatoprotective principles of *coleus aromaticus* should be carried out in future.

ACKNOWLEDGEMENT

Authors are thankful to the managing trustee of S.T.E.T women's college, Mannargudi for the facilities provided to complete the project work in a successful way.

REFERENCES

1. Slater T.F.En; Free radical mechanism in tissue injury J.W.Arrow smith Ltd., Bristol, P. 1972; 118 – 163.
2. John Timbrell. Principles of Biochemical toxicology.British library cataloguing in publication Data., Philadelphia, 2000; PP: 259 – 348.
3. Chaudhri., Herbal drug industry edition, 1996; 1: 1-5.
4. King EJ, Armstrong AR. Calcium, phosphorus and phosphatases, In: Practical clinical biochemistry, CBS publishers, New Delhi, 1988; PP. 458.
5. King EJ, Armstrong AR. Canad. Med.Asso. J, Vol 31, 1934; PNO: 376.
6. Slater EC, Bonner WD. Biochem J 1952; 52: 185.
7. Minakami S, Ringer RL, Singer TP. J Boil chem. 1962; 237: 569.
8. Pearl W, Cancercao J, Zweifach BW, J Histochem cytochem 1963; 11: 102.

9. Szczeklik V, and oralowski M. Serum gamma glutanyl peptidase activity in liver disease gastro enterology 1961; 41: 353 – 359.
10. Wilkinson J.H. The principles and practice of Diagnostic Enzymology. Edward Arnold (Publishers) Ltd., London 1976; P.P: 305 – 348.
11. Plaa and Zimmerman. Quantitative evaluation of Indices of hepato toxicity Toxicology of the liver Raven Press, New York, 1997; PP: 103 – 120.
12. Tanaka T, Sakai E and Hisot Y. Cultivation and sapoxin contents lef guangxi Bupleurum, shoy aakvgakuzassh, 1968; 42: 236 – 239.
13. Anandan R, Devaki T. med sci. Res 1998; 26: 349.
14. Jikk A, Jaki Y, Nakanura N, Tanaka J, Kamiyana Y, Oxawa K.J. Surg Res, 1984; 37: 361 – 368.
15. Miyahara M, Enzan H, Shirashi N, Kawase M, Yahamoto M, hara H, Utsumi. Biochem Biophyr acta 1982; 77: 81.
16. Padma P, Setty OH, Indian J. Biochem Biophys 1997; 34: 296.