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The Phytochemical Composition of *Allium Cepa*/*Allium Sativum* and the Effects of Their Aqueous Extracts (Cooked and Raw Forms) on The Lipid Profile and other Hepatic Biochemical Parameters in Female Albino Wistar Rats.

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ABSTRACT

This research was carried out to compare the effects of the extracts of cooked and raw forms of Allium cepa (onions) and Allium sativum (garlic) on blood cholesterol (CHOL)l, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels in female adult albino Wistar rats applying spectrophotometric technique. Alkaloids, flavonoids, cardiac glycosides, terpenes and resin were detected in both forms of the plants. Fifteen rats were divided into five groups. Aqueous extracts of the cooked and raw forms of both vegetables were then administered orally to all the groups but control. The animals had almost identical weights; hence, isovolumetric quantity of the extracts was administered. Treatment resulted in increased levels of CHOL, TG, HDL, LDL and VLDL levels compared to control group values ($P<0.05$) albeit the elevated concentrations of HDL, TG and VLDL was not statistically significant ($P>0.05$) relative the control group values. Generally, extracts of cooked forms of both plants resulted in higher effects on the parameters than the extracts of their raw forms; activity of alkaline phosphatase in test group was however lower than the control values. Results obtained showed changes in the levels of the parameters. Increase in the activities of the transaminases could suggest adverse effects on the liver owing to the treatment. Heat did not affect the phytochemical composition of both plants.

Key Words: *Cholesterol, garlic, onions, triglycerides, lipoproteins.*

INTRODUCTION

Cholesterol is a primary sterol synthesised by animals and an essential structural component of mammalian cell membranes found mainly in all lipoproteins of plasma but mainly in the beta-lipoproteins. Although an important and necessary molecule for animals, a high level in serum indicates cardiac disease [1]. Onion, *Allium cepa,* belongs to the Lilly family, it shows only a single vertical shoot above the ground and is used for energy storage and as a spice; it could be in various shapes and sizes [2]. Garlic, *Allium sativum,* also of the Lilly family has a strong characteristic odour and taste and the bulb is used as flavouring agent [2]. Both plants are of medicinal values including common cold, heart disease, osteoporosis and other diseases [3]. Some studies have also shown that increased consumption of both plants reduces the risk of head and neck cancers [4]. Application of raw *Allium cepa* is also said to be helpful in reducing swelling from bee stings. It has been reported that *Allium cepa* extracts could be used in the treatment of topical scars [5]. Phytochemicals present in these plants include flavonoids whose active principle is quarcetin which possesses antioxidant attributes; also anthocyanins, polyphenols and diallyl disulphide among others. The antimicrobial properties of garlic *(Allium sativum*) is traced to allicin, a highly reactive compound formed when allinase catalyses its synthesis from alliin. Allicin oxidises haemoglobin to methemoglobin irreversibly thereby inhibiting the binding of oxygen leading to disastrous consequences and even death. This work was designed to monitor the effects of their extracts on some hepatic parameters.

MATERIALS AND METHODS

Equipments

The equipments used were for the laboratory of the Department of Biochemistry, Animal House unit of the Department of Pharmacology, University of Jos, Prestige Medical Laboratory and Jos University Teaching Hospital (JUTH).

Chemicals and reagents

Chloroform, Fortress diagnostics test kits for lipid profile, Randox diagnostic test kits for liver function test, Biuret reagent, sodium chloride solution, Dragendorf reagent, Sodium hydroxide, Ferric chloride, Conc. H_2SO_4, Benzene and Ammonia solution.

Animals used

Fifteen adult female albino Wistar rats with an average weight of 116.51g were used in this study. The rats were acclimatised to laboratory condition for two weeks and were fed vital growers mash obtained from Grand Cereal and Oil Mills Limited, Jos; water was given *ad libitum* before commencement of experiment.

Experimental design

The rats were grouped into five groups of three rats each as follows:

1. Control; fed standard feed and water only,
2. Test group; administered 1ml of raw onion extract only
3. Test group; given 1ml of cooked onion extract only
4. Test group; fed 1ml of raw garlic extract only
5. Test group; fed 1ml of cooked garlic extract only.

In all instances, feeding was oral and frequency of feeding was once daily.

Collection of Samples

Fresh bulbs of onion and cloves of garlic were obtained from terminus market, Jos, Plateau State.

Preparation of sample

Fresh bulbs of onion were washed in distilled water, sliced, boiled (in the case of cooked samples), blended and sieved to get the extract. This was then stored in a clean plastic bottle at room temperature. Same protocol was followed for garlic samples.

Preparation of feed

The standard feed was mixed with little quantity of water and molded into small balls and fed to the animals, water was given *ad libitum*.

Administration of onion and garlic extract

1ml of the onion and garlic extracts in each complete case was administered to the rats orally using a canular.

Collection of blood samples

At the end of two weeks of experimentation, blood from the animals was collected from the jugular veins into washed and plain tubes. Samples were left to clot at room temperature. They were thereafter dislodged and centrifuged at 3000G for 15minutes. Sera were then separated using Pasteur pipette and kept in sample bottles and refrigerated at 20^0C for subsequent liver function and lipid profile tests.

Phytochemical Screening

The phytochemical screening of the extract of the plant by organic solvent was carried out using standard qualitative procedures: Dragendorf test for alkaloids, sodium hydroxide test for flavonoids, ferric chloride test for tannins, Salkowski test for cardiac glycosides, Liebermann-Burchard test for terpenes, general test for saponins, phenols and balsam.

Cholesterol Determination

Cholesterol levels were also estimated colorimetrically. Absorbance of standard and samples were measured at 546nm after prior mixing and incubation in oven at 37^0Cfor 5 minutes.

Triglyceride determination

Triglycerides were determined colorimetrically at 546nm after prior mixing and incubation in oven at 37^0Cfor 5 minutes.

High density lipoprotein (HDL) determination

Low density lipoproteins were determined colorimetrically at 510nm after prior mixing and incubation in oven at 37^0Cfor 5 minutes.

Low Density Lipoprotein (LDL) Determination

This was estimated as a calculated value from the other fractions using the Friedewald calculation.

Very Low Density Lipoprotein (VLDL) Determination

VLDL concentration was estimated thus:

VLDL = 0.45 x TG (mmol/L); where TG = triglycerides.

Assay of the transaminases

colorimetric method for the determination of serum glutamic oxaloacetate transaminase and serum glutamic pyruvic

transaminase was applied to assay for these enzymes [6].

Assay of alkaline phosphatases

colorimetric method for the assay of serum alkaline phosphatase activity was applied [7].

Statistical Analysis

All grouped data were evaluated statistically. ANOVA was used and P value of 0.05 was considered significant. All results expressed as the mean ± standard error of mean for three animals in each group.

RESULTS AND DISCUSSION

The thrust of this work was to monitor the effects of temperature differentials on the phytochemicals and lipid profiles of *Allium cepa* (onion) and *Allium sativum (garlic)* using experimental animals. Results obtained showed the presence of phytochemicals such as alkaloids, flavonoids, cardiac glycosides, terpenes and steroids, and resins in both forms of the plants. However, tannins, saponins, balsam and phenols were not found in both the raw and boiled forms of the vegetables. Alkaloids are involved in relaxation of muscles and relieve nasal congestion, also components of quinine and aspirin. Cardiac glycosides are antidotes for heart failure, irregular heartbeats. Terpenes are psychoactive chemicals found in cannabis. [8] has reported that flavonoids possess both bacteriostatic and bacteriocidal effects on some strains of bacteria; further, they inhibit the activity of reverse transcriptase and proteases. Both vegetables contain them; hence their consumption in moderation is beneficial to the body.

Table 1: Phytochemical components of the raw and cooked form of garlic and onions

	Raw garlic	Cooked garlic	Raw onion	Cooked onion
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	_	_	_	_
Saponins	_	_	_	_
Balsam	_	_	_	_
Cardiac glycosides	+	+	+	+
Terpenes and steroids	+	+	+	+
Resin	+	+	+	+
Phenols	_	_	_	_

Key: + = present; - = absent

Table 2 shows the result for the effect of raw and boiled forms of the aqueous extracts of the vegetables on the activities of the transaminases: aspartate oxaloacetate (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Compared to the control values, the effects of aqueous extract of onion on the activity of AST were higher whether cooked or raw although the effect of cooked form was lower. As for garlic, raw aqueous extract caused decreased activity of AST whereas aqueous extract of the cooked form of garlic led to increased activity of AST compared to control group values ($p<0.05$). In the case of ALT, results indicated higher activity for groups administered aqueous extract of raw form of onion compared to control group values ($P<0.05$). Extracts of the cooked form of onion also led to increased activity of ALT over control group values albeit to a lesser magnitude relative the raw form. A similar pattern was also obtained for garlic which was also the trend for the activity of ALP.

Table 2: Activities of the serum aminotransferases and alkaline phosphatase for the tests and control groups

Groups	AST	ALT	ALP
$Control_1$	389.33 ± 2.15	109.67 ± 1.69	170.67 ± 1.30
$Control_2$ Raw onion	395.03±1.14 445.23 ± 1.52[a]	113.26±1.05 143.2 ± 2.69[a]	179.03±0.84 190 ± 1.98[a]
Cooked onion	1077.3 ±1.25[a, b]	242.2 ± 1.87[a, b]	122 ± 0.62[a, b]
Raw garlic	305.4 ± 1.17[a]	178.87 ± 0.93[a]	221.67 ± 0.96[a]
Cooked garlic	952.07 ± 1.13.5[a, c]	240.77 ± 1.20[a, c]	87 ± 1.57[a, c]

Results are expressed as mean (±SEM); n = 3. a = statistically significant compared to control
b = statistically significant compared to raw onion c = statistically significant when compared to raw garlic
$Control_1$ = Onion extract $Control_2$ = Garlic extract.

Results in table 3 indicated higher level of cholesterol for both forms of aqueous extracts of garlic and onions compared to control groups values ($p<0.05$). But aqueous extracts of the cooked form of the vegetables showed higher magnitude than the raw extracts. In the case of triglycerides (TG), treatment was observed thus: aqueous extracts of cooked garlic having higher values than cooked onions, raw onion and raw garlic compared to control group values ($p<0.05$). Triglycerides are composed of three fatty acids esterified to a glycerol backbone and are transported in the blood as core constituents of all lipoproteins, but are major components of triglycerides-rich chylomicrons and very low-density lipoproteins. Measuring triglycerides levels, in conjunction with other lipids assays, is useful in the diagnosis of primary and secondary hyperlipoproteiemia, dyslipidemia and triglyceridemia. Triglycerides level is useful in diabetes mellitus, nephrosis and liver obstruction disease. For low density lipoproteins (LDL), results compared to control group values, trend observed in order of decreasing effect was: aqueous extracts of cooked onion greater than cooked garlic. Both raw onions and garlic extracts had result similar with that of control groups. Results for high density lipoproteins (HDL) indicated a medley of trends as follows: extract of cooked garlic was highest compared to control group values ($p<0.05$), followed by extract of raw onion, raw garlic and cooked onion extract. For very low density lipoproteins (VLDL), the results compared to control group values were: extract of cooked garlic greater than cooked onions; extracts of raw onion and garlic had identical levels.

Table 3: Lipid profile of the various groups of rats

Groups	Cholesterol (mmol/L)	HDL (mmol/L)	TG (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
$Control_1$	2.19 ± 0.69	1.77 ± 0.19	1.58 ± 0.11	1.67 ± 0.22	0.75 ± 0.04
$Control_2$ Raw onion	2.54 ± 0.11 3.30 ± 0.26^{a}	1.86 ± 0.58 2.21 ± 0.26^{b}	1.59 ± 0.16 1.67 ± 0.10^{b}	1.66 ± 1.12 1.64 ± 0.32^{a}	0.88 ± 0.31 0.75 ± 0.04^{b}
Cookedonion	$3.59 \pm 0.22^{a, e}$	$1.70 \pm 0.36^{b, c}$	$1.73 \pm 0.03^{b, c}$	$2.32 \pm 0.09^{a, e}$	$0.78 \pm 0.01^{b, c}$
Raw garlic	2.66 ± 0.09^{a}	1.72 ± 0.08^{b}	1.66 ± 0.04^{b}	1.36 ± 0.04^{a}	0.75 ± 0.02^{b}
Cooked garlic	$3.94 \pm 0.21^{a, f}$	$2.44 \pm 0.11^{b, d}$	$1.84 \pm 0.13^{b, d}$	$1.89 \pm 0.07^{a, f}$	$0.83 \pm 0.06^{b, d}$

Results are expressed as mean (±SEM); n = 3.
a = statistically significant compared to control
b = not statistically significant compared to control
c = not statistically significant compared to raw onion
d = not statistically significant compared to raw garlic
e = statistically significant compared to raw onion
f = statistically significant compared to raw garlic
$Control_1$ = Onion extract, $Control_2$ = Garlic extract.

Both *Allium cepa* (onion) and *Allium sativum (garlic)* contain very important compounds with medicinal values. Onions contain quercetin derivatives regarded as the most important flavonoids to improve diabetic status in cells and animal models. Regular consumption of onions is reported to lower cholesterol levels thus preventing incidence of atherosclerosis and diabetic heart disease. This is generally speculated to be effected by the organosulfur compounds present, which lower high homocysteine levels, risk factor for heart attack and stroke [3]. In this report, the levels of cholesterol for the test groups was higher than the control values ($p<0.05$) for both forms of the plants. However, extracts of the cooked form of the vegetables had higher magnitude of cholesterol increase over the extracts of the raw form of the plants. [9] reported onions to be hypoglycaemic and allyl propyl disulfide is implicated to be the active principle; it lowers blood sugar levels by increasing the amount of free insulin available. Onion extracts are used in the treatment of topical scars [10]. Garlic on the hand contains the sulphur containing compounds allinn, ajoene, diallylsulfide, dithiin, S-allylcysteine, vitamin B, proteins, flavonoids, saponins and minerals. Furthermore allixin (3-hydroxy-5-methoxy-6-methyl-2-penthyl-4*H*-pyran-4-one) was found a non-sulfur compound with a γ-pyrone skeleton structure with anti-oxidative effects, anti-microbial effects, anti-tumor promoting effects, inhibition of aflatoxin B_2 DNA binding, and neurotrophic effects [11]. These chemical components in each plant constitute the active principles in drugs such as allicin (component of garlic) in antibiotics, organo-sulphur compounds in onion believed to confer antioxidant, anti-inflammatory, anti-allergic, antithrombic activity by inhibiting cyclooxygenase [12]. Allicin affects atherosclerosis through modification of lipoprotein and as an inhibitor of HMG-coA reductase activity__ the rate-limiting enzyme of cholesterol biosynthetic pathway. Quarcetin possesses hypolipidemic effects and protects against lindane induced toxicity in liver by restoring the altered levels of lipids.

But these useful active principles can be affected by temperature variations which could lead to the lost of their medicinal significance. Fresh aqueous extract of garlic containing 324μg/ml allicin inhibited the growth and killed *Salmonella serovars;* in some cases, it led to static effect on the bacteria. At 4^0C, garlic extract did not lose its antibacterial activity and can stand for several days. However, excessive high temperatures have been reported to cause the lost of the potency of garlic (allicin) against *Salmonella serovars* [13]; allicin is garlic's defence mechanism

against attacks by pests and is produced through the catalytic action of allinase which catalyses conversion of allin to allicin. [14] have reported allicin to inhibit the growth of *Helicobacter pylori in vitro* and that the bacteriostatic properties of allicin were active to at least 10 months for samples maintained at 6^0C. [15] have reported that boiled garlic had little effect on the activity of cyclooxygenase which is related to the fact that the active principle of raw garlic is destroyed upon heating. Also, 45 minutes (1 minute of microwave heating) of oven heating of garlic blocked the ability of garlic to inhibit *in vivo* binding of mammary carcinogen (7,12-dimethylbenzene(a) anthracene metabolites to rat mammary epithelial cell DNA; heating destroys garlic's active allyl sulphur formation which could relate to its anticancer attributes. The route of administering the extracts of both vegetables to the animals is also another factor that affects the bioavailability of the active principles present in them. In this work, the aqueous extracts of both the raw and cooked forms of the plants were administered orally to the rats and therefore the extracts had to pass through the gastrointestinal tract before reaching the circulation. Hence, the exact quantity of the extracts and therefore the active principle may not be delivered to organs and tissues which could give abnormally low or high magnitude of the effects of the beneficial active principles.

Generally, animals in the test groups showed higher activities of the transaminases as well as alkaline phosphatase; this means that the experiment may have caused some levels of damage to the liver. In the case of the lipid profile results, there was no significant difference in the TG, HDL and VLDL levels compared to control group values ($p>0.05$). This agrees with the report of [3], that regular consumption of onion and garlic reduces blood cholesterol levels leading to a reduced risk of developing cardiovascular diseases including atherosclerosis.

CONCLUSION

Heat did not affect the presence of the phytochemicals screened in the two vegetables but affected the levels of the hepatic parameters analysed. The elevated activities of the enzymes assayed shows that the extract may potentiate some adverse effects on the liver. Hence, consumption in moderation is advised; cooking them at high temperatures should be avoided as some active medicinal principles present in them could be destroyed. Further work should consider a longer time frame of treatment; levels of phytochemicals, mineral elements and anti-nutritional factors present in the samples. This will further enrich and concretise the basis for drawing conclusions.

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