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Comparative restorative effects of plant extracts against acetaminophen-induced liver toxicity

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ABSTRACT

In view of the increase in liver toxicity caused by acetaminophen overdose in the past few decades, this present study was designed to compare the restorative effects of three plant extracts against acetaminophen-induced liver toxicity in rats. One hundred and eight (108) adult male albino rats comprising of nine normal and ninety-nine acetaminophen hepatotoxic rats were used for the study and acetaminophen liver toxicity was induced by single administration of acetaminophen at 750mg/kg ip on the first day of the experiment. The summary of comparative restorative effects of A. cepa, A. sativum and Z. officinale extracts showed that Z. officinale at 450mg/kg produced the best cumulative percentage decrease (-212.34) on all the restorative parameters assessed and ranked 9 on a 10 points scale, followed by Z. officinale at 300mg/kg (-206.79), ranked 8 and then Z. officinale at 200mg/kg (-191.79) and ranked 7. They were closely followed by A. sativum at 450mg/kg (-190.3), ranked 6, A. sativum at 300mg/kg (-184.86), ranked 5 and A. cepa at 300mg/kg (179.28), ranked 4. Similarly A. cepa at 450mg/kg (-179.05) ranked 3, A. sativum at 200mg/kg (-170.33) ranked 2 and A. cepa at 200mg/kg (-164.56) ranked 1 on a 10 points scale. Normal control did not have significant percentage change; acetaminophen control had a cumulative percentage increase of about 57.05 whereas silymarin decreased the parameters cumulatively with -250.46 percent. Zingiber officinale produced the best significant restorative effect when compare to Allium cepa and Allium sativum extracts.

Keywords: Comparative, restorative effects, *Allium cepa*, *Allium sativum*, *Zingiber officinale*, Acetaminophen, Liver toxicity.

INTRODUCTION

Acetaminophen (also known as paracetamol) is a commonly used, effective analgesic and antipyretic agent for the relief of mild and moderate pain. Drug induced liver toxicity (DILT) is a health problem worldwide and is expected to increase as the number of drugs being consumed increases, both prescription and non-prescription drugs. DILT is the most commonly cited reason for withdrawal of already approved drugs from the market [1]. In the United States, drug-induced liver toxicity accounts for more than 50% of acute liver failures, with liver toxicity caused by overdose of acetaminophen accounting for 39% [2]. DILT accounts for approximately half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease [3]. Despite the frequency of DILT being relatively low, data from the Centers for Disease Control and Prevention in the United States reported approximately 1600 new acute cases of liver failure annually, of which acetaminophen liver toxicity accounts for approximately 41% [4]. Many research efforts have been directed towards the provision of empirical proof to back up the use of many tropical plants in trado-medicinal practices [5, 6]. Extracts of 50 plants have been reported to ameliorate liver toxicity in animal models [7, 8, 9, 10]. There are few drugs that stimulate liver function, offer protection to the liver from toxicity or help regeneration of hepatic cells [11]**.** In the face of the paucity of reliable liver protective drugs in

modern medicine, herbs will play a good role in the management of various liver toxicity most of which speeds up the natural healing processes of the liver. To rationalize the use of herbs in management of liver toxicity, a comparative scientific study on them is necessary to pin point the strength and weakness of each plant extracts in the management of liver toxicity. This present study was designed to compare the restorative effects of increasing dosage of *Allium cepa (*onions), *Allium sativum* (garlic) and *Zingiber offticinale* (ginger) extracts against acetaminophen induced liver toxicity in rats viz-a-viz alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total serum bilirubin (TSB).

EXPERIMENTAL SECTION

Experimental Animals

One hundred and eight (108) adult white wistar strain male albino rats (*R. norvegicus*) weighing 200 to 250g were used for the study. They were fed *ad libitum* with 18% crude protein (Guinea feed) commercial feed. They were allowed to acclimatize for two weeks under standard photoperiodic condition in a clean rat cage with three rats per cage in the research laboratory. Analyses were carried out at Physiology and Biochemistry Laboratories, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. All animals were maintained under the standard laboratory condition for temperature (26 ± 2^0C), humidity (50±5%) and light (12 hours day length) and were allowed free access to food and water.

Preparation and Extraction

Fresh healthy each of *A. cepa*, *A. sativum* and *Z. officinale* (2000g) were washed, cut into small pieces and homogenized in a warring blender. The resulting mixture was soaked 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^oC using a rotary evaporator under reduced pressure. The dried extracts were weighed and then stored in a refrigerator.

Acetaminophen-induced Liver Toxicity in Rats

The minimum dose of acetaminophen that causes death in rats is 1060mg/kg and the median lethal dose (LD_{50}) is 765mg/kg [12]. Acetaminophen liver toxicity was induced by single administration of solution of acetaminophen at 750mg/kg intraperitoneally. After 4 days only rats with ALT levels above 65U/l were considered hepatotoxic and used for the study. Several researchers have induced liver toxicity in rats by single administration of solution of acetaminophen on rats at 750mg/kg ip [13]**.**

Experimental Methodology

The study was carried out on acetaminophen- induced hepatotoxic rats for three months. The experimental design was the three by three Latin square design. One hundred and eight rats used were divided into two major groups:

Group I: Nine non hepatotoxic rats (non-hepatotoxic control).
Group II: Ninety-nine acetaminophen induced hepatotoxic rats.

The group I rats were three rats each in three different cages and each received 1ml/kg of 5% methanol solution daily throughout the duration of the study.

The Group II rats (acetaminophen induced hepatotoxic rats) were divided into 5 subgroups (IIa, IIb, IIc, IId, IIe). The subgroups IIa was the acetaminophen hepatotoxic control (three rats) and were replicated thrice and had three rats each which received 750mg/kg of acetaminophen only [15, 16]. Subgroups IIb, IIc and IId were divided into 3 replicates (IIb_1, IIb_2, IIb_3, IIc_1, IIc_2, IIc_3 and II_{d1}, II_{d2}, II_{d3}) respectively each replicate had 3 rats and received 200mg/kg, 300mg/kg or 450mg/kg of *A. cepa*, *A. sativum and* Z. *officinale* extracts daily. The subgroups IIa and IIe was the acetaminophen hepatotoxic control and standard (nine rats each) and were each replicated thrice, had three rats each and received 750mg/kg of acetaminophen [14, 15] and 100mg/kg of antihepatotoxic drug silymarin [16]. The different biochemical parameters assessed were determined first before the start of the study and subsequently monthly for the duration of the study. Blood samples were collected from the rat through the eye monthly for analysis. Serum was obtained by centrifugation (5000rpm for 10 mins) and stored at -20^oC prior to analysis.

Biochemical Studies

Serum alanine aminotransferase and aspartate aminotransferase levels were determined by colorimetric method of [17] and absorbance was read at 505nm using spectrophotometer. Alkaline phosphatase level in serum was determined by the method of [18]. Serum was incubated with disodium phenylphosphate as substrate buffered at P^H 10 for 15 minutes at 37^oC. The hydrolytic products, phenol was condensed with 4-amino antipyrine and then

oxidized with alkaline ferrcyanide and the red complex developed was read at 510nm using spectrophotometer. Total serum bilirubin was determined following the method of [19]**.** Diazotised sulphonilic acid reacts with bilirubin in diluted serum and forms purple colored azobilirubin which was read at 540nm using spectrophotometer.

Statistical Analysis

The data collected was pooled and analyzed for their central tendencies using descriptive statistic, values were given as mean ± 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 and table.

RESULTS AND DISCUSSION

A. cepa reduced alanine aminotransferase level in a dose dependent fashion across the duration of study with *A. cepa* at 200mg/kg reducing alanine aminotransferase level by 15.79%, at 300mg/kg it was reduced by 20.67% whereas at 450mg/kg it was lowered by 21.99% after the duration of treatments when compared to acetaminophen control at week 4. *A. sativum* reduced alanine aminotransferase level in a dose independent fashion across the duration 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% after the duration of treatments compared with acetaminophen control at week 4 (Figure 1). *Z. officinale* reduced alanine aminotransferase level in a dose dependent fashion across the duration of study with *Z. officinale* at 200mg/kg reducing alanine aminotransferase level by 20.58% at 300mg/kg it was reduced by 21.94% whereas at 450mg/kg it was reduced by 22.23% after the duration of treatments. Silymarin at 100mg/kg reduced alanine aminotransferase level by 30.15% after the duration of treatment compared with acetaminophen control at week 4 (Figure 1). Normal control had no significant effect on alanine aminotransferase level whereas the acetaminophen treated control raised alanine aminotransferase level by 8.19%. Among the extracts studied, after the duration of treatment, *A. sativum* at 300mg/kg bw ip was the most potent in reducing alanine aminotransferase level reducing it by 24.28%, *A. sativum* at 200mg/kg followed with 23.77%, *Z. officinale* at 450mg/kg was next with 22.23% and *A. cepa* at 450mg/kg with 21.98%. The comparative restorative effects of plant extracts studied, compared with normal control, acetaminophen control and silymarin, indicated that *A. sativum* at 300mg/kg bw ip caused significant percentage reduction in alanine aminotransferase level quite comparable to that of silymarin standard drug at 100mg/kg

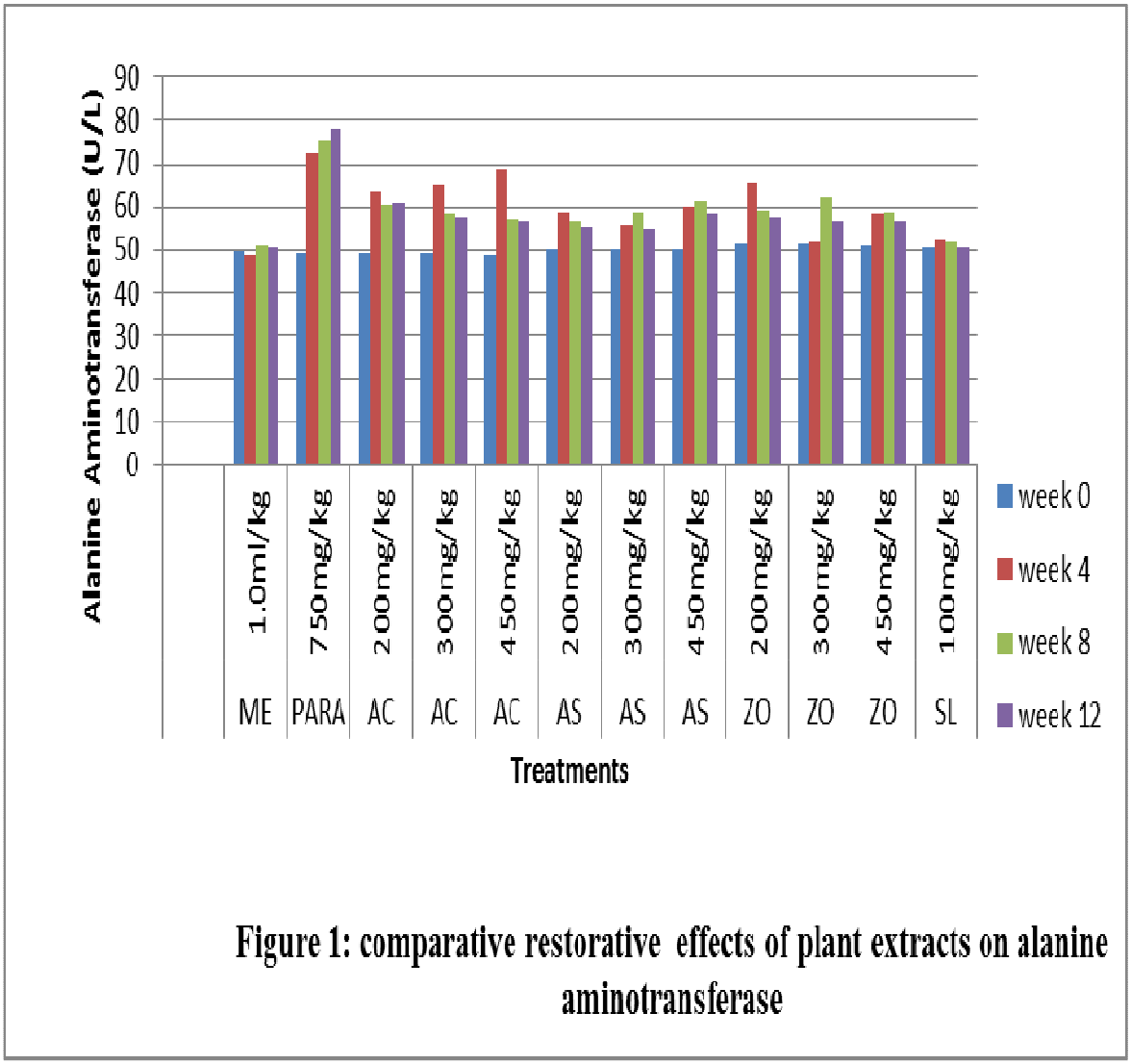

Figure 1: comparative restorative effects of plant extracts on alanine aminotransferase

ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Acetaminophen negative control, AC = Allium cepa, AS = Allium sativum, ZO = Zingiber officinale and SL= Silymarin representing hepatotoxic control.

A. cepa reduced aspartate aminotransferase level in a dose independent fashion across the duration of study with *A. cepa* at 200mg/kg reducing aspartate aminotransferase level by 41.77% at 300mg/kg it was reduced by 39.57% whereas at 450mg/kg it was lowered by 38.59% after the duration of the treatments when compared to acetaminophen control at week 4. *A. sativum* reduced aspartate aminotransferase level in a dose independent fashion across the duration 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 the duration of treatments compared with acetaminophen control at week 4 (Figure 2). *Z. officinale* reduced aspartate aminotransferase level in a dose independent fashion across the duration of study with *Z. officinale* at 200mg/kg reducing aspartate aminotransferase level by 52.72% at 300mg/kg it was reduced by 59.60% whereas at 450mg/kg it reduced it by 51.38% after the duration of treatment. Silymarin at 100mg/kg reduced aspartate aminotransferase level by 62.26% after the duration of treatment compared with acetaminophen control at week 4 (Figure 2). Normal control had no significant effect on aspartate aminotransferase level whereas the acetaminophen treated control raised aspartate aminotransferase level by 8.97%. Among the extracts studied, after the duration of treatment, *Z. officinale* at 200mg/kg was the most effective in reducing aspartate aminotransferase level, reducing it by 59.72%, *Z. officinale* at 300mg/kg followed with 59.60%, *Z. officinale* at 450mg/kg was next with 51.38% and *A. sativum* at 200mg/kg with 44.93%. The comparative effects of the increasing dosage of the plant extracts studied, compared with normal control, acetaminophen control and silymarin, indicated that *Z. officinale* at 200mg/kg caused significant percentage reduction in aspartate aminotransferase level quite comparable to that of silymarin standard drug at 100mg/kg.

Figure 2: comparative restorative effects of plant extracts on aspartate aminotranferase level

ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Acetaminophen negative control, AC = Allium cepa, AS = Allium sativum, ZO = Zingiber officinale and SL= Silymarin representing hepatotoxic control.

A. cepa reduced alkaline phosphatase level in a dose independent manner across the duration of study with *A. cepa* at 200mg/kg reducing alkaline phosphatase level by 52.94%, at 300mg/kg it was reduced by 53.77% whereas at 450mg/kg it was lowered by 50.68% after the duration of treatments when compared to acetaminophen control at week 4. *A. sativum* reduced alkaline phosphatase level in a dose dependent manner across the duration of the 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 the duration of treatments compared with acetaminophen control at week 4 (Figure 3). *Z. officinale* reduced alkaline phosphatase level in a dose independent manner across the duration of the study with *Z. officinale* at 200mg/kg reducing alkaline phosphatase level by 54.63%, at 300mg/kg it was reduced by 56.90% whereas at 450mg/kg it was reduced by 56.85% after the duration of treatment. Silymarin at 100mg/kg reduced alkaline phosphatase level by 75.70% after the duration of treatment compared with acetaminophen control at week 4 (Figure 3). Normal control had no significant effect on alkaline phosphatase level whereas the acetaminophen treated control raised alkaline phosphatase level by 6.00%. Among the extracts studied, after the duration of treatment, *Z. officinale* at 300mg/kg was the most potent in reducing alkaline phosphatase level reducing it by 56.90%, *Z. officinale* at 450mg/kg followed with 56.85%, *A. sativum* at 450mg/kg was next with 54.75% and *Z. officinale* at 200mg/kg with 54.63%. The comparative effects of the increasing dosage of the plant extracts studied, compared with normal control, acetaminophen control and silymarin, indicated that *Z. officinale* at 300mg/kg caused significant percentage reduction in alkaline phosphatase level that is close to that of silymarin standard drug at 100mg/kg.

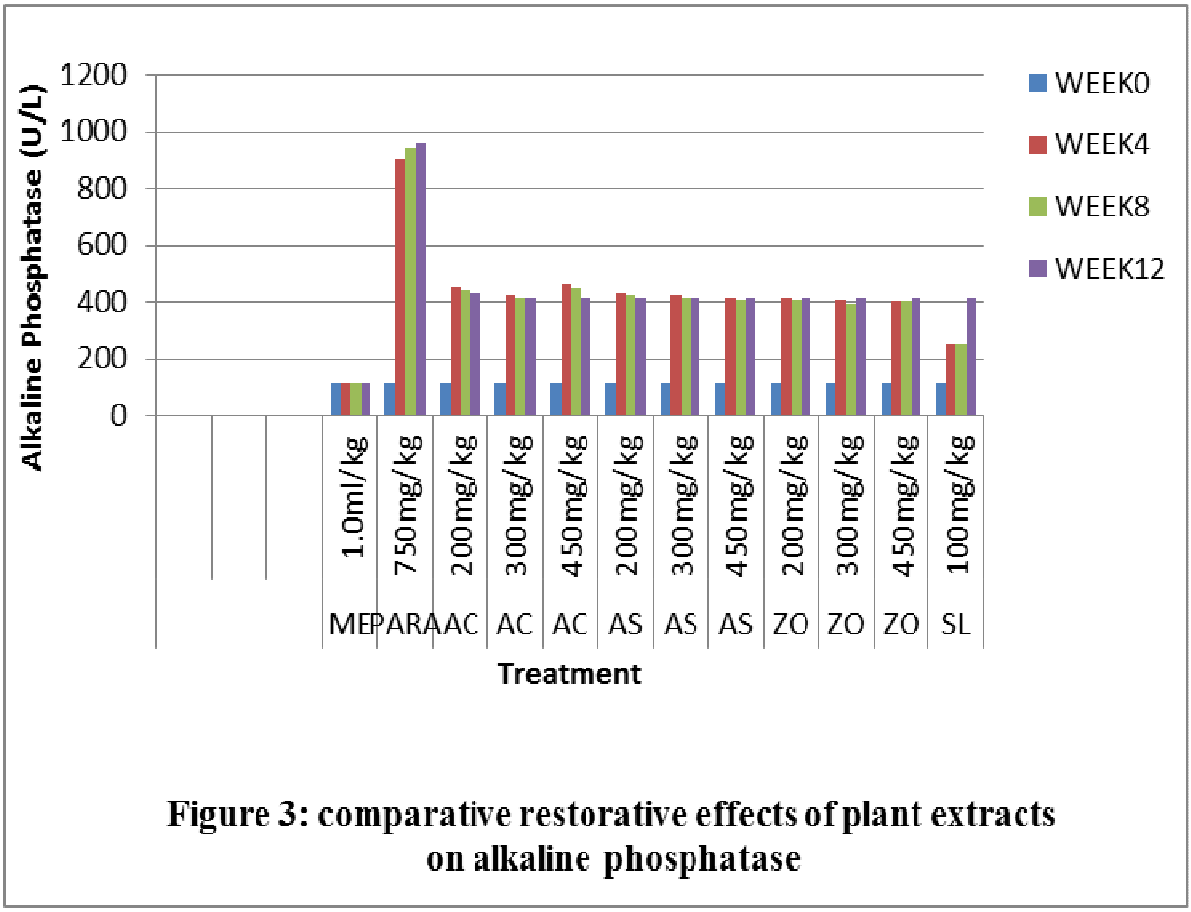

Figure 3: comparative restorative effects of plant extracts on alkaline phosphatase

ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Acetaminophen negative control, AC = Allium cepa, AS = Allium sativum, ZO = Zingiber officinale and SL= Silymarin representing hepatotoxic control.

A. cepa reduced total serum bilirubin level in a dose dependent manner across the duration of study with *A. cepa* at 200mg/kg reducing total serum bilirubin level by 54.06%, at 300mg/kg it was reduced by 65.27% whereas at 450mg/kg it was lowered by 67.79% after the duration of treatments when compared to acetaminophen control at week 4. *A. sativum* reduced total serum bilirubin level in a dose dependent manner across the duration of the study with *A. sativum* at 200mg/kg reducing total serum bilirubin level by 48.46%, at 300mg/kg it was reduced by 62.75% while at 450mg/kg it was reduced by 71.15% after the duration of treatments compared with acetaminophen control at week 4 (Figure 4). *Z. officinale* reduced total serum bilirubin level in a dose dependent manner across the duration of study with *Z. officinale* at 200mg/kg reducing total serum bilirubin level by 56.86%, at 300mg/kg it reduced it by 68.35% whereas at 450mg/kg it was reduced by 73.95% after the duration of treatment. Silymarin at 100mg/kg reduced total serum bilirubin level by 82.35% after the duration of treatment compared with acetaminophen control at week 4 (Figure 4). Normal control had no significant effect on total serum bilirubin level whereas the acetaminophen treated control raised total serum bilirubin level by 33.89%. Among the extracts studied, after the duration of treatment, *Z. officinale* at 450mg/kg was the most potent in reducing total serum bilirubin, reducing it by 73.95%, *A. sativum* at 450mg/kg followed with 71.15%, *Z. officinale* at 300mg/kg was next with 68.35% and *A. cepa* at 450mg/kg followed with 67.79%. The comparative effects of the increasing dosage of the plant extracts studied, compared with normal control, acetaminophen control and silymarin, indicated that *Z. officinale* at 450mg/kg caused significant percentage reduction in total serum bilirubin level similar to that of silymarin standard drug at 100mg/kg.

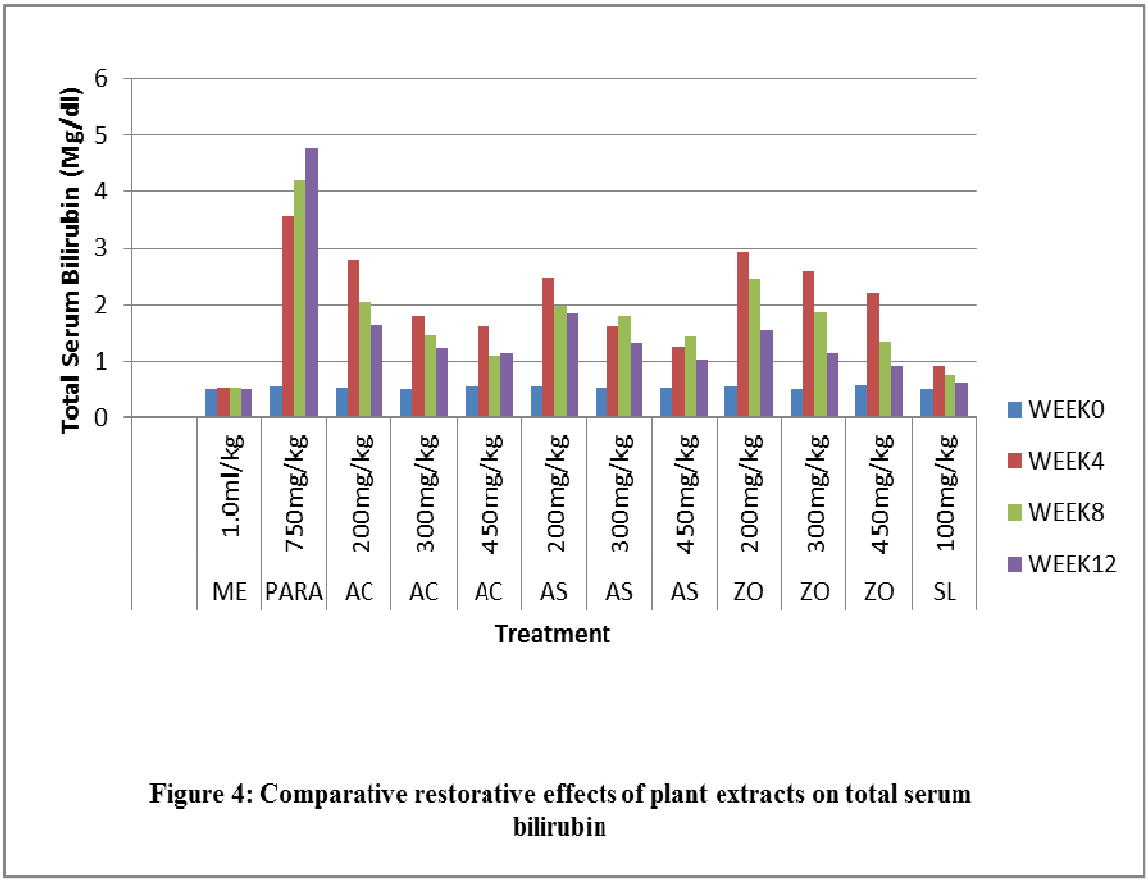

Figure 4: Comparative restorative effects of plant extracts on total serum bilirubin

ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Acetaminophen negative control, AC = Allium cepa, AS = Allium sativum, ZO = Zingiber officinale and SL= Silymarin representing hepatotoxic control.

The summary of comparative restorative effects of the increasing dosages (200, 300 and 450mg/kg) of *A. cepa*, *A. sativum* and *Z. officinale* extracts showed that *Z. officinale* at 450mg/kg produced the best cumulative percentage decrease (-212.34) on all the restorative parameters assessed and ranked 9 on a 10 points scale (Table 1), followed by *Z. officinale* at 300mg/kg (-206.79), ranked 8 and then *Z. officinale* at 200mg/kg (-191.79) and ranked 7 after the study. They are closely followed by *A. sativum* at 450mg/kg (-190.3), ranked 6, *A. sativum* at 300mg/kg (-184.86), ranked 5 and *A. cepa* at 300mg/kg (179.28), ranked 4. Similarly *A. cepa* at 450mg/kg (-179.05) ranked 3, *A. sativum* at 200mg/kg (-170.33) ranked 2 and *A. cepa* at 200mg/kg (-164.56) ranked 1 on a 10 points scale. Normal control did not have significant percentage change; acetaminophen control had a cumulative percentage increase of about 57.05 whereas silymarin decreased the parameters cumulatively with -250.46 percent.

Table 1: Summary of Restorative Effects of Plant Extracts on Acetaminophen Induced Hepatotoxic Rats

Dosage	Restorative Parameters				Total % Change	Rank	Restorative Effects (R. E)
	ALT	AST	ALP	TSB			
ME 1.0ml/kg	-	-	-	-	-	-	-
PARA 750mg/kg	8.19	8.97	6.00	33.89	57.05	-ve control	-Ve control
AC 200mg/kg	-15.79	-41.77	-52.94	-54.06	-64.56	1	Poor R. E
AC 300mg/kg	-20.67	-39.57	-53.77	-65.27	-179.28	4	Poor R. E
AC 450mg/kg	-21.99	-38.59	-50.68	-67.79	-179.05	3	Poor R. E
AS 200mg/kg	-23.77	-44.93	-53.17	-48.46	-170.33	2	Poor R. E
AS 300mg/kg	-24.28	-43.75	-54.08	-62.75	-184.86	5	Good R. E
AS 450mg/kg	-19.61	-44.79	-54.75	-71.15	-190.3	6	Good R. E
ZO 200mg/kg	-20.58	-59.72	-54.63	-56.86	-191.79	7	Good R. E
ZO 300mg/kg	-21.94	-59.60	-56.90	-68.35	-206.79	8	V. Good R. E
ZO 450mg/kg	-22.23	-51.38	-56.85	-73.95	-212.34	9	V. Good R. E
SL 100mg/kg	-30.15	-62.26	-75.70	-82.35	-250.46	standard	standard

Values represent the percentage change on each parameter after the duration of the study. Total represents the summation of the percentage change for each parameter after the duration of the study. Rank = grading of the hepatoprotective effects on a 10 points scale. Hepatoprotective effects were assessed as poor = (-183 & below), good = (-184 to -194), very good = (-200 to -220), Excellent = (-220 to -250), exceptional = (above -270). ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Acetaminophen negative control, AC = Allium cepa, AS = Allium sativum, Z0 = Zingiber officinale and SL= Silymarin representing hepatotoxic control. Negative % change denotes decrease, Positive % change denotes an increase.

The liver is the largest solid organ, the largest gland and the main metabolic organ of the body [1]**.** Its primary function is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system [20]. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. A total loss of liver function could leads to death within minutes, demonstrating the liver's great importance. Acetaminophen-induced liver toxicity is commonly used as an experimental model for the study of hepatoprotective effects of medicinal plant extracts and drugs [21; 22]**.** The significant increase observed in the level of serum aminotransferase in acetominophen 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 [23]**.** Silymarin and the plant extracts treatment reduced the level of aminotransferase enzymes in the serum. The mechanism of action of the plant extracts could be by prevention of intracellular enzyme release through its membrane stabilizing and antioxidant effects [24]**.** The reduction in ALP levels by plant extracts may suggest repairing of rat's liver by plant extracts and the possible mechanism responsible for the protection of acetaminophen induced liver damage may be by its ability to act as a free radical scavenger thereby intercepting those radicals involved in acetaminophen metabolism. Elevated total serum bilirubin observed in acetaminophen hepatotoxic rats suggested abnormal conjugation of bilirubin by the liver due to generalized hepatocellular damage [25]. Decrease in total serum bilirubin observed in this study was reported by previous works [26]**.** The possible mechanism of action of plant extracts that decreased total serum bilirubin may be through their antioxidative effects, because plant extracts have active ingredients that are capable of free radical scavenging in living system [27]**.** Among the three plant extracts used at different dosages 200, 300 and 450mg/kg, *Zingiber officinale* extracts at 450mg/kg produced the best restorative effect on acetaminophen induced hepatotoxic rats followed by *Zingiber officinale* at 300mg/kg, *Zingiber officinale* at 200mg/kg, *Allium sativum* at 450mg/kg, *Allium sativum* at 300mg/kg, *Allium cepa* at 300mg/kg, *Allium cepa* at 450mg/kg, *Allium sativum* at 300mg/kg and finally *Allium cepa* at 200mg/kg respectively.

CONCLUSION

Overall, the result of the present study indicated that *Allium cepa*, *Allium sativum* and *Zingiber officinale* demonstrated a significant restorative effect against acetaminophen induced liver toxicity in rats. Morever *Zingiber officinale* showed the best significant restorative effect in comparison to *Allium cepa* and *Allium sativum*.

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