

Biochemical study of *Ocimum sanctum* against carbon tetra chloride Induced hepatic damage

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Abstract—Chemical toxicity is a major difficulty, facing through out the world, those who are handling and consuming. Some chemicals are used as drug, in pharmaceutical related activities also cause the toxicity. Some chemical compounds are directly or indirectly enter into the human system and create many side effects. All kind of these effects are expressed in serum as abnormal enzymes, and free radicals. The present study to intended to evaluate the aqueous extract of *Ocimum sanctum*, against carbon tetra chloride induced toxicity in albino rats. In this study we performed the serum enzymatic assay of Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), 5' nucleotidase (5'NTase), Acid Phosphatase (ACP), Lactate Dehydrogenase (LDH) and enzymatic antioxidant, Superoxide dismutase, Catalase, Gluthione peroxidase, peroxidase, Glucose 6 phosphate Dehydrogenase, and also non enzymatic antioxidants Glutathione, vitamin C, Vitamin A, Total sulphhydryl groups, Protein sulphhydryl group, nonprotein sulphhydryl groups. From this study it can be concluded that *Ocimum sanctum* prevent the toxicity effects of carbon tetra chloride in rats.

Keywords—Chemical toxicity, *Ocimum sanctum*, Carbon tetra chloride, superoxide dismutase, antioxidants.

I. INTRODUCTION

Toxicity of chemicals majorly affects all kinds of plants and animals. Excess of any kind of compounds will be harmful to life [1]. Liver plays a major role in detoxification and is generally the major site for intense metabolism [2]. It is also a site of biotransformation, of toxic compounds were converted into less harmful form [3]. So in this study liver releasing enzymes and also the free radical enzyme activity are evaluated. Free radicals interact with the cellular macromolecules such as proteins, lipids and DNA leading to a cascade of oxidation and reduction reaction causing liver damage [4]. The free radicals of both enzymatic and non enzymatic antioxidants was analyzed in this study.

Ocimum sanctum commonly known as Tulsi in Hindi and Holy Basil in English a popular herb was used for this study. This herb is found throughout the semitropical and tropical parts of India. It is an Anti-oxidant, Anti carcinogenic, Anti-inflammatory, Antiulcerogenic, wound healing [5-9] properties.

Very few research have been done on the antitoxic effect of *Ocimum sanctum* on CCl_4 so far. Therefore this study was conducted to explore the antitoxic and antioxidant effect of leaf aqueous extracts of *Ocimum sanctum*. Therefore, in the present study the effect of *Ocimum sanctum* has been evaluated using carbon tetra chloride as toxicity producing agent.

II. MATERIALS AND METHODS

A. Plant extract

The crude leaf powder extract of the *Ocimum sanctum* was used in this study. The crude powder was subjected to standard chemicals tests to determine qualitatively the presence or absence of ALT, AST, ACP, LDH, Superoxide dismutase, Catalase, Gluthione peroxidase, peroxidase, Glucose 6 phosphate Dehydrogenase, and also non enzymatic antioxidants Glutathione, Vitamin C, Vitamin A, Total sulphhydryl groups, Protein sulphhydryl group, and nonprotein sulphhydryl groups.

B. LD₅₀ Determination

The LD₅₀ value calculation was referred by log-dose/Probit regression method [10]. LD₅₀ value of *Ocimum sanctum* (500mg/kg) and carbon tetra chloride (2ml/Kg/per day) was referred as earlier and followed the same [5].

C. Animals

This study was conducted on healthy male, albino rats weighing 100-150g obtained from Perundurai, Erode India. They were maintained under controlled laboratory conditions, fed standard animal food, tap water ad libitum purchased from Hindustan lever, Bangalore, India

D. Toxicity Induction

Animals were subcutaneously injected with a single dose of CCl_4 (2ml/ kg body weight/ day) for the induction of necrosis for a period of one week. This dosage was proved to be effective from the earlier report [11].

E. Experimental Design

The rats were divided into following four groups of six each: Control(Group-I), Toxicity induction group(Group-II) treated with CCl₄ for seven days(2ml/Kg/day), Preventive Group (Group-III) animals were pretreated with *Ocimum sanctum* leaf powder(500mg/ kg body weight/ day) for a period of 30 days and on the next day CCl₄ toxicity induced for seven days. Curative group(Group-IV) animals received CCl₄ for seven days and treated with *Ocimum sanctum* leaf powder for 30 days.

After the period has been over, animals were exposed to mild chloroform anaesthesia, blood was collected on decapitation and serum was separated by centrifugation (2500 rpm for 20 min at 4 °C) and stored.

F. Biochemical Assay

The biochemical enzymes and antioxidants such as the Aspartate Transaminase(AST), Alanine Transaminase (ALT)[12], Alkaline Phosphatase(ALP), Acid Phosphate (ACP)[13], Lactate Dehydrogenase (LDH)[14], 5'nucleotidase[15] and enzymatic antioxidant, Superoxide dismutase[16], Glutathione Peroxidase[17], Peroxidase, Catalase[18], Glucose 6 phosphate Dehydrogenase[19], and also non enzymatic antioxidants Glutathione, vitamin C, Vitamin A, Total sulphhydryl groups, Protein sulphhydryl group, non protein sulphhydryl groups has been evaluated in this study. when any abnormality are found in any organ or tissues these enzymes are expressed in serum as well as in the damaged tissues or organ.

G. Stastical analysis

The results of biochemical estimation have been expressed as mean \pm standard error mean (n=6). Data were analyzed using ANOVA, Scheffe's multiple range test, and the levels of significant were set at 0.05. Over all group comparison was carried out using ANOVA and significant at 0.0001 level.

III. RESULTS

A. Effects of serum enzyme level (Table-I)

A highly significant p(< 0.05) increase levels of serum enzymes, Aspartate Transaminase(AST), Alanine Transaminase(ALT), Alkaline phosphatase(ALT), Acid Phosphatase(ACP), Lactate dehydrogenase(LDH), 5'nucleotidase(5'NTase) were found due to CCl₄ treatment(Group-II). Whereas in group-III (*Ocimum sanctum*+ CCl₄), group-IV(CCl₄+ *Ocimum sanctum*) rats showed a significant decline in hepatic enzyme activity when compared to group-II. Group-I was the control shows the normal value.

TABLE. I EFFECT OF OCIMUM SANCTUM ON THE ACTIVITIES OF SERUM ENZYMES IN MALE ALBINO RATS.

(Values are expressed as Mean \pm SEM) (n = 6)

Enzymes	Group I	Group II	Group III	Group IV
AST ^a	66.39 \pm 0.04	95.66 \pm 0.02	74.88 \pm 0.02	68.24 \pm 0.35
ALT ^a	20.56 \pm 0.02	34.94 \pm 0.02	29.53 \pm 0.03	26.09 \pm 0.03
ALP ^b	548.92 \pm 0.04	962.53 \pm 0.03	745.23 \pm 0.02	686.68 \pm 0.02
ACP ^b	15.55 \pm 0.02	44.50 \pm 0.20	25.29 \pm 0.03	21.24 \pm 0.02
LDH ^a	340.91 \pm 0.02	771.25 \pm 0.03	650.63 \pm 0.02	544.90 \pm 0.02
5'NTase	10.14 \pm 0.01	32.57 \pm 0.03	20.66 \pm 0.02	12.04 \pm 0.06

a- n moles of pyruvate liberated/min/mg protein

b- n moles of phenol liberated/min/mg protein

B. Effects of antioxidants levels :

1). Enzymatic antioxidants (Table II)

The enzymatic antioxidants Superoxide Dismutase(SD), Catalase(Case), Glutathione Peroxidase (GP), Peroxidase(Pase), Glucose 6 phosphate Dehydrogenase (G6D) levels were significantly reduced in group-II, group-III, group-IV, when compared to control(Group-I). When significant increase were found in group-III and Group-IV as compared with Group-I.

TABLE. II EFFECTS OF OCIMUM SANCTUM ON THE ACTIVITIES OF ENZYMATIC ANTIOXIDANT LEVELS IN MALE ALBINO RATS. (VALUES ARE EXPRESSED AS MEAN \pm SEM) (N = 6)

Enzyme	Group I	Group II	Group III	Group IV
SD ¹	6.71 \pm 0.02	4.42 \pm 0.01	6.26 \pm 0.01	6.30 \pm 0.02
Case ²	73.86 \pm 0.05	51.01 \pm 0.04	62.18 \pm 0.05	63.80 \pm 0.06
Pase ³	68.12 \pm 0.02	44.52 \pm 0.03	55.71 \pm 0.03	59.78 \pm 0.02
GP ⁴	21.30 \pm 0.03	15.61 \pm 0.05	18.41 \pm 0.02	19.09 \pm 0.03
G6D ⁵	4.99 \pm 0.09	3.20 \pm 0.01	4.38 \pm 0.02	4.46 \pm 0.01

1. 50% inhibition of nitrate/min/mg protein

2. n –moles of H₂O₂ decomposed/min/mg peotein

3. μ Moles/min/mg protein

4. μ g of GSH/min/mg protein

5. 0.01 OD/min//mg protein

2). Non enzymatic antioxidants (Table III)

Non enzymatic antioxidants Glutathione(GLU), Vitamin A(Vit A), Vitamin C (VitC), Total Sulphydryl Groups(TSG), Protein Sulphydryl Group(PSG), Non Protein Sulphydryl Groups(NPSG) shows that their is significant decrease in CCl₄ treated groups(Group-II) as the same results as enzymatic antioxidants. When treated with *Ocimum sanctum* gives the significant increase (Group-III, Group-IV).

Table.III Effects of *Ocimum sanctum* on the activities of Nonenzymatic ANTIOXIDANT levels in male albino rats. (VALUES ARE EXPRESSED AS MEAN \pm SEM) (N = 6)

Non Enzyme	Group I	Group II	Group III	Group IV
GLU*	4.00 \pm 0.02	2.53 \pm 0.02	3.51 \pm 0.02	3.42 \pm 0.03
Vit A^	2.48 \pm 0.03	1.38 \pm 0.02	1.98 \pm 0.02	2.17 \pm 0.02
Vit C*	20.30 \pm 0.34	13.02 \pm 0.06	15.54 \pm 0.54	17.56 \pm 0.11
TSG*	18.17 \pm 0.02	12.54 \pm 0.01	14.76 \pm 0.02	15.73 \pm 0.04
PSG*	13.99 \pm 0.02	10.87 \pm 0.02	12.28 \pm 0.03	12.36 \pm 0.02
NPSG*	4.21 \pm 0.03	1.69 \pm 0.02	2.48 \pm 0.08	3.27 \pm 0.05

- *- μ g/g tissue
- ^- μ g/mg protein

These entire biochemical assay shows that *Ocimum sanctum* have the protective character.

IV. DISCUSSION

Hepatic dysfunction due to injection or inhalation of toxin is increasing worldwide. In the present investigation CCl₄ was used as toxin for inducing damage. The study of enzyme activities and constituents present in the serum has been found to be of great value in the assessment of clinical and experimental liver damage [20].

In the present study enzymes were assayed by induced hepatotoxin(CCl₄)group-II. The tendency of these enzymes to return towards near normal level in group-III group-IV rats, shows the clear manifestation of antihepatotoxic effects of *Ocimum sanctum*. The aspartate transaminase and alanine transaminase was increased in serum due to CCl₄ toxicity, may lead to hepatocellular necrosis, which cause increase in the permeability of the cell membrane[21].

The lipid peroxidative degradation of biomembrane is one of the principle causes of hepatotoxicity of CCl₄ [22,23]. This is evidenced by an elevation of liver marker enzyme namely acid phosphatase, alkaline phosphates. In the present study CCl₄ treated rats shown, increased activity of acid phosphatase and alkaline phosphatase in serum.

Increase in lipid peroxidation during galactosamine administration was reversed to normal by Picrorrhizza kurroa[24] was in agreement with our present findings.

A similar increase in acid phosphatase and alkaline phosphatase activities in lysosome suspension has been reported in CCl₄ induced hepatotoxicity [25 ,26]. The result of this study were incorporation with earlier reports on the hepatoprotective activity of aqueous extract of *Andrographis paniculata* and picroliv (herbal formation) against CCl₄ induced liver damage[27].

In the present study hepatic activity of lactate dehydrogenase was enhanced after treatment with CCl₄ (group-II).Group-III and group-IV treated with *Ocimum sanctum* found to restore the enzyme level to near normal when compared to CCl₄ treated animals. This finding were also in accordance with cassia occidentails levels normalized

the increased lactate dehydrogenase against paracetamol induced hepatotoxicity[28].

The site specific oxidative damage of some susceptible aminoacids of protein is now regarded as major cause of metabolic dysfunction during pathogenesis. Hypo albuminemia is most frequently observed in the presence of advanced chronic liver diseases [29]. In the present study proteins recorded in liver of CCl₄ treated revealed the severity of hepatopathy. Similar reports were found earlier [30].

There was a significant decreased in the activities of superoxide dismutase in CCl₄ treated as compared to control. A significant decrease was found in preventive (Group-IV) and curative group(Group-V) as compared to control. Superoxide Dismutase metabolizes superoxide anion radicals. It was an effective defense of the cells against endogenous and exogenous generation of oxygen [31] that showed the inhibition of superoxide dismutase in CCl₄ treated animals.

Glucose 6 phosphate Dehydrogenase caused decreased supply of reducing equivalent like NADPH. So the decreases in NADPH production decrease the catalase activity. Reduction of catalase was noted in rats intoxicated with CCl₄. On treatment with *O. sanctum* leaf powder of these enzymes was recovered normal. Catalase had been shown to be responsible for the detoxification of significant amount of hydrogen peroxide. Peroxidase enzymes activity decreased by CCl₄ intoxication [32]. Fridovich[33] had shown the inhibition of glutathione peroxidase activity, in CCl₄ treated animals liver. Anandan and Devaki[24] reported that pretreatment with the extract of picrorrhizza kurroa prevented the increase in activities of glutathione due to D-Galactosamine induced hepatitis in rats. The same results also evaluated in our study.

Vitamin C was an important water soluble antioxidant and it protects plasma lipid membrane[34]. Vitamin A played a role in trapping peroxy radicals in tissue at low partial pressure of oxygen. Vitamin C and Vitamin A was decreases when CCl₄ induction (Group-II). It was recovered with *Ocimum sanctum*(Group-III, Group-IV) when compared with group-II.

Thiols are water soluble antioxidants associated with membrane proteins and are important for the antioxidants system. Total sulphhydryl group, protein sulphhydryl group and non protein sulphhydryl group significantly decrease in the activities of CCl₄ intoxication. These sulphhydryl groups maintained the structural integrity.

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