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Research Article

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Effect of *Allium Sativum* on Some Serum Biochemical Changes in CCl_4-Induced Liver Injury in Wistar Albino Rats

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Abstract The study was carried out to investigate the effect of ethanolic extract of *Allium sativum* leaf on some biochemical parameters in carbon tetrachloride induced liver damage in albino rats. Twenty five male Wistar albino rats were used, comprising of five groups of five animals each. Rats in groups 2 and 3 were administered oral extracts of *Allium sativum* (100 and 200mg/kg) respectively for twenty five days while, group4 received 100mg/kg body weight of vitamin C. Animals in groups 2-5 were also, injected single dose of 0.5 mg/kg body weight/day of carbon tetrachloride i.p for the same period of study. Blood collected by cardiac puncture on the 26th day of study and was used to determine the total protein, total bilirubin, albumin and ALT concentrations using standard kits. The results showed significant increase ($p<0.05$) in the level of albumin in rat group1 (35.6 ±0.98), compared to rat group 5 (44.9 ± 1.50). Also, rats in *A. sativum* treated groups 2 and 3 showed significant decrease in the level of albumin (36.2±6.57 and 39.4±1.51) respectively compared to positively control group5 rats. Total protein level increased significantly ($p<0.05$), in rats group5 (98.6 ± 2.69) compared with rats groups 1, 2, 3 and 4 (74.9 ± 1.28, 79.7 ± 3.36, 76.5 ± 3.86 and 77.0 ± 2.99) respectively. Level of ALT increased significantly ($p<0.05$) in untreated rat group5 (25. 8 ± 17.35) compared rats in group1 (18.8±15.25). However, there was significant decrease in the ALT levels in the extract treated groups 2 and 3 (19.0±3.60 and 21.5±17.04) compared to the untreated rat group5. Total bilirubin level increased significantly in positively control rat group5 (13.57±3.03) compared to normal control rat group1 (1.458±0.32). There was significant decrease in the total bilirubin levels in *A. sativum* extract treated groups 2 an3 (1.763 ± 0.24 and 1.623 ±0.41) respectively, compared untreated rat group 5. The results showed that ethanolic extract of *A. sativum* offer protective effect to CCl_4-induced liver damage in albino rats.

Keywords Carbon Tetrachloride, *Allium Sativum*, Total Protin, Total Bilirubin

Introduction

Allium sativum, commonly known as garlic has been used as both food and medicine in many cultures for thousands of years; garlic is a species in the onions genus, which is native to central Asia and a frequent seasoning in Asia, Africa and Europe [1]. The used of herbs is a time approach to strengthening the body and treating diseases. It was known to ancient Egyptians and has been used throughout its history for both culinary and medicinal purposes [2]. A number of favorable therapeutic effects and biological activities of the garlic have been reported. Black garlic has been shown to exhibited a wide range of biological activities, such as antioxidant [3], anticancer [4], hypoglycemic [5], hypolipidemic [6], anti-inflammatory [7], hepatoprotective [8] and immunostimulatory properties [9]. Garlic is a potential adjunct to conventional medical care for liver damage [10]. In Europe, many cultures have used garlic for protection or white magic, perhaps owing to its reputation as a potent preventative medicine [11]. However, garlic should be consumed in appropriate amount because cytotoxicity was reported at high doses [3]. For these reasons, herbs should be taken with care, under the supervision of a health care provider qualified in the field or botanical medicine. Garlic is generally recognized as safe (GRAS) by the U.S. food and drug administration.Side effects from garlic include upset stomach bloating, bad breath, body odor, and a stinging sensation on the skin from handling too much fresh or tried garlic [12-13]. Handling garlic may also cause skin lesions. Other, rare side effects that have

been reported by those taking garlic supplements include headache, fatigue, loss of appetite, muscle aches, Dizziness described as vertigo, and allergies such as an asthmatic reactions or skin rash [14]. Given the importance of this vegetable as much in feeding as in therapeutic uses the present study is carried out to ascertain its effect on some biochemical parameters in carbon tetrachloride induced liver damage in albino rats.

Materials and Methods

Plant Collection/ Preparation

Fresh *Allium sativum* was purchased from local market in Yenagoa, Bayelsa State, Nigeria. It was identified by Prof. K. Ajibeshin in the Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria.

Plant Extraction

The fresh garlic (*Allium sativum*) was weighed (200g), rinsed in clean water and then crushed with mortar and pestle. 500ml of Ethanol were added into the crushed garlic in a 1000ml container and was kept for 24 hours. The mixture was constantly stirred, after which suspension was filtered and the filtrate was concentrated using water bath set at 40 °C. Total weight of extract obtained (16g) was kept inside glass container and stored in a refrigerator until needed.

Experimental Animals

Twenty five (25) male wistar rats (average weight of 180g) were bought from the Department of Pharmacology Animal House and transferred to Department of Biochemistry Animal House all in College of Health Sciences, Niger Delta University Wilberforce Island, Bayelsa State. The animals were randomly divided into five groups of five animals per group and housed in stainless-steel cages under standard laboratory conditions of 27 ± 2°C, relative humidity 50 ± 15% and normal photo period (12h dark/12h light), for acclimatization for one week. The animals were allowed free access to growers mash and water *ad libitum*.

Experimental Design/*Allium sativum* administration

25 male wistar rats were randomly shared into 5 groups of 5 rats per group and ethanolic extract of *Allium sativum* administered orally through orogastric tube on daily basis for twenty five days as shown below;

Group 1: growers match+ tween 80
Group 2: growers match+ 100mg/kg extract
Group 3: growers match+ 200mg/kg extract
Group 4: growers match + 100mg/kg Vitamin C
Group 5: growers match

Animals in groups 2-5 were injected single dose of 0.5 mg/kg body weight/day of carbon tetrachloride *i.p.* for the same period of study. The animals were sacrificed on the 26^{th} day of study and blood collected by cardiac puncture into well labeled universal bottles. Blood samples were centrifuged at 2500 rpm for 10 minutes and the serum collected was used for biochemical studies.

Biochemical Assay

Total protein was determined by colorimetric method (Biuret method). Serum transaminase (ALT) was determined by method of Reitman-Frankel). Bilirubin was estimated by colorimetric method and albumin was estimated using standard assay kits (Coral Clinical System, Goa, India) with the help of clinical serum biochemistry analyzer (Photometer 5010 V5+, Dynalab Enterprize).

Statistical Analysis

Values were represented as mean ± SD. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-kramer multiple comparison Test using graph pad Instat® software. P values of <0.05 were considered statistically significant.

Results

Result of the present study as presented in Table 1, showed that ethanolicextract of *Allium sativum*has significant effect ($p<0.05$) on some serum biochemical parameters in the CCL_4 –induced rats. Biochemicalvalues obtained from group1 (normal control) are compared to group5 (positive control) and values of the extract treated groups 2 and 3 are compared to group 5. There was significant increase ($p<0.05$) in the level of albumin in rat group1 (35.6 ±0.98), compared to rat group 5 (44.9 ± 1.50). Also, rats in *A. sativum* treated groups 2 and 3 showed significant decrease in the level of albumin (36.2±6.57 and 39.4±1.51) respectively compared to positively control group5 rats.

Total protein level increased significantly ($p<0.05$), in rats group5 (98.6 ± 2.69) compared with rats groups 1, 2, 3 and 4 (74.9 ± 1.28, 79.7 ± 3.36, 76.5 ± 3.86 and 77.0 ± 2.99) respectively.

Level of ALT increased significantly ($p<0.05$) in untreated rat group5(25. 8 ± 17.35)

compared rats in group1(18.8±15.25). However, there was significant decrease in the ALT levels in the extract treated groups 2 and 3(19.0±3.60 and 21.5±17.04) compared to the untreated rat group5.
Total bilirubin level increased significantly in positively control rat group5 (13.57±3.03) compared to normal control rat group1(1.458±0.32). There was significant decrease in the total bilirubin levels in *A. sativum*extract treated groups 2 an3 (1.763 ± 0.24 and 1.623 ±0.41) respectively, compared untreated rat group 5.

Table 1: Effect of *A. sativum* on serum biochemical changes in CCl_4-induced liver injury.

Experimental Group /Treatment	***Weight Change(g)***	***Albumin (g/l)***	***Total protein (g/l)***	***ALT (U/L)***	***Total Bilirubin (mg/dl)***	***Direct Bilirubin (mg/dl)***
Group1(Normal control) + tween 80 (0.5mg/kg)	20.6± 23.01	35.6±0.98*	74.9 ±1.28*	18.8 ± 15.25*	1.458±0.32*	0.016±0.03
Group2(100mg/kg *A.sativum*+0.5mg/kg CCl_4)	17.6±1.59	36.2±6.57*	79.7 ±3.36*	19.5 ± 12.92*	1.763±0.24*	0.0140±0.003
Group3(200mg/kg *A.sativum* +0.5mg/kg CCl_4)	17.6±21.54	39.4 ±1.51**	76.5 ± 3.86*	19.0 ± 3.60*	1.623±0.41*	0.016±0.003
Group4 (100mg/kg Vit C+0.5mg/kg CCl_4)	21.0±9.53	43.6 ± 0.78***	77.0 ±2.99*	21.5 ± 17.04*	10.64±9.10**	0.025±0.005
Group5 +0.5mg/kg CCl_4 (positive control)	20.4±7.70	44.9 ± 1.50***	98.6 ±2.69**	25. 8± 17.35**	13.57±3.03***	0.0242±0.003

Values are means of five determinations± SD
Values with different superscript in the column differ significantly (P<0.05)

Discussion

The purpose of this study was to explore the antioxidant action ofethanolic extracts of *Allium sativum* leaf in the hepatic damage caused by CCl_4. Administration of CCl_4 to normal rats significantly increased serum levels of albumin, total protein, ALT and bilirubin. This may be due to enzymes leaking out from damaged liver cells into circulating blood which indicates the damage to hepatic cells. It is well established that the toxic metabolite of CCl_4, a free radical CCl_3^- is responsible for damage to liver cells [15]. Increase in ALT in oral acetaminophen-induced hepatotoxicity in rats has been reported as indication for significant liver damage [16]. Mizutani and co-workers (1999) also, reported an increase in serum ALT activity in methimazole-induced hepatotoxicity in mice [17]. However, there was significant decrease in the levels of albumin, ALT, total protein and bilirubin in rats treated with ethanolic extract of *Allium sativum* leaf. This result is in agreement with the earlier reportof the protective role of *A. sativum* against a number of toxic agents such as carbon tetrachloride and cisplatin [18-19].
Several authors have shown that *Allium sativum* is endowed with strong *in vitro* and *in vivo* anti-oxidant properties [20]. The antioxidant action of allicin (diallythiosulfinate) has been proposed as one of the major possible mechanisms for the protective actions of the *A. sativum* against toxicity [21]. Recent report indicated the isolation and identification of allicin from *A. sativum* which is endowed with strong anti-oxidant action both in vivo and in vitro, in addition to strong anti-inflammatory action [21]. This makes it a very effective agent for alleviation of ischemia-reperfusion-induced oxidative hepatic injury in rats [20]. Also, garlic oil has been reported to possess antioxidant properties and provides protection against ethanol induced gastric injury [22]. Recent studies have showed that garlic extract attenuates gentamicin-induced renal damage and oxidative stress in rats [23].

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