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Effect of the oral administration of *Allium Cepa* and *Allium Sativum* on some serum enzymes of normal and iodine treated albino wistar rats

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ABSTRACT

Biochemical effects following ingestion of *Allium cepa* (onion) and *Allium sativum* (garlic) by normal and iodine treated albino Wistar rats have been studied. The determination of the effect of the oral administration of onion and garlic extract on the activity of some serum enzymes specifically alanine aminotransferase, ALT, aspartate aminotransferase, AST, alkaline phosphatase, ALP, including the AST: ALT ratio was studied. 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. The mean values for aspartate aminotransferase (AST) were 89.50 ± 14.92 U/L for iodine treated high dose garlic group and 76.38 ± 10.39 U/L for the iodine treated high dose onion group. Oral administration of onion and garlic extract led to a statistically significant ($P < 0.05$) increase in the AST: ALT ratio of both normal and iodine treated rats. There was a significant ($P < 0.05$) decrease in alkaline phosphatase (ALP) activity in both normal and iodine treated rats when compared with the control due to oral administration of onion and garlic extract. The increase in serum AST activity upon administration of onion and garlic extracts respectively in high doses and a rise in the AST: ALT ratio suggests that some phytochemical components in onion and garlic may have heart directed toxicity and calls for caution on the use of these spices by people suffering from cardiac related disorders. The results are discussed in relation to the fight against iodine deficiency eradication.

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

INTRODUCTION

The *Alliums* are a large genus containing about 700 species including economically important vegetable and flowering ornamentals as well as wild species from Europe, Asia, and the Americas [1]. They are all odourless until the tissue is damaged, at which point all generate the volatile and reactive sulphur containing chemicals that are responsible for the expression of their best known characteristics.

Onion (*Allium cepa*) and garlic (*Allium sativum*) as well as other *Alliums* are important because of the culinary value of their flavours, and odours. Numerous health benefits have been attributed to these vegetables, including prevention of cancer and cardiovascular disorders [2-3]. Onions have a unique combination of three families of compounds that are believed to have salutary effects on human health- fructans, flavonoids and organo-sulfur

compounds [4]. Though many clinical trials showed a positive effect of garlic on almost all cardiovascular conditions, however a number of studies have cast doubt on the efficacy of garlic to lower cholesterol level in serum [5]. In Nigeria, iodination of table salts have been made compulsory in order to combat the scourge of goitre. Presently, little is known about the effect of some of the phytochemical components of these *Alliums* 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.

The dry scaly part of the onion bulbs was removed and the fresh bulbs were properly washed. A quantity of 300 g 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 455.7 g 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 300g of the garlic were weighed and macerated in 300ml of deionized water using an electric blender. The solution left behind 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 400mls of water. 0.8ml 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 80-120 g 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 $26\pm 2^{\circ}\text{C}$ and 46% respectively. Feed and water were provided *ad libitum*. Their 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

Extract administration, sample collection and biochemical assays.

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 1.14 g 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.

About 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. This is same as incorporating it into the diet fed to the animals, but on account of likely nutrient-chemical interaction that may affect bioavailability, oral solution of potassium iodide was preferred.

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



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:

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 [6-7] using enzymatic colorimetric diagnostic kits obtained from Human Laboratories (Human Gesellschaft für Biochemica und Diagnostica mbH Max-Planck-Ring 21-D-65205 Wiesbaden Germany).

Principle:

p-Nitrophenylphosphate reacts reversibly in the presence of water in a reaction catalysed by alkaline phosphatase to form phosphate and p-nitrophenol. Change in absorbance after every minute for 4mins was measured. This was used to determine the alkaline phosphatase activity. 20 µl 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. Loading the experimental animals with iodine led to a statistically significant (P < 0.05) decrease in the serum ALT activity when compared to normal rats. Oral administration of onion and garlic extract either to normal or iodine treated rats led to a statistically significant (P<0.05) decrease in the serum ALT activity when compared with the control.

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. Treatment with iodine led to a statistically significant (P<0.05) decrease in serum AST activity. Except the high dose onion group treated with iodine, oral administration of onion and garlic extract led to statistically significant (P<0.05) increase in AST when compared with the control. The changes in the AST activity due to the administration of the onion or garlic extracts were dose dependent in the all animals expect 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. There was a statistically significant (P<0.05) increase in AST:ALT ratio due to the treatment with iodine. Oral administration with onion and garlic extract led to statistically significant (P<0.05) increases in the AST:ALT ratio of both normal and iodine treated rats which were not dose dependent. In general, the oral administration of onion and garlic extract led to an increase in the AST:ALT ratio of both normal and iodine treated rats.

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. The treatment with iodine led to a statistically significant (P<0.05) decrease in the ALP activity of the experimental animals. The oral administration of onion and garlic extract led to statistically significant (P<0.05) decrease in the ALP activity of both normal and iodine treated rats which was least pronounced in the low garlic groups. Oral administration of onion and garlic decreased ALT activity in both normal and iodine treated rats when compared with the control.

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

Values are presented as mean ± SD; LDG= Low dose garlic; HDG= High dose garlic; LDO= Low dose onion; HDO=High dose onion; KI = Potassium 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.

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. This enzyme is a relatively specific indicator of acute hepatocellular damage. 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, when found in serum has correlation to heart tissue damage. Administration of high doses of the onion and the garlic extracts caused a statistically significant ($P < 0.05$) increase in the AST activity of normal rats which was also observed in the high dose garlic group of the iodine treated rats.

The computed AST:ALT ratio provided additional evidence. The oral administration of the onion and the garlic extracts caused statistically significant ($P < 0.05$) increases in both the normal and the iodine treated rats when compared to the control group. Although results on the effect of onion and garlic extract administration on the aminotransferase activities of tissues have not been reported in literature, the use of AST: ALT ratio generally to monitor pathologies involving the liver or heart has been reported by [8-9]. According to these reports, an increase in AST: ALT ratio points to pathology involving the heart while a reverse implicates the liver. This increase in the ratio observed may point to some unestablished effect on the heart due to consumption of onion and garlic. Diseases affecting the heart or liver with resultant increased permeability or breakdown of membrane architecture of the cells lead to spillage of these cytosolic enzymes into plasma and their concentration in the latter rises.

In this study, the increase in AST activity in the serum due to administration of onion and garlic extract especially in high doses and a rise in the AST: ALT ratio provided important evidence that onion and garlic may have a toxicological effect directed towards the heart. The liver was not affected probably because of its metabolic competence to degrade metabolite. It is therefore advocated that these spices be consumed with a little bit of caution. 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 and calls for caution on the use of these spices by people suffering cardiac related disorders. These results are discussed in relation to the fight against iodine deficiency

CONCLUSION

The results of the enzyme assay suggest that some phytochemicals in both onion and garlic may have heart directed toxicity but not in the liver. This may be due to interaction with organic sulphur compounds. Hence, inhibiting enzyme activity. The present study however corroborate with the report of Zhang 2008 [2], and therefore calls for caution on its use by people suffering from heart related disorders.

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