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Changes in some Serum Enzymes due to Oral Administration of *Allium Cepa* and *Allium Sativum* to normal and Iodine treated Wistar Albino Rats

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ABSTRACT

Biochemical effects following ingestion of *Allium cepa* (onion) and *Allium sativum* (garlic) during iodine fortification are yet to be ascertained. This study determined the effect of the oral administration of these *Alliums* extracts on the activity of some serum enzymes specifically alanine aminotransferase, ALT, aspartate aminotransferase, AST, alkaline phosphatase, ALP, of normal and iodine treated Wistar albino rats. Oral administration of the aqueous extracts of garlic and onion at high dose (1.5 ml containing 1.70 g of the sample) and low dose (1 ml containing 1.14 g of the sample), was done to eight groups of wistar albino rats. Four of these were iodine treated using potassium iodide solution (0.8 mg/kg body weight) while the other groups were normal. Two control groups one for the normal rats, the other for the iodine treated rats which were not administered with the spices. Oral administration of onion and garlic led to a statistically significant ($P < 0.05$) decrease in the serum ALT activity of the experimental animals when compared with the control. There was no statistically significant change ($P < 0.05$) in the activity of AST due to either the administration of the extracts on both normal or iodine treated rats. There was a significant ($P < 0.05$) decrease in ALP activity in both normal and iodine treated rats when compared with the control due to oral administration of onion and garlic extract. The results of this study show that oral administration of Allium cepa and Allium sativum extract may not be toxic to normal or iodine treated rats at the doses investigated.

KEYWORDS: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) *Allium cepa* and *Allium sativum*, iodine

INTRODUCTION

Onion *(Allium cepa)* and garlic *(Allium sativum)* which were important because of the culinary value of their flavours, and odours (Jones *et al.*, 2004) have also been found to have numerous health benefits, including lowering hypertension (Mikaili *et al.*, 2013) and ischemic heart disease (Hara *et al.*, 2013) various forms of cancer (Bisen and Emerald, 2016) and many forms of infectious diseases (Bisen and Emerald, 2016). This has their use more popular.

In 2005, Nigeria became the first African country to be certified as USI compliant after having achieved 98 % household coverage of iodized salt (IDD, 2013). With the popularity of the *Alliums*, there was need to investigate the effect of these substances on some serum enzyme activities. Enzymes are organic molecules that can speed up biochemical reactions without being affected at the end of the reaction (Esani, 2014). In the living body certain enzymes are known to be found in certain organs (Newman, 2018). This phenomenon is of great use as a diagnostic tool. Abnormal levels of serum enzymes are indicative of damaged cells and provide clues to parts of the body that may be involved in the disease processes (Esani, 2014). Presently, little is known about the effect of some of the phytochemical components of these *Allium*s on the serum enzymes of iodine treated rats and consequently the effect of these *Alliums* in the presence of iodine fortification. It is on the basis of this that a clear picture of the effect of the consumption or exposure to garlic and onion on biochemical processes and particularly on the Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) is desired. Based on the afore mentioned, the present study is designed to assess the effect of *Allium cepa* and *Allium sativum* on the enzyme activities of normal wistar albino rats and wistar albino rats exposed to iodized salts.

MATERIALS AND METHODS

The onion and garlic sample used for this study were purchased from Ika Ika Qua Market in Calabar, capital city of Cross River State of Nigeria.

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The dry scaly part of the onion bulbs was removed and the fresh bulbs were properly washed. A quantity of 300g of the onions were weighed and macerated in 300ml of deionized water using an electric blender. The homogenous mixture obtained after maceration was filtered through a chesse cloth and the residue removed, dried and weighed. The solution left behind weighing 455.7g was used as whole onion extract and from this stock; high and low doses were obtained for the experiments.The dry scaly outer part of the garlic cloves was removed and the fresh cloves were properly washed. A quantity 300 g of the garlic were weighed and macerated in 300 ml of deionized water using an electric blender. The solution was used as whole garlic extract of 455.7 g was used as whole garlic extract and from this; high and low doses were obtained for the experiment. A quantity of 400 mg of potassium iodide was dissolved in 400 mls of water. 0.8 ml of the solution corresponding 0.8 mg/kg body wt was administered to the animals.

Animal Grouping, Extract Administration and Biochemical Assay

A total of 100 albino rats of the Wistar strain consisting of both male and females were obtained from the disease-free stock of the departmental animal house of Biochemistry Department, Faculty of Basic Medical sciences, University of Calabar, Nigeria. These animals weighing between 80g-120g were used for the experiment.

The animals were housed in Perspex cage, (North Kent Plastic Cages Ltd, England) with bottom grid and a stainless steel top. The animals were kept under adequate ventilation at temperature and relative humidity of $26^{\pm}2\ ^{0}C$ and 46% respectively. Feed and water were provided *ad libitum.* There weights were taken 3 times during the course of the 14 days.

The animals were randomly allocated into ten study groups of ten animals each based on their average weight and litter origin. The groups were treated as stated below:

Group 1 - control –deionized water (placebo)
Group 2 - positive control – potassium iodide solutions only
Group 3 - low dose of garlic only
Group 4 - high dose of garlic only
Group 5 - low dose of onion only
Group 6 - high dose of onion only
Group 7 - low dose of garlic + potassium iodide solution
Group 8 - high dose of garlic + potassium iodide solution
Group 9 - low dose of onion + potassium iodide solution
Group 10 -high dose of onion + potassium iodide solution

Administration of the extracts

The administration of the aqueous extracts to the different groups of animals was done for 14 days.1ml of both the onion and the garlic extracts containing 1.14g of each sample of onion and garlic extract was designated as low dose while 1.5 ml containing 1.70 g of each sample was designated as high dose. A quantity of 0.8mg/kg body wt of potassium iodide was administered orally to the animals taking potassium iodide at least 4hours before the respective extracts were administered to ensure iodine had been absorbed into the plasma(this is to ensure an iodine loaded state). All these were administered using orogastric tubes.

Twenty-four hours after the last administration, the animals were removed, placed in a dessicator glass jar, anaesthetized in chloroform vapour and dissected. Whole blood sample obtained by cardiac puncture from each animal was collected into a sterile tube. The whole blood sample collected was allowed to stand for one hour to clot and serum was neatly separated from the clot by a gentle tap with syringe and needle down the side of the tubes. The serum obtained was further subjected to centrifugation using an MSE-table top centrifuge (Minor, England) set at 8000 revolutions per minute (rpm) for 15 minutes, and a clear serum devoid of any trace of hemoglobin obtained. The serum sample obtained from the animals was used for assays.

Aspartate aminotransferase-AST (Glutamate oxaloacetate transaminase-GOT) EC 2.6.1.1: Aspartate: 2-oxo glutarate aminotransferase

Aspartate aminotransferase activity in the serum was measured using enzyme end point colorimetric diagnostic kit obtained from Randox Laboratories (Randox Laboratories Ltd., Admore, Diamond Road, Grumlin, Co. Antrim United Kingdom BT 294 QY).

Principle:

L-aspartate reacts with alpha-ketoglutarate in a reaction step catalysed by aspartate aminotransferase to yield oxaloacetate and glutamate according to the equation

L-Aspartate + α-ketoglutarate →oxaloacetate + glutamate

The oxaloacetate that forms is reacted with 2,4-dinitrophenyl hydrazine. The resulting hydrazone of oxaloacetate is highly coloured and the absorbance at 530-550nm is proportional to AST activity. Thus, AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4 dinitrophenyl hydrazine. Aliquot (0.05ml) of serum was used for the assay of AST activity.

Alanine aminotransferase-ALT (Glutamate pyruvate transaminase, GPT) EC 2.6.1.2-Alanine: 2-oxo glutarate amino transferase.

Alanine aminotransferase activity in the serum was similarly measured by end point colorimetric assay method with kit obtained from Randox Laboratories England.

Principle:

L-Alanine + α -ketoglutarate → pyruvate + Glutamate

The pyruvate that forms in the above reaction is treated with 2,4 dinitrophenyl hydrazine.

The resulting hydrazone of pyruvate is highly coloured and its absorbance at 530-550nm is proportional to ALT activity. As in AST assay, 0.05ml of serum was used for ALT activity determination.

Alkaline phosphatase; EC 3.1.3.1

The activity of serum alkaline phosphatase was measured by optimized standard method of Klin (1970) and Klin (1972) using enzymatic colorimetric diagnostic kits obtained from Human Laboratories (Human Gesellschaft fur Biochemica und Diagnostica mbH Max-Planck-Ring 21-D-65205 Wiesbaden Germany.

Principle:

ρ-Nitrophenylphosphate reacts reversibly in the presence of water in a reaction catalysed by alkaline phosphatase to form phosphate and ρ –nitrophenol. Change in absorbance after every minute for 4mins was measured. This was used to determine the alkaline phosphatase activity.20µl of serum was used for ALP activity determination.

Statistical Analysis: Results of all the studies were expressed as mean± standard deviation. Data between groups were analysed using SPSS 2003(version 13).

RESULTS

The effect of onion and garlic extract on the activities of some of the serum enzymes are presented on Table 1. The mean ± SD values for serum alanine aminotransferase (ALT) activity obtained for the experimental animals ranged between 44.50 ±7.65 U/L for the control and 29.50 ± 4.11 U/L for low dose onion loaded with iodine.

The mean values for serum aspartate aminotransferase (AST) activity obtained for the experimental animals ranged between 89.59 ±4.92 U/L for high dose garlic loaded with iodine and 76.38 ±10.39 U/L for the high dose onion group loaded with iodine.

The AST/ALT ratio for the group with high dose garlic supplemented with iodine was the highest having a value of 3.22 ± 0.42 while the control group (placebo) had the least value of 1.96 ± 0.44.

The mean values for serum alkaline phosphatase (ALP) activities obtained for the experimental animals ranged between 44.10±3.60 U/L for the control (placebo) group and 35.84±5.71 U/L for the low dose garlic group normal rats

Table 1: Effect of oral administration of onion and garlic extract on the activity some serum enzymes of normal and iodine treated wistar rats

Enzyme/Treatment groups	ALT (U/L)	AST (U/L)	AST:ALT	ALP(U/L)
Control (placebo)	44.50 ±7.65[a]	84.75 ±11.44[a]	1.96 ± 0.44[a]	44.10 ± 3.60[a]
LDG	33.50 ± 9.13[b]	79.00 ± 12.05[a]	2.52 ± 0.79[a]	35.84 ± 5.71[b]
HDG	38.47 ± 5.47[b]	95.25 ± 12.05[a]	2.52 ± 0.58[a]	32.74 ± 3.44[b]
LDO	30.00 ± 3.78[b]	76.50 ± 11.93[a]	2.57 ± 0.38[a]	26.19 ± 9.55[b]
HDO	36.50 ± 6.11[b]	87.75 ± 4.80[a]	2.35 ± 0.65[a]	31.64 ± 3.47[b]
KI treated (positive control)	36.25 ± 7.61[b]	77.75 ± 11.47[a]	2.20 ± 0.32[a]	33.08 ± 2.08[b]
KI + LDG	38.50 ± 6.09[b]	84.50 ± 11.22[a]	2.24 ± 0.44[a]	35.22 ± 11.63[b]
KI + HDG	25.75 ± 5.97[c]	89.50 ± 14.92[a]	3.22 ± 0.42[b]	28.26 ± 8.17[b]
KI + LDO	29.50 ± 4.11[c]	83.75 ± 8.08[a]	2.87 ± 0.36[b]	29.64 ± 5.26[b]
KI + HDO	29.75 ± 3.33[c]	76.38 ± 10.39[a]	2.58 ± 0.29[a]	32.40 ± 6.38[b]

Mean values along the same column with different superscripts are significantly different at ($P < 0.05$)

Values are presented as mean ± SD; LDG= Low dose garlic; HDG= High dose garlic: LDO= Low dose onion: HDO=High dose onion; KI = Potassuim iodide

DISCUSSION

Low and high doses of onion and garlic extracts were administered orally to the experimental animals and some enzyme activities were measured in the serum. The results of the enzyme activity in this study were within the reference ranges of AST, ALT and ALP are 50 to 150 IU/L, 10 to 40 IU/L and 30 to 130 IU/L respectively (Sharp and LaRegina, 1998).

There was a statistically significant ($P < 0.05$) decrease in the activity of the alanine aminotransferase (ALT) in serum of both the normal and the iodine treated rats due to the administration of the onion and the garlic extract. ALT, one of the two enzymes that catalyze a reversible amino group transfer reaction in the Krebs cycle primarily appears in hepatocellular cytoplasm with lesser amounts in the kidneys, heart and skeletal muscles (Esani,2014). This enzyme is a relatively specific indicator of acute hepatocellular damage (Ruhl and Everhart, 2009). When such damage occurs, ALT is released from the cytoplasm into the bloodstream, resulting in abnormally high serum levels. The absence of any elevation in the activity of ALT in the treatment groups when compared with the control groups point to the fact that oral administration of onion and garlic extracts either to the normal or the iodine treated rats had no noticeable effect on the integrity of the hepatocytes. Conversely, aspartate aminotransferase (AST) also found in the liver, heart, skeletal muscle, kidneys and present in the pancreas, AST levels are also significantly increased in skeletomuscular disorders and pulmonary embolism (Esani,2014) . AST is also increase in the presence of myocardial injury (Shen *et al.,* 2015). Oral administration of onion and garlic extract did not have any statistically significant ($P<0.05$) effect on the AST activity of both the normal and iodine treated rats. Hence, the oral administration of garlic and onion did not cause any obvious myocardial injury on the experimental animals. The AST/ALP ratios in all the experimental animals were above 2.0. There was a significant ($P <0.05$) increase in AST/ALP ratio in the iodine treated groups due to the oral administration of the onion and garlic extracts which was not dose dependent. However the other groups showed difference in AST/ALP ratio which were not significant ($P < 0.05$) when compared to the control group. There was a significant ($P < 0.05$) decrease in the activity of alkaline phosphates (ALP) in both the normal and the iodine treated rats when compared to the control group. Increased activity of serum alkaline phosphatase (ALP) is usually correlated to mild biliary obstruction and is a primary indicator of space-occupying hepatic lesions. In this study, a decrease in the activity of serum ALP was observed in all the experimental groups when compared to the control group. This further confirms that these spices (onion and garlic) had no noticeable effect on the liver cytoarchitecture.

CONCLUSION

The study was carried out to determine the changes in the activity of some serum enzymes due to oral administration of the aqueous extracts of *Allium cepa*

(onion) and Allium sativum (garlic) to normal and iodine treated rats. It was established through the activities of these enzymes that these *Alliums* at the doses used for this study have no noticeable effect on the liver cytoarchitecture.

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