

Effects of *alliums sativum* extract on paracetamol – induced hepatotoxicity in albino rats

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In the present study, an attempt has been made to evaluate the presence of antioxidant property and hepatoprotective activity in ethanol extract of *Allium sativum* on paracetamol- induced hepatotoxicity in albino rats. A total of 28 rats were used for the study. The rats were grouped into four with four rats in each group. Group A was the control, group B received paracetamol over dose (500mg / kg / ml) without extracts, and group C received paracetamol over dose plus extract of *A.sativum* at three different concentrations of 200, 400 and 800 ug / body weight. Oral administration of *Allium sativum* extract significantly decreased the level of liver enzymes (ALT, AST and ALP), Total protein and bilirubin to near normal levels. The increased levels of lipid peroxidation in tissues were reverted significantly. The treatment also resulted in a significant increased in GSH, SOD and Catalase in the liver. These results clearly show the antioxidant and hepatoprotective property of *Allium sativum* extract.

Keywords: Paracetamol, Antioxidant Activity, Hepatoprotective property, *Allium sativum* extract.

INTRODUCTION

Plant has been the source of help, survival and good health. Plants are able to be functioning in this capacity because they have many important chemical substances found all over their various parts such as alkaloids, carbon compounds, nitrogen, glycosides, essential oils, fatty oils, resins, mucilage, tannins, gums and others (Osmund, 2001). Most of these are potent bioactive compounds found in medicinal plant parts that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora, 1993).

The Liver is the center of drug metabolism, in the case of an overdose, the liver is overwhelmed by the drug and this can lead to oxidative stress and toxicity. Paracetamol is a drug commonly used for the treatment of mild pain. The metabolism of paracetamol is an excellent example of toxicity. Paracetamol is quite safe at therapeutic doses and normally undergoes glucuronidation and sulfation to the corresponding conjugates. When acetaminophen intake far exceeds therapeutic doses, the glucuronidation and sulfation pathways are saturated and the cytochrome

P450 pathway becomes increasingly important. However, with time hepatic glutathione is depleted faster than it can be regenerated and accumulation of a reactive and toxic metabolite (NAPQI) occurs. In the absence of intracellular antioxidants such as glutathione, this reactive metabolite reacts with nucleophilic groups present on cellular macromolecules such as proteins or lipids to produce toxic free radicals and cause hepatotoxicity (Chen, 2007). *Allium sativum* (garlic) is a bulbous perennial plant of the lily family (liliaceae). *Allium sativum* like other medicinal plants has potential medicinal values which includes antimicrobial and bacterial properties. *Allium sativum* has being proven powerful natural antibiotics, effective in reducing high blood pressure and provides antioxidant protection to cells (Zimmerman, 1996).

In the present study we investigated the effects of the ethanol extracts of *A.sativum* on, some antioxidants indices, ALT, AST, ALP, direct bilirubin and total bilirubin levels in albino rats.

MATERIALS AND METHODS

Collection and Preparation of *Allium sativum*

Extracts

The cloves of *Allium sativum* (200 g) were purchased from Abakpa Market in Abakaliki, Ebonyi State and identified by a plant taxonomist in the Department of Botany, Ebonyi State University, Abakaliki, Nigeria. They were washed thoroughly under running tap water, shade dried and pulverized, using a grinding machine. Ethanol extract was prepared by soaking about 100 g of the powdered leaves in 1500 ml ethanol for 24 hours. The extract was filtered and the solvents removed with rotary evaporations to obtain crude active ingredient.

Experimental Animal

Twenty eight (28) albino rats of both sexes each weighing 122.50 – 165.50g were procured from The Pharmacy Department of University of Nigeria, Nsukka, Nigeria. The animals were acclimatized for 7 days under standard environmental conditions and fed *ad-libitum* on their normal diets. The first group A served as the control group, group B and C were used as test groups, each had 5 rats. Hepatic damage was induced by administering 500 mg of paracetamol per kg rat. The control (group A) was fed with rat feed and water for 7 days. Group B was fed with rat feed, water and paracetamol orally at 500 mg/kg/day without any extract. Group C made up of 15 rats of 5 in each sub groups received paracetamol overdose plus extract of *Allium sativum* at 3 different doses of 200mg/kg, 400 mg/kg and 800 mg/kg for 7 days.

Experimental design

In this experiment, a total of 25 albino rats (20 paracetamol induced Hepatic damage surviving rats, 5 normal rats) were used. Hepatic damage was induced alongside the treatment of the rats with the extracts for 7 days. Varying concentrations of the crude extracts of *Allium sativum* were administered via oral intubation to the animals in groups C (C1, C2 and C3) for a period of 7 days. These served as paracetamol- induced hepatic damage experimental groups while those in group B did not receive the extracts and served as paracetamol-induced hepatic damage control group.

Group A: Normal untreated rats.

Group B: Paracetamol- induced hepatic damage untreated rats

Group C1: Paracetamol- induced hepatic damage rats given ethanol extract of *A.sativum* (200mg/kg body weight) daily using a canular for 7 days

Group C2: Paracetamol- induced hepatic damage rats given ethanol extract of *A. sativum* (400mg/kg body weight) daily using a canular for 7 days.

Group C3: Paracetamol- induced hepatic damage rats given ethanol extract of *A.sativum* (800mg/kg body weight) daily using a canular for 7 day.

After the treatment period (7 days), the animals of all groups were sacrificed. The rats were dissected and 5 ml of blood drawn directly from the heart using syringe. The blood was poured into test tubes, placed inside the centrifuge and centrifuged at 5000 rpm for 10 minutes. After centrifugation, the serum was then separated and used for assay of serum aspartate transaminase activity (AST), alanine transaminase activity (ALT) and serum alkaline phosphatase ALP was measured using the method of Gosh et al, 2007. Direct and total Bilirubin were measured using the method of Malloy and Evelyn, 1937. Total protein was measured using the method of Tietz, 2000. The antioxidants properties were determine as follows:

Determination of Antioxidant Activity

The livers were excised, rinsed in normal saline, followed by Tris-HCl (pH 7.2), blotted dry under air and weighed with weighing balance. A 2g portion of the liver tissue was sliced and then homogenized with 10 ml of normal saline. A part of the homogenate after precipitation of protein using 10 % TCA was used for the following estimation

Determination of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS in tissues was estimated by the method of Fraga *et al* (1981). To 0.5 ml tissue homogenate, 0.5 ml normal saline and 1 ml of 10 % TCA were added, mixed well and centrifuged at 3000 rpm for 20 min. To 1.0 ml of the protein –free supernatant, 0.25 ml of Thiobarbituric acid (TBA) reagent was added; the contents were thoroughly mixed and boiled for 1 hr at 95°C. The test tubes were then cooled to room temperature under running water and absorbance measured at 532 nm.

Determination of Reduced Glutathione (GSH)

GSH was determined by the method of Ellman *et al* (1958). About 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml precipitating reagent (1.67 g of meta phosphoric acid, 0.2 g of EDTA, 0.2 g disodium salt, 30 g sodium chloride in 1 liter of distilled water) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2.0 ml of the filtrate, 4.0 ml of disodium hydrogen phosphate solution and 1.0 ml of DTNB (5, 5-dithio-bis-2-nitrobenzoic acid) reagent were added and read at 412 nm.

Assay of Super Oxide Dismutase (SOD)

The activity of SOD in tissue was assayed by the method of Kakkar *et al* (1984). The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3), 0.1 ml phenazine methosulphate, 0.3 ml NTB (Nitro blue tetrazoline), 0.2 ml NADH and approximately diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30 °C for 90 sec, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against n-butanol.

Assay of Catalase (CAT) Activity

Catalase was assayed according to the method of Maehly and Chance (1954). The estimation was done spectrophotometrically at 230 nm. The tissue was homogenized in 2 ml phosphate buffer (pH 7.0) at 1-4 °C and centrifuged at 5000 rpm. The reaction mixture contained 0.01M phosphate buffer, 2 ml Hydrogen peroxide and the enzyme extract. The specific activity of catalase is expressed in terms of (mole of H₂O₂ consumed/min/mg of protein).

STATISTICAL ANALYSIS

Data for antioxidant activity, reduced GSH, SOD, CAT, AST, ALT, ALP, Total Protein, Albumin, Globulin and Bilirubin were expressed as mean $\pm$ SD and analyzed statistically using One Way Analysis of Variance (ANOVA). The minimum level of significance was fixed at $P < 0.05$.

RESULTS

The effect of the ethanol extracts of *A. sativum* on ALT, AST, ALP, total protein, total and direct bilirubin and some antioxidants indices levels in albino rats as shown in Table 1 and 2 respectively. They were significant increase ($p < 0.05$) in glutathione and catalase levels in the treated groups than the controls as shown in table 1. From the results in Table 1, a significant decrease ($p < 0.05$) was observed in the levels of TBARS and SOD in the liver of rats treated with extract of *A. sativum* as compared to paracetamol treated. And control. The significant increase ($p \leq 0.05$) in AST, ALT, ALP and Bilirubin levels in rats treated with paracetamol overdose show that the paracetamol affected the liver cells causing the enzymes to leak into circulation. However, treatment with ethanol extract of *A. sativum*, caused a significant

decrease ($p < 0.05$) in the elevated levels of AST, ALT, ALP and Bilirubin in a dose dependent order, showing that the extracts contains compounds that can be useful in the treatment of liver damage.

DISCUSSION

Oxidative stress is any condition that consistently interferes with normal body function. Although oxygen is essential for life, its transformation to reactive oxygen species (ROS) may provoke uncontrolled reactions (Sofowora, 1993). Antioxidants offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and other forms of toxicity (Sofowora, 1993).

The significant increase ($p < 0.05$) in glutathione level observed after treatment of animals with extract of *A. sativum* suggests hepatoprotection. The actual mechanism of hepatoprotection of these extracts is not well understood; however chemical constituents of plant extracts have been shown to exhibit antioxidant properties. For example, flavonoids have been reported to contain antioxidant properties (Ghosh *et al.*, 2007). These antioxidant properties of extracts may have contributed to hepatoprotection displayed in this work. Further, cells have a number of mechanisms to protect themselves from the toxic effect of reactive oxygen species (ROS). Glutathione is an intracellular reductant, widely distributed in cells and plays major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds (Fairhurst, 1982). In the present study, the effectiveness of these extract were demonstrated using paracetamol induced rats which is a known model for both hepatic glutathione depletion and injury. Therefore, the level of glutathione is of crucial importance in liver injury caused by paracetamol. These results are in line with a research work by Gosh *et al* (2007), because we found that after *A. sativum* administration, the GSH level increased significantly ($p < 0.05$) in a dose dependent manner as compared with paracetamol treated showing that the extracts may contain principles that might provide a means to recover reduced GSH levels and to prevent tissue disorders and injuries (Ghosh *et al.*, 2007).

From the results (Table 1), a significant decrease ($p < 0.05$) was observed in the level of TBARS, the end products of lipid peroxidation in the liver of rats treated with extract of *A. sativum* as compared to paracetamol treated (Table 1). The increase in TBARS levels in liver suggests enhanced lipid peroxidation leading to tissue damage. Pretreatment with ethanolic extract of *A. sativum* significantly reversed these changes in a dose dependent pattern. Hence it may be possible that the mechanism of hepatoprotection of the extracts is due to

Table 1. Effect of ethanol extract of *Allium sativum* on TBARS, SOD, CAT and GSH in liver of paracetamol – induced hepatic damage in rats.

TREATMENT	CONC.	TBARS ^a	SOD ^b	CAT ^c	GSH ^d
CONTROL		1.17 ± 0.20	12.59 ± 0.05	64.40 ± 1.25	61.59 ± 2.11
PARACETAMOL TREATED		6.66 * ± 0.75	4.49 * ± 0.01	43.31 * ± 3.25	32.42 * ± 1.30
PARACETAMOL + EXTRACT	200mg/kg C ₁	4.69 ± 0.50	4.45 ± 0.00	44.15 ± 3.25	44.42 ± 1.30
	400mg/kg C ₂	4.23 ± 0.42	7.23 ± 0.09	50.75 ± 3.50	50.78 ± 0.95
	800mg/kg C ₃	3.20 ** ± 0.52	10.39 ** ± 0.02	58.48 ** ± 2.31	54.66 ** ± 1.11

Table 2. Effect of ethanol extract of *Allium sativum* on AST, ALT, ALP and some hematological parameters (Bilirubin, Total Protein, Albumin and Globulin) on paracetamol – induced hepatic damage in rats.

Group	Treatment	Concentrations	AST (U/ml)	ALT (U/ml)	ALP (U/ml)	BILIRUBIN		Total Protein
						Total	Direct	
A	Control		37.96 ± 1.88	21.71 ± 0.62	89.23 ± 4.45	0.61 ± 0.02	0.08 ± 0.01	7 1.34
B	Paracetamol treated		123.60* ± 1.94	104.93* ± 1.83	200.57* ± 3.46	5.40* ± 0.31	0.96* ± 0.15	19.85
C	Paracetamol + Extract (<i>A. Sativum</i>)	200µg/ml C ₁	86.65 ± 2.22	84.02 ± 1.05	188.18 ± 3.54	4.30 ± 0.07	0.70 ± 0.03	26.68
		400µg/ml C ₂	66.81 ± 2.46	66.91 ± 0.89	155.63 ± 2.55	2.87 ± 0.48	0.40 ± 0.08	31.40
		800 µg/ml C ₃	50.11** ± 1.07	47.64** ± 1.75	131.41** ± 0.55	0.93** ± 0.01	0.18** ± 0.40	34.34

their ability to reduce or prevent lipid peroxidation. Chen *et al.*, 2007 reported the same decrease or prevention of lipid peroxidation as a possible mechanism to hepatoprotection of the liver by *A. sativum*.

The result on super oxide dismutase (SOD)) showed a significant decrease ($P < 0.05$) in the activity of SOD in the rats treated with paracetamol relative to the control group. This increased significantly ($p < 0.05$) after treatment of animals with extract of *A. sativum* in a dose dependent manner, showing a reversal in the toxicity effect and antioxidant action. Phenols are very important plant constituents because of their free radical scavenging ability and antioxidant action due to their hydroxyl groups. These extracts contain phenolic compounds in significant amount and hence possess antioxidant activity (Sofowora, 1993).

Significant increase ($p < 0.05$) in the level of catalase (CAT) activity after treatment of animals with extracts was observed. This implies that the extract poses antioxidant

activity. It has been documented by, Ghosh *et al.*, 2007 that high levels of the hepatocellular enzymes, (SOD and CAT), serve as biomarkers of hepatocellular injury due to alcohol and drug toxicity. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide (free radicals) into less reactive gaseous oxygen and water molecules. There was a significant difference ($p < 0.05$) produced between the lowest dosage (200 µg/ml) and the highest dosage of (800 µg/ml) of the extracts showing that the extracts are more effective at high concentrations. Our results are in agreement with the report of Gosh *et al.*, (2007) on hepatoprotection and antioxidant activity of some medicinal plants.

The significant increase ($p < 0.05$) in AST, ALT, ALP and Bilirubin levels in rats treated with paracetamol overdose show that the paracetamol affected the liver cells causing the enzymes to leak into circulation. However, treatment with ethanolic extract of *A. sativum*, caused a significant decrease ($p < 0.05$) in the elevated

levels of AST, ALT, ALP and Bilirubin in a dose dependent order, showing that the extracts contains compounds that can be useful in the treatment of liver damage. Serum transaminases are important liver enzymes because they indicate the condition of the liver (Nelson, and Cox, 2000). Liver degeneration due to drug toxicity is accompanied by leakage of liver enzymes from injured hepatocytes into circulation. These transaminases are most useful for monitoring the degree of recovery of a damaged liver (Nelson and Cox, 2000). Effective control of alkaline phosphatase activity and Bilirubin levels points towards an early improvement in secretory mechanism of hepatic cells (Chen, 2007). This is in line with the work of Sorimuthu *et al* (2005), where the antioxidant property of *A. barbadensis* was observed in streptozotocin – induced diabetes in rats.

The extract of *A. sativum* produced a significant increase ($p < 0.05$) in albumin level in the groups; these effects were found to be dose dependent. The reversal of this hypoalbuminaemia by the extract is currently poorly understood. However, albumin performs an important function in binding many substances, reducing their availability and toxic actions. Thus, the binding (conjugation) of bilirubin protects the newborn child against the toxic action of the substance, preventing it from penetrating the blood-brain-barrier. The binding ability of albumin to drugs is reduced in hypoalbuminaemia when binding sites are blocked by various metabolites. Although these hepatoprotection cannot be ascribed to a particular chemical constituent of the extracts, some of their phytochemicals such as flavonoids and phenols are known to poses antioxidant properties.

In summary, the results have demonstrated that overdose of paracetamol intake (500 mg / kg body weight) could be dangerous to the liver. From the findings, the ethanol extracts of *A. sativum* had dose dependent effects on the parameters analyzed, hence its use as antioxidants and hepatoprotective agents may have bases.

In conclusion, this research work has shown the damaging effects of paracetamol overdose and that the medicinal plant *A. sativum* posses high antioxidant activity which can enhance the body defense mechanism

in conditions of oxidative stress and also a hepatoprotective activity which can rid the liver of toxic metabolites.

Further studies regarding the isolation and characterization of the active principles responsible for the antioxidant and hepatoprotective activity of these medicinal plants is recommended. Partial purification of the extracts is also recommended.

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