

## Investigation of the Antihepatotoxic Effects of *Allium sativum* Extracts Against Acetaminophen Intoxicated *Rattus norvegicus*

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**Abstract:** This present study was undertaken to establish the hepatoprotective effects of *Allium sativum* methanolic extracts on paracetamol induced hepatotoxic rats. Fifty-four (54) adult male albino rats comprising of nine normal and forty-five paracetamol hepatotoxic rats were used for this study. The experimental design was the three by three Latin square design. Paracetamol hepatotoxicity was induced by single administration of paracetamol at 750mg/kg on the first day of the experiment. The different biochemical parameters assessed were determined before the start of the study and subsequently monthly for the duration of the study. Blood samples were collected from the rat through the retro orbital plexus for analysis and serum was obtained by centrifugation (5000rpm for 10mins) and stored at -20°C prior to analysis. The effects of duration and increasing dosages (200, 300 and 450mg/kg) of *A. sativum* methanolic extracts produced a duration dependent significant ( $p < 0.05$ ) reductions in the alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total serum bilirubin (TSB) of paracetamol hepatotoxic rats after the duration of the study when compared with those of the paracetamol, normal and silymarin control rats. *A. sativum* reduced alanine aminotransferase and total serum bilirubin in a dose dependent fashion whereas it reduced aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase level in a dose independent manner. The result of this experimental study suggested that the administration of *A. sativum* extracts protected against paracetamol liver damage in rats and therefore may be a potent hepatoprotective agent. These encouraging results may have future clinical implications because of the increased use of natural herbs worldwide and Nigeria in particular. Further studies are needed to unravel the exact mechanism of action of *A. sativum*.

**Key words:** *Allium sativum* • Paracetamol • Hepatoprotective effects • Alanineaminotransferase • Aspartate aminotransferase • Alkaline phosphatase • Lactate dehydrogenase • Total serum bilirubin

### INTRODUCTION

Paracetamol (acetaminophen) produces severe hepatotoxicity if an over dose is consumed in experimental rats [1]. It is safe in recommended doses but deliberate or

accidental overdose is very common because of its wide availability and this often leads to liver damage. Paracetamol overdose has been reported to cause acute centrilobular hepatic necrosis which is now one of the most common causes of liver failure amongst British

populations [2]. Paracetamol is mainly metabolized in the liver to excretable glucuronide and sulphate conjugates [3, 4]. The hepatotoxicity of paracetamol is due to the formation of toxic metabolites formed when a part of paracetamol is activated by hepatic cytochrome P-450 [5], to a highly reactive metabolic N-acetyl-P-benzoquinone imine (NAPQ1) [6]. NAPQ1 is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid [7]. When the rate of NAPQ1 formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of protein and alters the homeostasis of calcium after depleting GSH. *Allium sativum* commonly known as garlic is a bulb-forming herb of Liliaceae family and genus *Allium* [8]. It is cultivated in some parts of Nigeria and used as meat tenderizer and spice in many delicacies [9]. The traditional medical practitioners have considered garlic as an excellent medicinal plant that has a lot of therapeutic potentials. Garlic is used as anti-hypertensive herb [8] and in the treatment of various ailments like asthma, cold, paralysis, forgetfulness, tremor, colicky pain and chronic fever [9, 10], it has been found that garlic lowered blood pressure and cholesterol level [11, 12] and possesses strong anti-microbial level [13]. Garlic aqueous extracts has anti-diabetic [14], hypolipidaemic effects [15], hypoglycaemic effects [16] and haematological effects [17]. Garlic has been used as a traditional medicine in the treatment of heart diseases, tumors and headaches and exhibits medicinal properties including immunomodulation, antioxidant, antimutagenic, antibacterial and anticarcinogenic effects [18]. Moreover, garlic has also been reported to possess antifungal effects [19]. The active principle present in garlic is organosulphur compounds such as allicin, allin, alliinase, S-allyl cysteine diallyl disulphide and allyl methyl trisulphide [20]. These active compounds are mainly responsible for protecting from tissue damage and various disorders. Herbal based therapies for hepatoprotection has been in use for a long time and has been popularized world over by leading pharmaceuticals [21]. Given our earlier experimental studies on the hepatoprotective effects of *Allium cepa* (onions) against paracetamol induced liver damage in rats [21], it becomes necessary to extend more research on more members of the Genus *Allium*. This present experimental study was then designed to evaluate the hepatoprotective effects of *Allium sativum* (garlic) methanolic extracts against paracetamol induced liver damage in rats viz - a - viz biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline

phosphatase (ALP), lactate dehydrogenase (LDH) and total serum bilirubin (TSB) level of paracetamol induced hepatotoxic rats.

## MATERIALS AND METHODS

**Plant Materials:** The *A. sativum* used for the study was bought from Ogige market, Nsukka, Enugu State, Nigeria. The plant was identified [22], to species level at the Herbarium Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

**Animal Model:** Fifty-four (54) adult white wistar strain male albino rats (*R. norvegicus*) weighing 180 to 200g were used for the study. They were fed *ad libitum* with 18% crude protein (Guinea feed) commercial feed and allowed to acclimatize for two weeks under standard photoperiodic condition in a clean rat cage with three rats per cage in the research laboratory. All animals were maintained under the standard laboratory condition for temperature ( $26 \pm 2^\circ\text{C}$ ), humidity ( $50 \pm 5\%$ ) and light (12 hours day length) and were allowed free access to food and water.

**Preparation of Plant Extracts:** Fresh healthy *A. sativum* were washed, cut into small pieces and homogenized in a warring blender. The resulting mixture was extracted in two litres of 80% methanol. The mixture was allowed to stand for twenty four hours with intermittent shaking. Following filtration, the filtrate obtained was concentrated to dryness at  $40^\circ\text{C}$  using a rotary evaporator under reduced pressure. The extract was kept in refrigerator thereafter, dissolved in distilled water and used for the study.

**Induction of Paracetamol Hepatotoxicity in Rats:** The minimum dose of paracetamol that causes death in rats is 1060mg/kg and the median lethal dose ( $\text{LD}_{50}$ ) is 765mg/kg [23]. Paracetamol hepatotoxicity was induced by single administration of solution of paracetamol at 750mg/kg intraperitoneally. After 4 days only rats with ALT levels above 65U/l were considered hepatotoxic and used for the study. The normal ALT standard reference range for experimental studies is 10 - 40U/l.

**Experimental Design:** The study was carried out on paracetamol-induced hepatotoxic rats for three months. The experimental design was the three by three Latin square design. Fifty-four rats used were divided into two major groups:

**Group I:** Nine non-hepatotoxic rats (Normal control)

**Group II:** Forty-five paracetamol induced hepatotoxic rats.

The group I rats were three rats each in three different cages and each received 1ml/kg of distilled water daily throughout the duration of the study. The Group II rats (paracetamol induced hepatotoxic rats) were divided into three subgroups (IIa, IIb, IIc). The subgroup IIa was the paracetamol control, three rats in a cage and was replicated thrice and had 3 rats each which received 750mg/kg of paracetamol only [24, 25]. Subgroup IIb was divided into 3 replicates (Iib1, Iib2 and Iib3) respectively each replicate had 3 rats and received 200 mg/kg, 300 mg/kg or 450 mg/kg of *A. sativum* methanolic extracts orally daily. The subgroup IIc, three rats each in a cage and replicated thrice received the standard drug silymarin at 100mg/kg [26]. The different biochemical parameters (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase and total serum bilirubin) assessed were determined first before the start of the experiment and subsequently monthly for the duration of the study. Blood samples were collected from the rat through the retro orbital plexus monthly for analysis. Serum was obtained by centrifugation (5000rpm for 10 mins) and stored at -20°C prior to analysis.

**Evaluation of Biochemical Parameters:** Serum alanine aminotransferase and aspartate aminotransferase levels were determined by colorimetric method of [27] and absorbance was read at 505nm using spectrophotometer. Alkaline phosphatase level in serum was determined by the method of [28]. Serum was incubated with disodium phenylphosphate as substrate buffered at PH 10 for 15 minutes at 37°C. The hydrolytic products, phenol was condensed with 4-amino antipyrine and then oxidized with alkaline ferricyanide and the red complex developed was read at 510nm using spectrophotometer. Lactate dehydrogenase level was estimated by the method of [29], where the reduction of nucleoside derived amino acids (NAD) was coupled with the reduction of tetrazolium salt and the produced formazan was measured using spectrophotometer at 503nm. Total serum bilirubin was determined following the method of [30]. Diazotised sulphonic acid reacts with bilirubin in diluted serum and forms purple colored azobilirubin which was read at 540nm using spectrophotometer.

**Data Analysis:** The data collected was pooled and analyzed for their central tendencies using descriptive statistic, values were given as mean  $\pm$  standard deviation

of the observations. Analysis of variance and LSD was employed to test the significant differences ( $p < 0.05$ ) among treatment means. All analyses were performed using SPSS for windows statistical software package version 16. The resulting outputs were presented in figures.

## RESULTS

**Alanine Aminotransferase Level:** *Allium sativum* methanolic extracts produced a time dependent significant ( $p < 0.05$ ) reductions in the alanine aminotransferase levels of paracetamol hepatotoxic rats after twelve weeks of treatment when compared with those of the paracetamol and silymarin control rats. Alanine aminotransferase levels were significantly higher in paracetamol control groups throughout the 12 weeks of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group (1ml/kg of distilled water). *A. sativum* reduced alanine aminotransferase level in a dose dependent fashion across the 12 weeks of study with *A. sativum* at 200mg/kg reducing alanine aminotransferase level by 23.77%, at 300mg/kg it was reduced by 24.28% while at 450mg/kg it was lowered by 19.61% compared with paracetamol control at week 4 (Figure 1). Silymarin at 100mg/kg reduced alanine aminotransferase level by 30.15% after twelve weeks of treatment compared with paracetamol control at week 4 (Figure 1). Normal control had no significant effect on alanine aminotransferase level whereas the paracetamol treated control raised alanine aminotransferase level by 8.19%.

**Aspartate Aminotransferase Level:** *A. sativum* methanolic extracts produced a time dependent significant ( $p < 0.05$ ) reductions in the aspartate aminotransferase level of paracetamol hepatotoxic rats after twelve weeks of treatment when compared with that of the paracetamol and silymarin control rats. Aspartate aminotransferase level was significantly higher in paracetamol control groups throughout the 12 weeks of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group (1ml/kg of distilled water). *A. sativum* reduced aspartate aminotransferase level in a dose independent fashion across the 12 weeks of study with *A. sativum* at 200mg/kg reducing aspartate aminotransferase level by 44.93% at 300mg/kg it was reduced by 43.75% while at 450mg/kg it reduced it by 44.79% after 12 weeks of treatments compared with

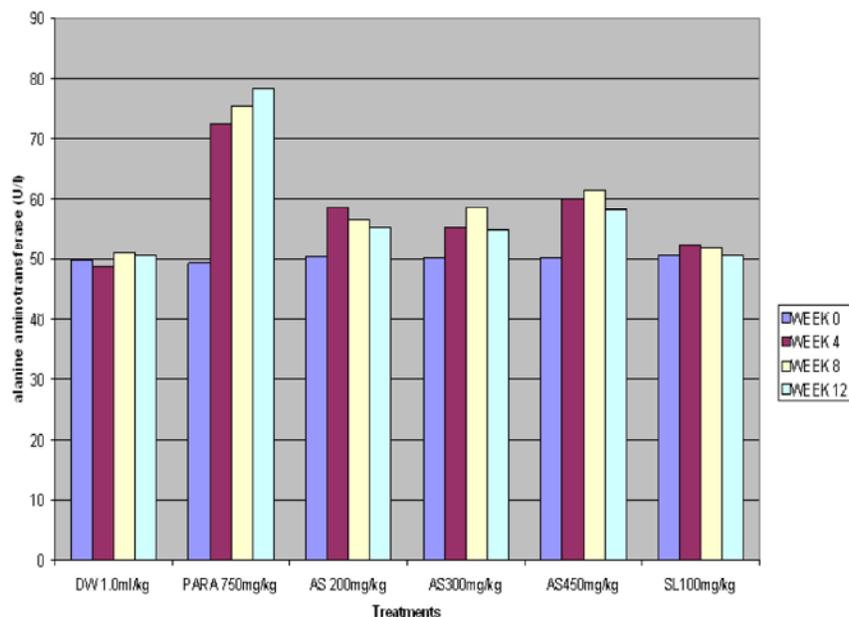


Fig. 1: Effects of *Allium sativum* methanolic extracts on alanine aminotransferase of paracetamol-induced hepatotoxic rats

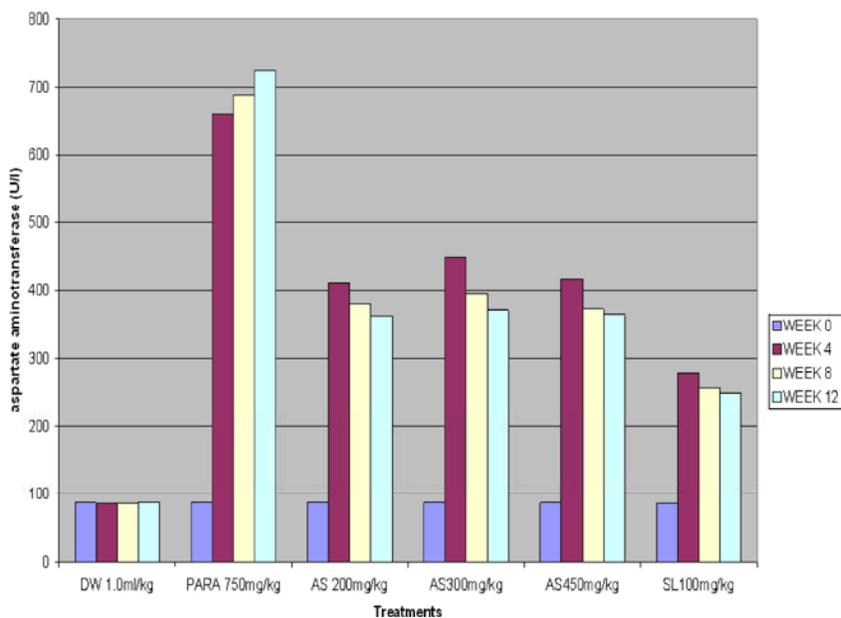


Fig. 2: Effects of *Allium sativum* methanolic extract on aspartate aminotransferase of paracetamol hepatotoxic rats

paracetamol control at week 4 (Figure 2). Silymarin at 100mg/kg reduced aspartate aminotransferase level by 62.26% after twelve weeks of treatment compared with paracetamol control at week 4 (Figure 2). Normal control had no significant effect on aspartate aminotransferase level whereas the paracetamol treated control raised aspartate aminotransferase level by 8.97%.

**Alkaline Phosphatase Level:** *Allium sativum* methanolic extracts produced a time dependent significant ( $p < 0.05$ ) reductions in the alkaline phosphatase level of paracetamol hepatotoxic rats after twelve weeks of treatment when compared with that of the paracetamol and silymarin control rats. Alkaline phosphatase level where significantly higher in paracetamol control groups

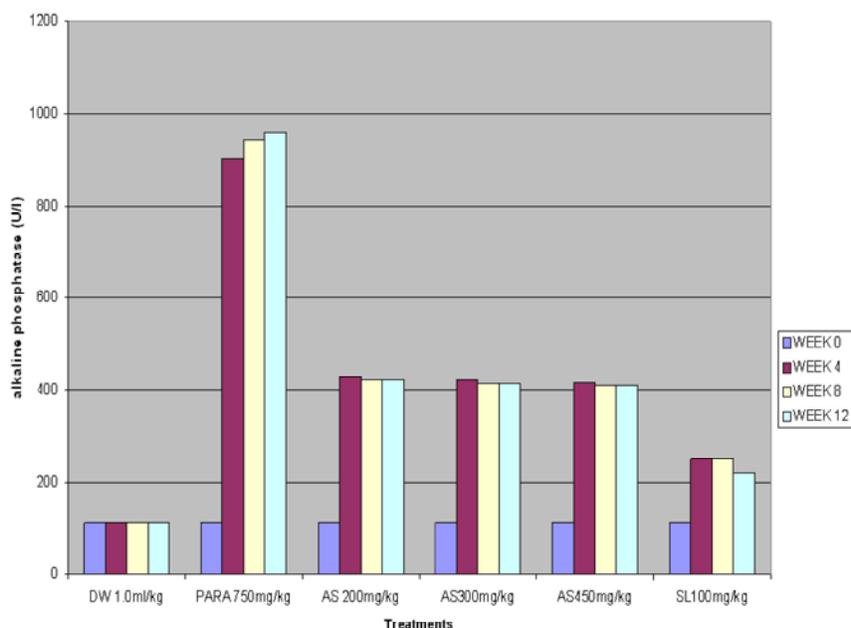


Fig. 3: Effects of *Allium sativum* methanolic extracts on alkaline phosphatase of paracetamol induced hepatotoxic rats

throughout the 12 weeks of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group (1ml/kg of distilled water). *A. sativum* reduced alkaline phosphatase level in a dose dependent manner across the 12 weeks of study with *A. sativum* at 200mg/kg reducing alkaline phosphatase level by 53.17% at 300mg/kg it was reduced by 54.08% while at 450mg/kg it was reduced by 54.75% after 12 weeks of treatments compared with paracetamol control at week 4 (Figure 3). Silymarin at 100mg/kg reduced alkaline phosphatase level by 75.70% after twelve weeks of treatment compared with paracetamol control at week 4 (Figure 3). Normal control had no significant effect on alkaline phosphatase level whereas the paracetamol treated control raised alkaline phosphatase level by 6.00%.

**Lactate Dehydrogenase Level:** *Allium sativum* methanolic extracts produced a time dependent significant ( $p < 0.05$ ) reductions in the lactate dehydrogenase level of paracetamol hepatotoxic rats after twelve weeks of treatment when compared with that of the paracetamol and silymarin control rats. Lactate dehydrogenase level where significantly higher in paracetamol control groups throughout the 12 weeks of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group (1ml/kg of distilled water). *A. sativum* reduced

lactate dehydrogenase level in a dose independent manner across the 12 weeks of study with *A. sativum* at 200mg/kg reducing lactate dehydrogenase level by 45.08% at 300mg/kg it was reduced by 45.94% while at 450mg/kg it was reduced by 44.43% after 12 weeks of treatments compared with paracetamol control at week 4 (Figure 4). Silymarin at 100mg/kg reduced lactate dehydrogenase level by 63.08% after twelve weeks of treatment compared with paracetamol control at week 4 (Figure 4). Normal control had no significant effect on lactate dehydrogenase level whereas the paracetamol treated control raised lactate dehydrogenase level by 6.79%.

**Total Serum Bilirubin Level:** *Allium sativum* methanolic extracts produced a time dependent significant ( $p < 0.05$ ) reductions in the total serum bilirubin level of paracetamol hepatotoxic rats after twelve weeks of treatment when compared with those of the paracetamol and silymarin control rats. Total serum bilirubin level where significantly higher in paracetamol control groups throughout the 12 weeks of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group (1ml/kg of distilled water). *A. sativum* reduced total serum bilirubin level in a dose dependent manner across the 12 weeks of study with *A. sativum* at 200mg/kg reducing total serum bilirubin level by 48.46% at 300mg/kg it was

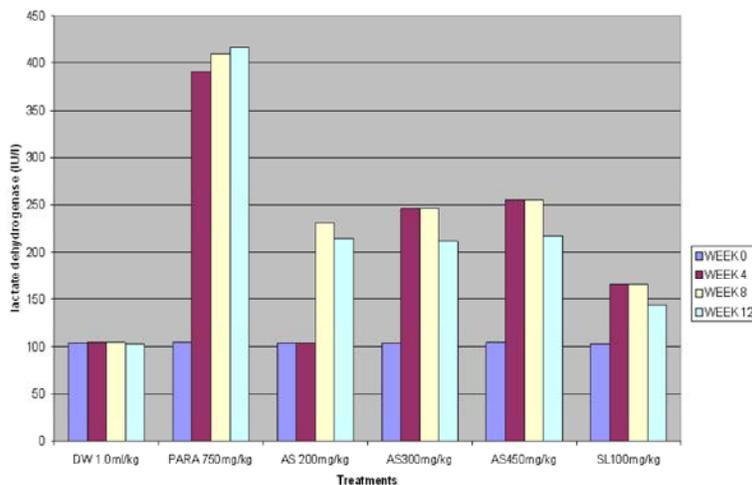


Fig. 4: Effects of *Allium sativum* methanolic extracts on lactate dehydrogenase of paracetamol hepatotoxic rats

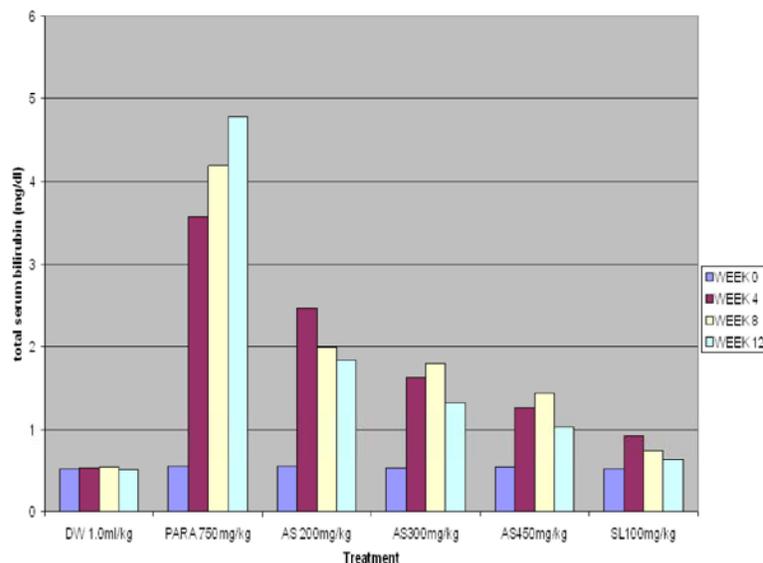


Fig. 5: Effects of *Allium sativum* methanolic extracts on total serum bilirubin of paracetamol -induced hepatotoxic rats

reduced by 62.75% while at 450mg/kg it was reduced by 71.15% after 12 weeks of treatments compared with paracetamol control at week 4 (Figure 5). Silymarin at 100mg/kg reduced total serum bilirubin level by 82.35% after twelve weeks of treatment compared with paracetamol control at week 4 (Figure 5). Normal control had no significant effect on total serum bilirubin level whereas the paracetamol treated control raised total serum bilirubin level by 33.89%.

## DISCUSSION

*Allium sativum* (garlic) has been found to have an important dietary and medicinal role for centuries. Most of its prophylactic and therapeutic effects were ascribed to

specific oil and water soluble organosulfur compounds like thiosulfinates and secondary metabolites of garlic, including steroids, terpenoids, flavonoids and phenols which may have been responsible for its reported therapeutic effects. When the liver gets damaged after paracetamol hepatotoxicity, it leads to leakage of cellular enzymes into the plasma [31]. The increased levels of serum enzymes such as ALT, AST, ALP and LDH observed in hepatotoxic rats, resulted in liver damage, increased permeability and necrosis of hepatocytes [32]. These significant increase observed in the level of serum aminotransferase (AST and ALT) in paracetamol treated rats compared to the normal rats in this study could be due to hepatocellular damage because these enzymes are normally located in the cytoplasm and released into the

circulation after cellular damage [33]. The mechanism of action of *A. sativum* could be by the prevention of the intracellular enzyme release and its membrane stabilizing effects [34]. This is because *A. sativum* are rich in strong antioxidant [35, 36]. The reduction in ALP and LDH levels by extracts may suggest repairing of rats liver by *A. sativum* extracts. The possible mechanism of action may be by the active ingredients in *A. sativum* (allyl propyl disulfide) which could have increased the levels of glutathione which binds to the toxic metabolites of paracetamol such as N-acetyl-p-benzoquinone imine (NAPQI) and increased its rate of excretion from the body. It might also have inhibited the levels of the cytochrome P-450 enzyme system which decreased the formation of NAPQI from ingested paracetamol. Administration of silymarin on paracetamol treated rats reduced the level of liver enzymes (AST, ALT, ALP and LDH) in the serum and this could be due to its strong antioxidant properties. The elevation in total serum bilirubin observed in paracetamol hepatotoxic rats suggested abnormal conjugation of bilirubin by the liver due to generalized hepatocellular damage [37]. Total serum bilirubin was decreased in paracetamol hepatotoxic rats after treatment with silymarin and *A. sativum* extracts. The possible mechanism of action of *A. sativum* extracts may be through their antioxidative effects. This is because *A. sativum* has active ingredients that are capable of free radical scavenging in living system [38].

### CONCLUSIONS

The results of this experimental study suggested that the administration of *A. sativum* extracts protected against paracetamol liver damage in rats and therefore it could be a potent hepatoprotective agent. These encouraging results may have future clinical implication because of the increased use of natural herbs worldwide and Nigeria in particular. Further studies are needed to unravel the exact mechanism of action of *A. sativum*.

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