EFFECTS OF NIGELLA SATIVA EXTRACT AGAINST CARBON TETRACHLORIDE INDUCED LIVER INJURY IN SWISS ALBINO MALE MICE

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ABSTRACT

Objective: *To observe the effect of Nigella Sativa (N. Sativa) in Carbon tetrachloride (CCl4) induced liver injury by studying the enzymatic changes in Swiss albino male mice.*

Materials and Methods: *An experimental study was conducted in the Department of Pharmacology, ISRA University, Hyderabad, and Agriculture University, Tandojam using a non-probability sampling technique (n=30): Swiss Albino male mice. The animals were divided into 03 groups comprising of 10 animals each. All the groups were given CCl4 alone and in combination with N. Sativa for three weeks.*

i) Group A (n=10): Control group

ii) Group B (n=10): received Carbon tetrachloride

iii) Group C (n=10): received Carbon tetrachloride + Nigella Sativa

Results: *It was found that the liver injury caused by CCL4 toxicity can be reversed by the hepatoprotective effect of N. Sativa extract. Thus by measuring the serum enzyme levels of ALT, AST, ALP, and ɣ-GT in venous blood samples of Swiss albino mice treated with CCl4 and control group, it is proved that N. Sativa protects against the harmful biochemical effects of CCL 4 induced injury to the liver.*

Conclusion: *Nigella Sativa is protectively related to the biochemical changes in Carbon tetrachloride-induced hepatotoxicity.*

Keywords: *Carbon tetrachloride CCl4. Liver injury, ALT, AST, ALP, ɣ-GT, etc*

INTRODUCTION

Carbon tetrachloride (CCl4) is one of the environmental pollutants that has well known hepatotoxic effects.[1] CCl4 causes centrilobular hepatocellular vacuolar degeneration (necrosis), which ultimately leads to liver steatosis. These effects increase the absolute and relative liver weight, serum levels of Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and Gamma-glutamyl transferase (ɣ-GT).[2]

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Metabolic activation of CCl4 by cytochrome P450 system causes the production of free radicals, namely trichloromethyl and trichloromethyl peroxy radicals, both these radicals reportedly enhance heme degradation in the liver, and lipid peroxidation of the hepatocyte membrane, fatty acids resulting in hepatocytic necrosis.[3,4] Oxidative stress is a common mechanism contributing to the initiation and progression of hepatic damage in a variety of liver disorders. Therefore, there is a great demand for the development of agents with potent antioxidant effect against the harmful effects of CCl[4.5]

Antioxidants or free radical scavengers are very important in protecting the living cells against any damage induced by free radicals.[6] Antioxidants are important endogenous components against injury caused by lipid peroxidation and cytotoxic reactions induced by reactive oxygen species (ROS).[7]

Thymoquinone (T.Q.), the main constituent of the volatile oil from Nigella (N.) Sativa seeds are reported to possess strong antioxidant properties. N. Sativa is a very famous herbal black seed commonly named Kalongi in South Asia and Black cumin in the western world.[8] The seeds belong to the botanical family of Ranunculaceae, grown mostly in the middle east, central Europe, and Western Asia.[9] Studies have shown that T.Q. has a considerable protective effect against oxidative damage induced by a variety of free radical-generating agents.[10] Other pharmacological investigations of the seed extracts reveal a broad spectrum of activities including antihistaminic, antimicrobial[11] antidiabetic,[12] anti-hypertensive,[13] anti-inflammatory,[14] analgesic,[15] and anticancer.[16] Many of these activities have been attributed to the Quinone constituents of the seed.[11] In various animal studies, the beneficial effects of N. Sativa on diabetic control have been documented.[17, 18]

Liver enzymes Aspartate amino transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are the markers for liver injury. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells and is commonly measured clinically as a marker for liver health. As with all transaminases, it operates via dual substrate recognition; it can recognize and selectively bind two amino acids (Asp and Glu) with different side-chains. ALT and AST are both associated with liver parenchymal cells, while ALT is found predominantly in the liver with clinically negligible quantities found in the kidneys, heart, and skeletal muscle. AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may also be elevated in conditions affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma.[19]

ALT is commonly measured clinically as part of a diagnostic evaluation of the hepatocellular injury, Significantly elevated levels of ALT often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. Elevated serum levels are found in ALT dietary choline deficiency. However, elevated levels of ALT does not always mark the existence of medical problems. The fluctuation of ALT levels is normal over the course of the day, and ALT levels can also increase in response to strenuous physical exercise.[20]

ALP is a hydrolase enzyme that catalyzes the removal of a phosphate group from many molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called de-phosphorylation. ALP is most effective in an alkaline environment. It is present in all tissues throughout the body but is particularly concentrated in the liver, bile duct, kidneys, bones, and the placenta. The normal range is 20 to 140 IU/L. High serum ALP levels indicate blockage of bile ducts.[21] Serum ALP levels are significantly higher in children and pregnant women. ALP levels also rise during active bone formation, as ALP is a byproduct of osteoblast activity (such as the case in Paget's disease of bone).[22]

Gamma-glutamyltransferase or gamma-glutamyl transpeptidase (also Ɣ-glutamyltransferase, GGT, GGTP, gamma-GT) is an enzyme that catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate).[23] GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification.[24]

In this study, protective effects of Nigella sativa

and zinc sulfate shall be observed and evaluated in Swiss albino male mice exposed to carbon-tetrachloride by measuring serum enzyme levels of ALT, AST, ALP, and Ɣ-GT.

MATERIALS AND METHODS

Swiss albino male mice were kept in the animal house of ISRA University. Animals were labeled with numbers, weighted, and kept in separate cages. CCl4 was purchased from the chemical store, and Olive oil was used as a vehicle for CCl4 administration. Seeds of N. Sativa (black seed) were purchased from a local herb store and authenticated by the Department of Botany, Agriculture University Tandojam. The seeds were cleaned, air-dried, and then powdered mechanically to prepare a suspension in isotonic saline solution. The suspension (1.25 g powder of N. Sativa + 100 ml isotonic saline) was prepared a few minutes before its administration. Gavage was used to administer the drug in liquid form orally to the mice.

Group A: (control group): All albino mice were given 0.9% isotonic saline solution, orally at a dose of 4 ml/kg (by wt.) on every other day for three successive weeks and blood samples were drawn from the jugular vein at the end of their respective period of administration.[25]

Group B: All albino mice were given orally CCl4 dissolved in olive oil at a dose of 1.9 ml/kg (by wt.) on every other day for three successive weeks, and blood samples were drawn from the jugular vein at the end of their respective period.[25]

Group C: All albino mice were given orally CCl4 dissolved in olive oil at a dose of 1.9 ml/kg (by wt.) along with the suspension of N. Sativa at a dose level of 4 ml/kg by wt. (50 mg/kg by wt.) on every other day for three successive weeks, and blood samples were drawn from the jugular vein at the end of their respective period.[25]

All blood samples were analyzed for the levels of ALT, AST, ALP, and Ɣ –G.T. in serum by using the standard kit method.

RESULTS

In the present study, venous blood samples were drawn and carried to the laboratory for biochemical evaluation at 1st, 2nd, and 3rd week, respectively. One-way ANOVA was applied to analyze the significance of the results. P-value of <0.05 was considered to be significant.

At 1st and 2nd week: Blood samples of Swiss albino male mice 03 from each subgroup were taken. Biochemical evaluation of 03 Swiss albino male mice from each subgroup (09 mice) who were given isotonic saline showed normal levels of ALT, AST, Ɣ -G.T., and ALP(Table 1, 2, 3 and 4). Biochemical evaluation of 03 Swiss albino male mice from each subgroup (09 mice) who were given CCl4 showed elevated levels of ALT, AST, Ɣ -G.T., and ALP compared to the control group (Table 1, 2, 3 and 4). Biochemical evaluation of 03 Swiss albino male mice from each subgroup (09 mice) who were given CCl4 along with Nigella sativa showed a reduction in levels of ALT, AST, Ɣ -G.T. and ALP compared to the group B which were treated with CCl4 alone. (Table 1, 2, 3 and 4).

In 3rd week: Blood samples of 12 Swiss albino male mice were taken, 04 Swiss albino male mice from each subgroup. Biochemical evaluation of 04 Swiss albino male mice from each subgroup (12 mice) who were given isotonic saline showed normal levels of ALT, AST, Ɣ -G.T., and ALP (Table 1, 2, 3 and 4). Biochemical evaluation of 04 Swiss albino male mice from each subgroup (12 mice) who were given CCl4 showed elevated levels of ALT, AST, Ɣ -G.T., and ALP compared to the control group (Table 1, 2, 3 and 4). Biochemical evaluation of 04 Swiss albino male mice from each subgroup (12 mice) who were given CCl4 along with Nigella sativa showed a reduction in levels of ALT, AST, Ɣ -G.T., and ALP compared to the group B who were treated with CCl4 alone. (Table 1, 2, 3 and 4).

DISCUSSION

In the present study, it was found that serum ALT, AST, ALP, and Ɣ-GT levels were raised in CCl4 treated mice. Similar were the findings of Khalaf et al. in 2009.[1]

Significantly raised levels of ALT, AST, Ɣ -G.T., and ALP were recorded in CCl4 treated Swiss albino male mice in comparison with the control group. These results are consistent with the study done by Yun-Chen Tien et al. (2011).[27] Wang et al. (1997) also reported that plasma ALT and AST activities were significantly elevated by CCl4 as early as 3 hours after treatment with CCl4; our study also confirmed

Table: 1 Serum Alanine transaminase levels (I.U.)

	1st week (Mean±SD)	2nd week (Mean±SD)	3rd week (Mean±SD)
Group, A. (Control)	22.3±1.5	21.6±1.1	23.0±0.8
Group, B. (CCl_4)	33.0±1.0	60.3±29.1	57.5±3.5
Group, C. (CCl_4 + N.S)	28.3±2.0	22.3±5.1	28.2±21.2

Group A vs. Group B P-value < 0.0001
Group C vs. Group B P-value< 0.001

Table: 2 Serum Aspartate transaminase levels (I.U.)

	1st week (Mean±SD)	2nd week (Mean±SD)	3rd week (Mean±SD)
Group, A. (Control)	32.6±1.15	32.0±1.0	34.7±3.7
Group, B. (CCl_4)	53.0±2.0	64.6±4.7	88.0±7.2
Group, C. (CCl_4 + N.S)	43.0±1.0	40.6±7.0	43.0±36.7

Group A vs. Group B P-value <0.0001
Group C vs. Group B P-value < 0.01

Table: 3 Serum Gamma-Glutamyl Transferase levels (I.U.)

	1st week (Mean±SD)	2nd week (Mean±SD)	3rd week (Mean±SD)
Group, A. (Control)	22.3±1.5	21.6±1.1	23.0±0.8
Group, B. (CCl_4)	33.0±1.0	60.3±29.1	57.5±3.5
Group, C. (CCl_4 + N.S)	28.3±2.0	22.3±5.1	28.2±21.2

Group A vs. Group B P-value < 0.0001
Group C vs. Group B P-value < 0.001

Table: 4 Serum Alkaline Phosphatase levels (I.U.)

	1st week (Mean±SD)	2nd week (Mean±SD)	3rd week (Mean±SD)
Group, A. (Control)	23.0±1.00	23.3±2.5	24.7±2.9
Group, B. (CCl_4)	45.3±4.1	57.6±2.5	80.2±6.8
Group, C. (CCl_4 + N.S)	32.0±0.1	28.6±7.0	38.0±27.7

Group A vs. Group B P-value <0.0001
Group C vs. Group B P-value <0.02

similar findings.[28] Clarke et al. (1997) indicated that levels of liver enzymes were raised in CCl4 induced rats.[29] In the current study, Nigella sativa proved to have a hepatoprotective effect by reducing the toxic effects of CCl4. Tennekoon et al. (1991) reported that there were no histological changes seen in the animal model treated with N.sativa.[30] Mastour S Al-Ghamdi, (2003), have reported that Nigella Sativa seeds appeared to be safe and possibly protective against CCL4-induced hepatotoxicity, the study is consistent with the results of the present study.[9]

CONCLUSION

Nigella Sativa is protectively related to the biochemical changes in Carbon tetrachloride-induced hepatotoxicity.

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