

Assessment of the Effects of Garlic-Citrus-Tumeric Shot on Bilirubin, Alkaline Phosphatase and Transaminases Levels

Airhomwanbor, K.O. ^[1], Idehen, C.I. ^[2], Omolumen, L.E. ^[1], Akhere, P.I. ^[3], Asibor, E. ^[2], Animasaun, O.P. ^[4], Iyevhobu, K.O. ^{[5]*}, Omolumen, B. ^[6], Omachonu, P.U. ^[1]

^[1]Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

^[2]Department of Histopathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

^[3]Department of Obstetrics and Gynaecology, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria

^[4]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

^[5]Department of Public Health, National Open University of Nigeria, Uromi Community Study Centre, Uromi, Edo State, Nigeria

^[6]Student Outreach Support Staff, University of Chester, Chester, England, United Kingdom

Abstract. Medicinal plants are widely used in disease management all over the world. Plant-based traditional medicines are still of great importance to people living in developing countries and also serve as a source to discover new drugs for a variety of diseases affecting man. The aim of this study is to investigate the effect of garlic-citrus-tumeric shot on bilirubin, ALP, ALT and AST of adult albino wistar rats. The animals were divided into two groups; the control group and the experimental animals. A total of five (5) adult wistar rats were used for the control group, while fifteen (15) adult wistar rats were used for the experimental studies. During the period of acclimatization, the rats were fed growers' mash water provided ad libitum. At every stage of the weight determinations, the test groups (B, C and D) presented body weight reductions. Body weights were found to be significantly increased in the control and tests groups before and after acclimatization. However, variations in body reductions were observed between the control and test rats. Comparatively, these body weight variations were significant in group C (175.00 ± 0.01 g) and D (171.67 ± 24.83 g) after garlic-citrus-tumeric shot administration. The results showed that AST levels were significantly lower ($p < 0.05$) in rats (23.73 ± 7.93 U/L) when compared with the control (33.60 ± 6.23 U/L). Also, ALT levels were significantly lower ($p < 0.05$) in rats (19.67 ± 7.58 U/L) when compared with the control (28.40 ± 5.18 U/L). ALP levels were also lower ($p < 0.05$) in rats (17.13 ± 5.36 U/L) when compared with the control (24.80 ± 4.15 U/L). Furthermore, TB levels were also significantly lower ($p < 0.05$) in rats (0.38 ± 0.14 mg/dl) when compared with the control (0.80 ± 0.16 mg/dl). Also, CB levels were significantly lower ($p < 0.05$) in rats (0.21 ± 0.11 mg/dl) when compared with the control (0.38 ± 0.08 mg/dl). Garlic-citrus-tumeric shot inhibits elevations of biochemical markers of hepatic and renal functions. This suggests that combination of garlic-citrus-tumeric shot is not toxic to the liver. Conclusively, the increase in extract given to adult wister rat decreases the parameters of liver function test thereby increases liver normalcy or better the performance state of the liver, hence, when the extract is taking in the right proportion increases the performance of the liver

Keywords: Garlic, *Citrus*, Turmeric, Bilirubin, ALP, ALT and AST

* Corresponding Author: Iyevhobu Kenneth Oshiokhayamhe, ORCID: 0000-0001-6577-0637

Introduction

Garlic (*Allium sativum* L.), which belongs to the family Liliaceae, is used widely as a flavoring agent in dishes and in traditional medicine to treat many diseases. The medicinal use of garlic has a long history. Garlic is probably one of the earliest known medicinal plants. Its bulbs (cloves) had been used as a cureall in ancient Egypt (Lewis & Elvin-Lewis, 2003). Potential health benefits of vegetables, in particular garlic (*Allium sativum*), has its origin in antiquity, but still there is a need to unveil the details of these benefits (Lewis & Elvin-Lewis, 2003). Garlic has been reputed to possess therapeutic properties, and is probably the most widely investigated medicinal plant (Nrashan, Kumar, Kewal, Raisuddin & Sahu, 2010). Despite the numerous therapeutic effects attributed to garlic, the chemistry behind its health-promoting effect is poorly understood (Lewis & Elvin-Lewis, 2003). Garlic is probably one of the earliest known medicinal plants (Moyers, 1996). Over the centuries, garlic has acquired a special position in the folklore of many cultures as a formidable prophylactic and therapeutic medicinal agent (Moyers, 1996). Recent *in vitro* studies have confirmed the vasoactive ability of garlic's sulfur compounds, whereby red blood cells convert garlic's organic polysulphides into hydrogen sulphide, a known endogenous cardioprotective vascular cell-signaling molecule (Benavides *et al.*, 2007). Garlic may also help to prevent cognitive decline by protecting neurones from neurotoxicity and apoptosis, thereby preventing ischaemia or reperfusion-related neuronal death, and by improving learning and memory retention (Borek, 2006).

Turmeric (*Curcuma longa*) also called Indian saffron since ancient times is widely cultivated in India, Sri Lanka, Belgium, Indonesia and France. Only India accounts for more than 90% of total production of the world. Hence the turmeric powder is one of the most common food flavouring and colouring agent in Asian cuisines. Curcumin is the most active constituent present in turmeric and exert potent biological effects in-vitro and in-vivo (Wealth of India, 1995; Sharma, Ghescher & Steward, 2005; Agarwal, Shishodia & Takada, 2007). Curcumin (diferuloylmethane) is a yellow colouring ingredient of the spice turmeric obtained from the rhizome of *Curcuma longa* Linn (Zingiberaceae). It is a perennial herb distributed mainly throughout tropical and subtropical regions of the world (Joe, Vijaykumar & Lokesh, 2004). Curcumin possesses anti-inflammatory, immunomodulatory, and antiatherogenic activities and is a potent inhibitor of various reactive oxygen-generating enzymes (Ara'ujo & Leon, 2001; Chainani-Wu, 2003).

The genus *Citrus*, belonging to the Rutaceae or Rue family, comprises of about 140 genera and 1,300 species. *Citrus sinensis* (sweet orange), *Citrus paradise* (Grape fruit), *Citrus limon* (Lemon), *Citrus reticulata* (tangerine), *Citrus grandis* (shaddock), *Citrus aurantium* (sour orange), *Citrus medica* (Citron), and *Citrus aurantifolia* (lime) are some important fruits of genus *Citrus* (Mandalari, Bennett, Bisignano, Saija, Dugo, Faulds & Waldron, 2006; Golein, Nazeryan & Babakhani, 2012). *Citrus* are well known as one of the world's major fruit crops that are produced in many countries with tropical or subtropical climate. Brazil, USA, Japan, China, Mexico, Pakistan, and countries of the Mediterranean region, are the major *Citrus* producers (Anwar, Groom & Sadler-Bridge, 2009). Citrus fruits are recognized as an important component of the human diet, providing a variety of constituents important to human nutrition, including vitamin C (ascorbic acid), folic acid, potassium, flavonoids, coumarins, pectin and dietary fibers. Flavonoids in citrus have a broad spectrum of biological activities including antibacterial, antioxidant, antidiabetic, anticancer, analgesic, anti-inflammatory and antianxiety (Sidana, Saini, Dahiya, Nain & Bala, 2013).

Medicinal plants are widely used in management of disease all over the world. Plant-based traditional medicines are still of great importance to people living in developing countries and also serve as source to discovery of new drug for a variety of diseases affecting

man. The aim of this study is to investigate the effect of garlic-citrus-tumeric shot on bilirubin, ALP, ALT and AST of dult albino wistar rats.

Materials and Methods

A total of twenty (20) Adult Albino Wistar rats of comparable sizes and weights were procured from the histology laboratory, College of Medical Sciences, Ambrose Alli University Ekpoma, Edo State and transferred to the experimental Laboratory of the Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria, where they were allowed two (2) weeks of acclimatization.

The animals were divided into two groups; the control group and the experimental animals. A total of five adult wistar rats was used for the control group while fifteen (15) adults wistar rats was used for the experimental studies. During the period of acclimatization, the rats were fed growers' mash water provided ad libitum.

Animal Grouping for Toxicological Studies

The animals for toxicological studies were separated into four groups (A – D). Each group contains five rats ($n = 5$) each. Group A served as the control, while groups B – D served as the test groups.

Group A received only the normal feed (grower's mash) and water with no administration of garlic-citrus-tumeric shot while Group B - D received different doses of garlic-citrus-tumeric shot.

The preliminary studies, animal acclimatization, ingredients procurement (garlic-citrus-tumeric shot preparation and production), animal experiment and evaluation of results, lasted for a period of six months. However, the actual administration of garlic-citrus-tumeric shot to the test animals lasted for two (2) weeks.

Substance Preparation and Extraction

The fresh garlic, citrus and tumeric was obtained, washed, chopped and dried for two weeks. The size was reduced with mortar and pestle into fine powder. 50g of the powder was extracted by soaking for two days. The liquid extract was then concentrated using electrothermal heater to give a brownish solid extract which is then weighed directly. The concentrate was reconstituted by using 200ml of ethanol and the beaker was weighed empty. Then the weight of the empty beaker was subtracted from the weight of the concentrate.

Extract Administration for Toxicological Studies

The administration of the garlic-citrus-tumeric shot was performed through the oral route as described below:

Group A (Control): 100g of normal feed (growers' mash) and distilled water daily for three (3) weeks.

Group B: 100mg/kg body weight of the extract plus normal feed once for three (3) weeks.

Group C: 200mg/kg body weight of the extract plus normal feed once for three (3) weeks.

Group D: 500mg/kg body weight of the extract plus normal feed once for three (3) weeks.

Sample Collection

Blood samples (5mls) of each adult wistar rat were obtained through the intra orbital route at the end of the administration under light chloroform anaesthesia and dispensed into lithium heparin containers labeled appropriately. The samples were separated with a laboratory centrifuge for 5 minutes at 3000 rpm within two hours after collection into clean dry plain containers which are labeled corresponding to the initial blood sample container.

The plasma obtained was stored frozen at -20°C until the time of analysis. Laboratory analysis was carried out for liver function and bilirubin.

Analytical Method

Alanine Amino Transferase (ALT): The ALT activity in the sample was determined using the method described by Rietman and Frankel (1957).

Procedure: About 0.5ml of buffer ALT substrate solution was placed in test tubes labeled test and blank and incubated at 37° C for 5minutes. 0.1ml of plasma was added to test and mixed thoroughly. It was incubated for 30mins. 0.5ml of 2, 4-dinitrophenyl hydrazine (DNPH) was added to each test tube (test and blank). All tubes were mixed well and allowed to stand at room temperature for 20mins. 5.0ml of 0.4N sodium hydroxide (NaOH) was added to each of the test tube and allowed to stand at room temperature for 5mins. The absorbance was read at 546nm. The ALT activity was obtained from the calibration curve in the appendix.

Aspartate Amino Transferase (AST): The AST activity in the sample was determined using the method described by Rietman and Frankel (1957).

Procedure: About 0.5ml of buffer AST substrate solution was placed in test tubes labeled test and blank and incubated in water bath at 37° C for 5minutes. 0.1ml of plasma was added to test and mixed thoroughly. It was incubated for 30mins. 0.5ml of 2, 4-dinitrophenyl hydrazine (DNPH) was added to each test tube (test and blank). 0.1ml of plasma was added to each test blank containing 0.5ml of buffered substrate and this was used as sample blank. All tubes were mixed well and allowed to stand at room temperature for 20mins. 5.0ml of 0.4N sodium hydroxide (NaOH) was added to each of the test tube and allowed to stand at room temperature for 5mins. The absorbance was read at 546nm. The AST activity was obtained from the calibration curve in the appendix.

Alkaline Phosphatase (ALP): The ALP activity in the sample was determined using the Deutsche method described by Rec (1972).

Procedure: About 0.02ml of the sample was added to 1.0ml of the reagent in a test tube and mixed thoroughly. The initial absorbance was read immediately at 405nm wave length against reagent blank. This was read again after 1, 2, and 3 minutes of incubation at 37° C. The intensity of the colour is directly proportional to the activity of the enzyme.

Total Bilirubin Test: A total bilirubin working reagent was prepared by adding 0.05ml (50µl) of sodium nitrite reagent per 1ml of total bilirubin reagent and mix. Four test tubes labeled Blank, Standard, Control and Sample were provided. Each test tube requires a blank tube. 0.1ml of total bilirubin reagent was dispensed into the blank tubes. One millimeter (1ml) of the working reagent was dispensed into the labeled, except blank tubes. 0.1ml of the standard, control, and sample was added to each respective tube. After one minute, add 3.0 ml of Methanol Reagent to all tubes, mix by inversion and let stand for five (5) minutes. The tubes were allowed to stand for five (5) minutes at room temperature. Using the reagent blank, the absorbance of all the tubes was read at 550nm.

Calculations:

$$\text{Total bilirubin (mg/dl)} = \frac{\text{sample abs.} - \text{abs. of blank}}{\text{Abs of calibrator} - \text{Abs. of calibrator blank}} \times \text{Conc. of calibrator}$$

Abs = absorbance

Conjugated Bilirubin Test: Four test tubes labeled Blank, Standard, Control and Sample were provided. Into the labeled test tubes, 1ml of total bilirubin reagent (sulfanilic acid and HCl) was added. 50µL (1 drop) of sodium nitrite reagent was added into a sample test tube only. The solution was mixed and allowed to stand between 1-2 minutes. 50µL of

the sample was added to both the sample blank and the sample test tube. The test tubes are allowed to stand for 5 minutes. Using the reagent blank, the absorbance of all the tubes was read at 550nm.

Calculations:

$$\text{Total bilirubin (mg/dl)} = \frac{\text{sample abs.} - \text{abs. of blank}}{\text{Abs of calibrator} - \text{Abs. of calibrator blank}} \times \text{Conc. of calibrator}$$

Abs = absorbance

Statistical Analysis

Student's t-test and ANOVA was used to compare result in both the control and the test groups (all results was reported as mean $\pm$ standard deviation), using a computer program named SPSS for windows release 21. Confident interval of Values $P \leq 0.05$ was considered significant.

Results

Table 1 presented the body weight changes in the test and control groups. Although at every stage of the weight determinations, the test groups (B, C and D) presented body weight reductions. Body weights were found to be significantly increased in the control and tests groups before and after acclimatization. However, variations in body reductions were observed between the control and test rats. Comparatively, these body weight variations were significant in group C ($175.00 \pm 0.01\text{g}$) and D ($171.67 \pm 24.83\text{g}$) after garlic-citrus-tumeric shot administration.

The results in Table 2 showed that AST levels were significantly lower ($p < 0.05$) in rats (23.73 ± 7.93 U/L) when compared with the control (33.60 ± 6.23 U/L). Also, ALT levels were significantly lower ($p < 0.05$) in rats (19.67 ± 7.58 U/L) when compared with the control (28.40 ± 5.18 U/L). ALP levels were also lower ($p < 0.05$) in rats (17.13 ± 5.36 U/L) when compared with the control (24.80 ± 4.15 U/L). Furthermore, TB levels were also significantly lower ($p < 0.05$) in rats (0.38 ± 0.14 mg/dl) when compared with the control (0.80 ± 0.16 mg/dl). Also, CB levels were significantly lower ($p < 0.05$) in rats (0.21 ± 0.11 mg/dl) when compared with the control (0.38 ± 0.08 mg/dl).

The results in table 3 showed the comparison of AST, ALT, ALP, TB and CB between Group B, Group C, Group D and Group A (control) of rat subjects. AST levels were significantly lower ($p < 0.05$) in Group D of rat subjects (15.60 ± 6.39 U/L), when compared with Group C (25.80 ± 4.15 U/L), Group B (29.80 ± 5.31 U/L) and Group A (control) (33.60 ± 6.23 U/L). Also, ALT levels were significantly lower ($p < 0.05$) in Group D of rat subjects (12.20 ± 6.83 U/L), when compared with Group C (21.20 ± 2.95 U/L), Group B (25.60 ± 5.41 U/L) and Group A (control) (28.40 ± 5.18 U/L). ALP levels were also significantly lower ($p < 0.05$) in Group D of rat subjects (12.60 ± 3.97 U/L), when compared with Group C (18.00 ± 3.67 U/L), Group B (20.80 ± 5.26 U/L) and Group A (control) (24.80 ± 4.15 U/L). TB levels were significantly lower ($p < 0.05$) in Group D (0.26 ± 0.05 mg/dl), Group C (0.36 ± 0.09 mg/dl) and Group B (0.52 ± 0.13 mg/dl) when compared with the Group A (control) (0.80 ± 0.16 mg/dl). CB levels were significantly lower ($p < 0.05$) in Group D (0.12 ± 0.04 mg/dl), Group C (0.18 ± 0.08 mg/dl) and Group B (0.32 ± 0.08 mg/dl) when compared with the Group A (control) (0.38 ± 0.08 mg/dl).

Table 1: Body weight changes of rats fed with garlic-citrus-tumeric shot at various intervals

Weight (g)	A (Control) (n=5)	B (100mg/kg G-C-T shot) (n=5)	C (200mg/kg G-C-T shot) (n=5)	D (500mg/kg G-C-T shot) (n=5)
WBA	173.22±17.22	191.67±18.35	200.00±12.65*	205.00±26.65*
WAA	196.67±15.06	211.67±24.01	225.00± 12.25*	237.50±34.46*
W2WK	181.67±9.31	193.33±18.89	204.17±10.21	214.17±32.31*
W3WK	167.50±13.69	176.67±16.02	183.33±8.16	196.67±23.80*
FW	145.83±18.82	154.17±18.82	175.00± 0.01*	171.67±24.83*

KEY: G-C-T shot: Garlic-Citrus-Tumeric Shot; **WBA:** Weight before acclimatization; **WAA:** Weight after acclimatization; **W2WK:** Weight at second week of coffee administration; **W3WK:** Weight at third week of coffee administration; **FW:** Final weight before sacrificing. Values are mean ± Standard deviation; n: Number of sample. Values in a row with superscript (*) are significantly different at P <0.05.

Table 2: Comparison of Liver Function Tests levels in rat subjects and control

Parameters	Control (N=30)	Subjects (N=90)	t value	P value
AST (U/L)	33.60±6.23	23.73±7.93	2.518	0.021
ALT (U/L)	28.40±5.18	19.67±7.58	2.375	0.029
ALP (U/L)	24.80±4.15	17.13±5.36	2.904	0.009
TB (mg/dl)	0.80±0.16	0.38±0.14	5.568	0.000
CB (mg/dl)	0.38±0.08	0.21±0.11	3.206	0.005

KEY: n=Sample size; p>0.05= Not significant; p<0.05= Significant.

TB-Total Bilirubin; CB-Conjugated Bilirubin; AST- Aspartate aminotransferase

ALT- Alanine aminotransferase; ALP- Alkaline Phosphatase

Table 3: Comparison of Liver Function Tests levels in rat subjects between Group A (control), Group B, Group C and Group D

Parameters	Group A (Control) (n=5)	Group B (n=5)	Group C (n=5)	Group D (n=5)	F value	P value
AST (U/L)	33.60±6.23 ^a	29.80±5.31 ^a	25.80±4.15 ^{ab}	15.60±6.39 ^b	9.613	0.001
ALT (U/L)	28.40±5.18 ^a	25.60±5.41 ^a	21.20±2.95 ^{ab}	12.20±6.83 ^b	8.999	0.001
ALP (U/L)	24.80±4.15 ^a	20.80±5.26 ^a	18.00±3.67 ^{ab}	12.60±3.97 ^b	7.083	0.003
TB (mg/dl)	0.80±0.16 ^a	0.52±0.13 ^b	0.36±0.09 ^{ab}	0.26±0.05 ^{ab}	20.969	0.000
CB (mg/dl)	0.38±0.08 ^a	0.32±0.08 ^a	0.18±0.08 ^b	0.12±0.04 ^b	12.638	0.000

KEY: n=Sample size; p>0.05= Not significant; p<0.05= Significant.

Values in a row with the same superscript are not significantly different at p<0.05.

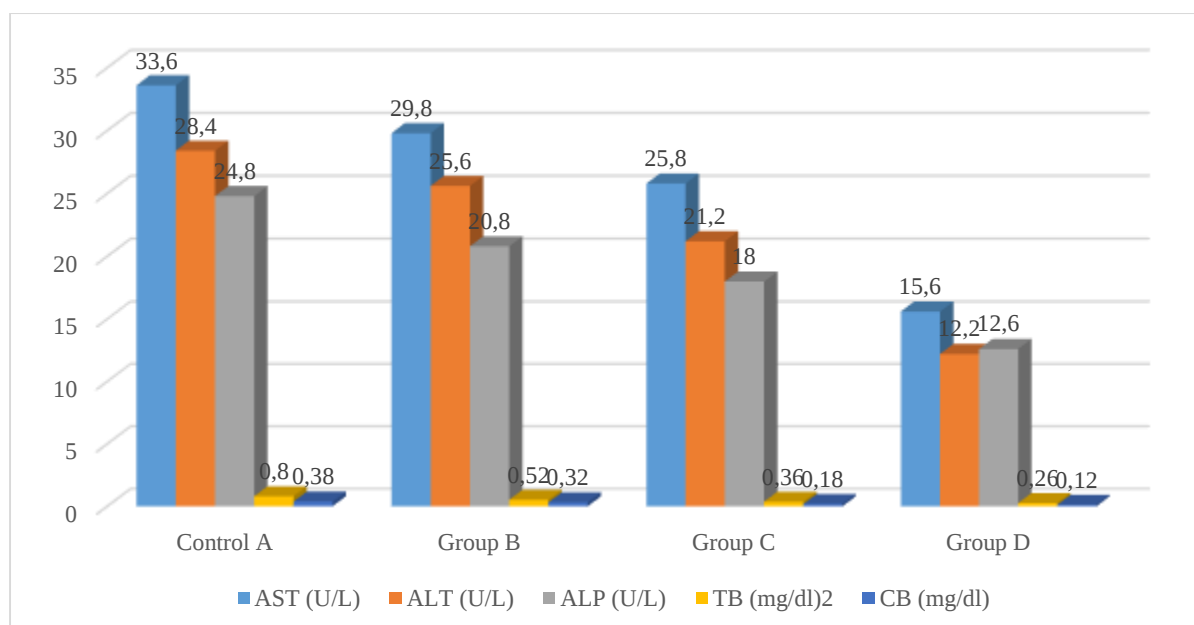


Figure 1: Comparison of Liver Function Tests levels in rat subjects between Group A (control), Group B, Group C and Group D

Discussion

Plants are important in our everyday existence. They provide our foods, produce the oxygen we breathe, and serve as raw materials for many industrial products such as clothes, foot wears and so many others. Plants also provide raw materials for our buildings and in the manufacture of biofuels, dyes, perfumes, pesticides and drugs (Adesuyi *et al.*, 2012). The use of plants in traditional medical practice has a long-drawn history, and remains the mainstay of primary health care in most of the third world. Traditional medicines are used by about 60% of the world population; in both developing and developed countries where modern medicines are predominantly used (Mythilypriya *et al.*, 2007). While an estimated 60-80% Africa's population depends solely on herbal remedies for its primary health care needs.

The clinical and diagnostic values associated with changes in blood enzymes concentrations such as AST, ALT, ALP and bilirubin have long been recognized (Martin, Freidman & Kaffee, 1998). Increased levels of these diagnostic markers of hepatic function in wistar rats are implicative of the degree of hepatocellular dysfunction (Mohammed, 2010).

The present study investigated the AST, ALT, ALP, TB and CB levels in Wistar rats. The liver is the main target organ of acute toxicity when exposed to the foreign substances. When these substances are absorbed in intestines, they are metabolized to other compounds which may or may not be hepatotoxic to the rats (Rhiouania, El-Hilalya, Israili & Lyoussia, 2008). In this study, a marked significant ($p < 0.05$) decrease in serum AST, ALT, ALP, TB and CB levels was observed. Bilirubin is a major breakdown product of hemoglobin. Increased bilirubin production results from impaired hepatic excretion or regulation of conjugated and unconjugated bilirubin from the hepatocytes. Our results indicated that garlic-citrus-tumeris shot dose 500 mg/kg b.w. decreased bilirubin level, although this dose is considered as low. Bhuiyan, Papajani, Paci & Melino (2015) found that garlic accelerates the RBC's turnover by inducing hemeoxygenase 1, which would then produce Fe 2+, biliverdin and CO.

Furthermore, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are biochemical markers of hepatic function. They are hepatic enzymes and their elevations in serum or plasma have been attributed to their leakage from liver cytosol into the blood stream as a result of hepatotoxicity (Bhattacharyya, Mukherjee, Pandit,

Das & Sur, 2003; Nyblom, Björnsson, Simrén, Aldenborg, Almer, & Olsson, 2006). ALT is specifically produced by hepatic cells (Dufour, Lott, Nolte, Gretch, Koff & Seeff, 2000; McClatchey, 2002), while AST is a less specific marker of liver function as it is produced by other organs as well. Elevation of ALT and AST is indicative of hepatic injury (Nyblom *et al.*, 2006), thus, elevation of plasma levels of these enzymes by alloxan is suggestive of toxicity to hepatic cells. The results obtained therefore give a strong indication that garlic-citrus-turmeric shot would induce hepatotoxic effect in rats.

The assessment of the serum AST, ALT, ALP, TB and CB could be used to reveal the deleterious effect of foreign compounds, toxins, chemicals and plant extracts on the blood constituents of animals. It is also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histology of the organs (Magalhaes, Appell & Duarte, 2008). The study also showed that daily administration of animals with various doses of garlic-citrus-turmeric shot significantly decreased serum AST, ALT, ALP, TB and CB levels. This showed that garlic-citrus-turmeric shot is toxic to red blood cells. This finding is in agreement with the results obtained by Banerjee & Maulik (2002) that prolonged feeding of rats with garlic-citrus-turmeric shot, and probably ginger, may cause anaemia, weight loss and stunted growth in rats.

However, in this study, the weight of the animals was determined. At every stage of the weight determinations, the test groups (B, C and D) presented body weight reductions. Body weights were found to be significantly increased in the control and tests groups before and after acclimatization. However, variations in body reductions were observed between the control and test rats. Comparatively, these body weight variations were significant in group C ($175.00 \pm 0.01\text{g}$) and D ($171.67 \pm 24.83\text{g}$) after garlic-citrus-turmeric shot administration. Ramjee, Berjak, Adhikari & Dutton, (1992) reported that aflatoxin has been directly related to underweight status in children in Benin and Togo. Bedi, Singh, John & Agarwal, (1996) reported decrease in body weight in Guinea fowl fed on aflatoxin B1. In present study the weight loss in group II may be due to the toxic and carcinogenic effects of DMBA. Turmeric, Garlic and citrus singly have antioxidant, hepatoprotective nature as well as anti-carcinogenic activity better but in combine shot, it has shown to be hepatotoxic.

Conclusion

The present study showed a significant decrease in serum AST, ALT, ALP, TB and CB levels due to the administration of garlic-citrus-turmeric shot. It was concluded that the consumption of garlic-citrus-turmeric shot decreased the liver enzyme activities, and it is not toxic to the liver, hence it should be encouraged and taken in moderation. In Conclusion, Garlic-citrus-turmeric shot inhibits elevations of biochemical markers of hepatic and renal functions. This suggests that combination of garlic-citrus-turmeric shot is not toxic to the liver. Conclusively, the increase in extract given to adult wister rat decreases the parameters of liver function test thereby increases liver normalcy or better the performance state of the liver, hence, when the extract is taking in the right proportion increases the performance of the liver

Considering the values of liver function obtained in this study, it is hereby recommended that;

1. Specific research should be carried out to determine the effect of garlic-citrus-turmeric shot on the liver using other doses.
2. The use of garlic-citrus-turmeric shot should be regulated and also specific research should be carried out to further determine the toxic dose.
3. However further research should be done on the long term effects of garlic-citrus-turmeric shot on the liver function

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

Acknowledgements

The authors would like to acknowledge the management of the department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University (AAU), Ekpoma, Edo State, Nigeria for creating the enabling environment for this study. Thanks to all the Laboratory and technical staff of St Kenny Research Consult, Ekpoma, Edo State, Nigeria for their excellent assistance and for providing medical writing/editorial support in accordance with Good Publication Practice (GPP3) guidelines.

References

- Agarwal, B.B., Shishodia, S., & Takada, Y. (2007). Curcumin suppresses the p38 mitogen-activated protein kinase induced nuclear factor pathway in breast Cancer. *Clinical Cancer Research*, 11, 7490-7498.
- Anwar, A., Groom, M., & Sadler-Bridge, D. (2009). Garlic: from nature's ancient food to nematocide. *Pesticide News*, 84(June), 18–20.
- Ara'ujo, C.C., & Leon, L.L. (2001). Biological activities of Curcuma longa L. *Mem'orias do Instituto Oswaldo Cruz*, 96(5), 723–728.
- Banerjee, S.K., & Maulik, S.K. (2002). Effect of garlic on cardiovascular disorders: a review. *Nutrition Journal*, 1, 4-14.
- Bedi, P.S., Singh, H., John, T.S., & Agarwal, R.K. (1996). Effects of aflatoxin on growth and feed consumption in Guinea fowl. *XX World Poultry Congress, Proceedings*, (III), 665- 667.
- Benavides, G.A., Squadrito, G.L., Mills, R.W., Patel, H.D., Isbell, T.S., Patel, R.P., Darley-Usmar, V.M., Doeller, J.E., & Kraus, D.W. (2007). Hydrogen sulfide mediates the vasoactivity of garlic. *Proceedings of the National Academy of Sciences of the U.S.A*, 104, 7977-7982.
- Bhattacharyya, D., Mukherjee, R., Pandit, S., Das, N., & Sur, T.K. (2003). Prevention of carbon tetrachloride induced hepatotoxicity in rats by Himmoliv (a poly herbal formulation). *Indonesia Journal of Pharmacology*, 35, 183-185.
- Bhuiyan, A.I., Papajani, V.T., Paci, M., & Melino, S. (2015). Glutathione-garlic sulfur conjugates: slow hydrogen sulfide releasing agents for therapeutic applications. *Molecules*, 20(1), 1731-1750.
- Borek, C. (2006). Garlic reduces dementia and heart-disease risk. *Journal of Nutrition*, 136(3 Suppl), 810-812.
- Chainani-Wu, N. (2003). Safety and anti-inflammatory activity of curcumin: a component of tumeric (Curcuma longa). *Journal of Alternative and Complementary Medicine*, 9(1), 161–168.
- Dufour, D.R., Lott, J.A., Nolte, F.S., Gretch, D.R., Koff, R.S., & Seeff, L.B. (2000). Diagnosis and monitoring of hepatic injury: II. Recommendations for use of laboratory tests in screening, diagnosis and monitoring. *Clinical Chemistry*, 46(12), 2050-2068.

- Golein, B., Nazeryan, M., & Babakhani, B. (2012). Assessing genetic variability in male sterile and low fertile citrus cultivars utilizing simple sequence repeat markers (SSRs). *African Journal of Biotechnology*, 11, 1632-1638.
- Joe, B., Vijaykumar, M., & Lokesh, B.R. (2004). Biological properties of curcumin -cellular and molecular mechanisms of action. *Critical Reviews in Food Science and Nutrition*, 44(2), 97-111.
- Kochmar, J.F., & Moss, D.W. (1976). *Fundamentals of Clinical Chemistry*. W.B. Saunders and company, Philadelphia, PA: 604.
- Lewis, W., & Elvin-Lewis, M. (2003). *Medical Botany: Plants Affecting Human Health* (2nd ed.). New York: Wiley. pp. 67 – 71.
- Magalhaes, P., Appell, H., & Duarte, J. (2008). Involvement of advanced glycation end products in the pathogenesis of diabetes complication: the protective role of regular physical activity. *European Review of Aging Physical Activity*, 5(1), 17-29.
- Mandalari, G., Bennett, R.N., Bisignano, G., Saija, A., Dugo, G., Faulds, C.B., & Waldron, K.W. (2006). Characterization of flavonoids and pectin from bergamot (*Citrus bergamia* Risso) peel, a major byproduct of essential oil extraction. *Journal of Agriculture and Food Chemistry*, 54, 197-203.
- Martin, P., Freidman, L.S., & Kaffee, E.B. (1998). Handbook of liver disease. *Philadelphia, Churchill Livingstone*, pp. 1-18.
- McClatchey, K.D. (2002). *Clinical laboratory medicine* (1st ed.). Philadelphia: Lippincott Williams & Wilkins; pp. 20-22.
- Mohammed, M.M.A. (2010). The Possible Protective Role of *Foeniculum vulgare* Mill. Against Radiation-Induced Certain Biochemical Changes in Albino Rats. *Master Degree of Science, Faculty of Science, Beni-Suef University*, 2, 10-15.
- Moyer, K.D., & Balistreri, W.F. (2011). Liver Disease Associated with Systemic Disorders. In Kliegman, R.M., Stanton, B.F., St Geme, J.W., Schor, N.F., Behrman, R.E. (Eds.), *Nelson Textbook of Pediatrics* (p. 1405). Saunders. ISBN 978-1-4377-0755-7.
- Moyers, S. (1996). *Garlic in Health, History and World Cuisine*. Suncoast Press, St. Petersburg, FL: pp. 1-36.
- Nrashan, T.S., Kumar, D., Kewal, L.S., Raisuddin, A., & Sahu, P. (2010). Adverse health effects due to arsenic exposure, modification by dietary supplementation of jaggey in mice. *Toxicology and Applied Pharmacology*, 242(3), 247-255.
- Nyblom, H., Björnsson, E., Simrén, M., Aldenborg, F., Almer, S., & Olsson, R. (2006). The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. *Liver International*, 26(7), 840-845.
- Ramjee, G., Berjak, P., Adhikari, M., & Dutton, M.F. (1992). Aflatoxins and Kwashiorkor in Durban, South Africa. *Annals of Tropical Paediatrics*, 12, 241-247.
- Rhiouania, H., El-Hilalya, J., Israili, Z.H., & Lyoussia, B. (2008). Acute and subchronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *Journal of Ethnopharmacology*, 118, 378-386.
- Sharma, R.A., Ghescher, A.J., & Steward, W.P. (2005). Curcumin: Story so far. *European Journal of Cancer*, 41, 1955-1968.
- Shishodia, S., Chaturvedi, M.M., & Agarwal, B.B. (2007). Role of Curcumin in cancer therapy. *Current Problems on Cancer*, 31, 243-305.
- Sidana, J., Saini, V., Dahiya, S., Nain, P., & Bala, S. (2013). A Review on Citrus - “The Boon of Nature”. *International Journal of Pharmaceutical Sciences and Review Research*, 18, 20-27.
- Wealth of India – Raw materials (1995). CL-CY, 2: 264-270, Published by CSIR, New Delhi, India.