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Effects of *Zingiber officinale* and *Allium sativum* on the Liver Enzymes of Albino Rats (*Rattus norvegicus*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author CMGO designed and supervised the study, while author TU wrote the protocol, and wrote the first draft of the manuscript, managed the analyses and literature searches of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the effect of ethanolic extract of *Zingiber officinale* and *Allium sativum* on the liver enzymes of albino rats.
Study Design: Experimental study.
Place and Duration of Study: CNed Medical Diagnostic Laboratory, Nsukka, Nigeria, between September – October, 2019.
Methodology: Adult Wistar rats of both sexes weighing 100-160g were obtained from an animal house of the Department of Animal Science, University of Nigeria Nsukka, Nigeria. The experimental animals were acclimatized for one week and fed with standard laboratory diet (finishers feed) and water *ad libitum.* The plant materials (Garlic and Ginger were extracted using the Soxhlet method. The rats were divided into seven groups of three rats each and were given different treatments. At the end of every stage of the experiment, all animals were fasted for 12 hours and thereafter sacrificed after mild anesthesia with chloroform. Blood samples (5 ml) were

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collected from the eye of each rat weekly and analyzed for liver enzymes, using spectrophotometric methods. Data generated were analyzed using Social Sciences (SPSS) of IBM Corporation, Armonk, USA) version 21. One way analysis of variance (ANOVA) was used to compare the experimental groups with Duncan multiple range tests used in partitioning the mean while the student t-test was used to compare the duration. Results were expressed as mean ± SEM and values with P<0.05 were considered statistically significant as described by Duncan, (1955).

Results: From the results of the acute toxicological screening, the oral LD50 was calculated and found to be: 1414 mg/kg for ginger extract 3162 mg/kg for garlic and ginger extract and >5000 mg/kg for garlic extract. Results for ALP showed a mean of 37.90 ± 3.48 µ/L and 55.97 ± 0.61 µ/L for ginger (100mg/kg) week 1 and 2 respectively, and there was a significant increase in ALP (*p=0.034).* Values for ALT showed significant increases (0.043) for ginger (200mg/kg) and garlic and ginger (both 100 and 50 mg/kg). All combinations of plants and concentrations were significant for AST, while only ginger at 100mg/kg showed significant difference when values for week 1 and 2 were compared for GGT.

Conclusion: This suggests that garlic extract exerts an acute effect in rats, contrary to ginger extract which exerts a chronic effect (that is to say, effect becomes more prominent with increase in duration of consumption).

Keywords: Zingiber officinale; Allium sativum; liver enzymes; albino rats (Rattus norvegicus).

1. INTRODUCTION

The use of “natural” or alternative medicines, especially common ones as garlic *(Allium sativum)* and ginger *(Zingiber officinale)* has increased markedly over the last few years [1]. “More and more older adults are using complementary and alternative medicine dietary supplements and herbal remedies without advice from a physician on the assumption that these substances will have a beneficial effect” [1]. “However, this might not be a safe or advisable practice. Regrettably, a great deal of the information regarding the effectiveness and safety of these remedies has been garnered from anecdotal or historical accounts, and much of the information offered is generally misleading and might even be detrimental” [2]. “Natural medicines are often tried for many conditions based on tradition, anecdotes or marketing, but not all of these indications are supported by reliable or credible scientific research” [3]. Previous studies have shown “the separate effects of *Allium sativum* and *Zingiber officinale* on various cardiovascular, haematological parameters and biochemical parameters but very little of their combined effects. Garlic has been known as one of the oldest known horticultural crops in the Old World and has been used since time immemorial as a culinary spice and medicinal herb which is very nutritious” [4]. Ginger is commonly used for relieving stomachaches, diarrhea, and vomiting. Ginger is used widely in Ayurveda to cure a many of the illness such as indigestion, tastelessness, loss of appetite, flatulence, intestinal, biliary colics, nausea, vomiting, allergic reactions, acute and chronic cough, common cold, fever, allergic rhinitis, sinusitis, acute and chronic bronchitis, respiratory troubles, pain, headache, backache or any kind of muscular catch, painful tooth and swelled gum (The Ayurvedic Pharmacopoeia of India, 1999). However, there is paucity of information in the available literature on the toxic or beneficial effects of using garlic and ginger collectively as a medicine on the liver enzymes. Results from the research of their combined effects on the liver enzymes can provide substantial information which may be beneficial to the therapy and prophylaxis of certain liver diseases. Therefore, the aim of this study was to determine the effect of ethanolic extract of *Zingiber officinale* and *Allium sativum* on the liver enzymes of albino rats.

2. MATERIALS AND METHODS

2.1 Procurement and Management of Experimental Animals

Strains of adult Wistar rats of both sexes weighing 100-160g were obtained from an animal house of the Department of Animal Science, University of Nigeria Nsukka, Nigeria. The experimental animals were acclimatized for one week and fed with standard laboratory diet (finishers feed) and water *ad libitum*. The animals were kept in metal cages under standard laboratory conditions (12 hours light and 12 hours dark cycle) and maintained under prevailing atmospheric conditions with frequent cleaning and observation.

2.2 Plant Collection and Extract Preparation

2.2.1 Plant collection

Fresh bulbs of garlic and ginger rhizomes (weighing 3kg each) were commercially obtained from the Ogige Central Market, Nsukka. The papery and epidermal skin of the garlic was removed to separate the bulbs into individual cloves. The garlic cloves were then cut longitudinally into smaller pieces in order to increase the surface area for quicker drying. The garlic slices were dried in an oven at about 60^0C. The result was dry and brittle garlic cloves with a brownish coloration. The properly dried garlic were ground into fine powder using a mechanical grinder and stored in an airtight container to prevent moisture penetration.

The ginger rhizomes were properly washed and grated using a manual grater in order to increase surface area for quicker drying. The grated ginger was spread on flat trays and allowed to air dry at room temperature. The result was a dry and crisp ginger with a brown colour. The dried ginger was weighed three consecutive times and was found to be of consistent weight. The dried ginger was then ground into fine powder using a mechanical grinder and stored in an airtight container.

Soxhlet apparatus was used to prepare the ethanolic extract of the powdered plant materials. 300g of the powdered material was loaded in the thimble of the 500 litres soxhlet apparatus. It was extracted with 500ml of 80% ethanol at 60^0C for three hours. The liquid extract was concentrated to dryness using a water bath at 40^0C. The extract was stored in a refrigerator until use.

2.2.2 Extract concentration preparation

The concentration of the garlic extract was obtained by first dissolving 100mg of the garlic extract in 10 ml of distilled water; which resulted in a 100 mg/ml stock solution. For the ginger extract, 500mg of ginger was dissolved in 6 ml of 3% tween 80 solution. This is because the ginger extract was not soluble in water, so tween 80 was used to make it more hydrophilic. the calculations proceeded as:

Concentration (ml) = Dose (mg/kg) x Weight of rat (kg) /Stock solution (mg/ml)

2.3 Acute Toxicity Study

Acute toxicity test was conducted based on Lorke's method [4]. The median lethal dose (LD_{50}) of the garlic and ginger extracts were carried out in order to determine a suitable dose for its evaluation and this was carried out using 9 rats. The rats were divided into three groups of three rats each, and were treated with 1000 mg, 2000 mg and 5000 mg of the extract per kg body weight orally. The rats were observed for 24 hours for signs of toxicity, including death. The LD_{50} was then calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of the consecutive doses, for which 0 and 100% survival rates were recorded.

2.4 Experimental Design

The twenty-one albino rats used for this experiment were divided into seven different cages labelled group 1-7 with three rats (n=3) in each group.

Group 1: Served as control; rat was orally administered with distilled water.

Group 2: Rat was orally administered with 200 mg/kg of garlic plant extract.

Group 3: Rat was orally administered with 100 mg/kg of garlic plant extract.

Group 4: Rat was orally administered with 200 mg/kg of ginger plant extract.

Group 5: Rat was orally administered with 100 mg/kg of ginger plant extract.

Group 6: Rat was orally administered with 100 mg/kg of garlic plant extract and 100 mg/kg of ginger plant extract.

Group 7: Rat was orally administered with 50 mg/kg of garlic plant extract and 50 mg/kg of ginger plant extract.

The rats were weighed at the end of each week and the experiment was performed over a duration of 14 days.

2.5 Sample Collection and Preparation

At the end of every stage of the experiment, all animals were fasted for 12 hours and thereafter sacrificed after mild anesthesia with chloroform. Blood samples (5ml) were collected from the eye of each rat weekly. The blood samples were kept for 30 minutes without disturbance then centrifuged for 5 – 10 minutes at 1000 rpm to separate serum. The clear supernatant serum was separated and collected by Pasteur pipette into a dry clean tube for liver function analysis.

2.6 Sample Analysis

2.6.1 Determination of liver enzymes

Liver enzymes (Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Gamma-glutamyl Transferase (GGT)) were analyzed using commercially available test kits.

2.6.1.1 Determination of Alanine Transaminase (ALT)

The Alanine Transaminase activity was determined using the method described by Reitman and Frankel, 1957 [5].

Principle: The amino group is enzymatically transferred by alanine transaminase present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate. Alanine transaminase activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine over a definite period of time in an alkaline solution.

2.6.1.2 Determination of Aspartate Transaminase (AST)

The Aspartate Transaminase activity was determined using the method described by Reitman and Frankel, 1957 [5].

Principle: The serum aspartate transaminase (AST) catalyzes the transfer of the amino group of glutamic acid to oxaloacetic acid in reversible reactions. The transaminase activity is proportional to the amount of oxaloacetate formed over a definitive period of time and is measured by a reaction with 2, 4-dinitrophenylhydrazine (DNPH) in alkaline solution.

2.6.1.3 Determination of Alkaline Phosphatase (ALP)

The Alkaline Phosphatase activity was determined using the method described by Huggins and Talalay, 1945 [6].

Principle: Serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline pH value (10.5-11.0), turns a pink colour that can be photometrically determined.

2.6.1.4 Determination of Gamma-glutamyl Transferase (GGT)

The Gamma-glutamyl Transferase activity was determined using the method described by Tietz, 1987 [7].

Principle: The substrate L-gamma-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by gamma-glutamyl transferase in the sample to 5-amino-2-nitrobenzoate which can be measured at 405 nm. The activity is based on the measurement of aniline liberated by the enzymatic cleavage of the substrate.

2.7 Statistical Analysis

Statistical Package for Social Sciences (SPSS) of IBM Corporation, Armonk, USA) version 21 was used for statistical analysis. One way analysis of variance (ANOVA) was used to compare the experimental groups with Duncan multiple range tests used in partitioning the mean while the student t-test was used to compare the duration. Results were expressed as mean ± SEM and values with $P<0.05$ were considered statistically significant as described by Duncan, (1955).

3. RESULTS AND DISCUSSION

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. This agrees with the work done by Rhiouania et al. [8]. The result of this experiment showed that the normal control group recorded the lowest significant mean serum ALT, AST, ALP and GGT levels in both week 1 and 2. The comparison of activities of serum levels of aspartate

transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) among the experimental groups showed that the level of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) increased significantly ($P < 0.05$) in groups dosed 200 mg/kg of garlic, 200 mg/kg of ginger and 100 mg/kg each of both garlic and ginger when compared with the normal control group; while the levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) activity of groups dosed 100 mg/kg of garlic, 100 mg/kg of ginger and 50 mg/kg each of both garlic and ginger were statistically similar with the normal control group. The increment of the ALT, AST, ALP and GGT levels showed a dose and time dependency in all experimental groups. This means that the ALT, AST, ALP and GGT mean serum levels increased with increased extract dosage and prolonged administration of extract. This corresponds to the result of the research carried out by Huzaifa *et al*, (2013) [9] but disagrees with the research carried out by Joshua *et al*, (2014). Elevations of alkaline phosphatase levels can indicate obstruction of the bile ducts or other problems impeding the flow of bile from the liver to the duodenum while increased levels of gamma-glutamyl transferase is much more sensitive and specific to liver function disruption. Aminotransferase levels are used primarily in the diagnosis of liver disease. Although not specific to liver disease, it can be used in combination with other enzymes to monitor the course of various liver diseases [10]. "Despite all these considerations, minor elevations of aminotransferases are often construed to be clinically insignificant, partly because of lack of a longitudinal study about the impact of abnormal aminotransferases on long-term outcome such as end-stage liver disease or premature mortality" [11].

Table 1. Effects of *Allium sativum* and *Zingiber officinale* on the serum alkaline phosphatase (ALP) of albino rat

Treatment groups / Concentrations (mg/kg)	Dose / Concentrations (mg/kg)	Weeks / Alkaline phosphatase, ALP (µ/L)		t	p-value
		1	2		
Normal control	0	33.37 ± 1.79^{a}	34.93 ± 1.50^{a}	-3.357	0.078
Garlic	200	57.17 ± 4.11^{c}	$63.03 \pm 0.69d^{e}$	-1.423	0.291
Garlic	100	39.83 ± 3.54^{ab}	48.87 ± 1.85^{b}	-2.818	0.106
Ginger	200	53.73 ± 4.99^{c}	65.33 ± 1.57^{e}	-2.663	0.117
Ginger	100	37.90 ± 3.48^{ab}	55.97 ± 0.61^{c}	-5.255	0.034
Garlic & Ginger	100 & 100	50.23 ± 2.56^{bc}	58.80 ± 2.98^{cd}	-4.029	0.056
Garlic & Ginger	50 & 50	39.03 ± 5.12^{ab}	54.50 ± 1.75^{c}	-3.027	0.094

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$).
*Level of significance along rows: * = $p < 0.05$; ** = $p < 0.01$*

Table 2. Effects of *Allium sativum* and *Zingiber officinale* on the serum alanine transaminase (ALT) of albino rats

Treatment groups / Concentrations (mg/kg)	Dose / Concentrations (mg/kg)	Weeks / Alanine transaminase, ALT (µ/L)		t	p-value
		1	2		
Normal control	0	5.00 ± 0.58^{a}	6.37 ± 0.73^{a}	-1.814	0.211
Garlic	200	17.00 ± 2.08^{d}	23.20 ± 0.44^{c}	-2.713	0.113
Garlic	100	10.17 ± 1.74^{b}	18.20 ± 0.70^{b}	-3.293	0.081
Ginger	200	16.37 ± 0.86^{d}	24.17 ± 1.01^{c}	-4.642	0.043
Ginger	100	7.90 ± 0.56^{ab}	16.67 ± 1.33^{b}	-8.159	0.015
Garlic & Ginger	100 & 100	14.40 ± 0.83^{cd}	23.17 ± 1.48^{c}	-3.887	0.060
Garlic & Ginger	50 & 50	11.17 ± 1.48^{bc}	19.00 ± 0.50^{b}	-5.391	0.033

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$).
*Level of significance along rows: * = $p < 0.05$; ** = $p < 0.01$*

Table 3. Effects of *Allium sativum* and *Zingiber officinale* on the serum aspartate transaminase (AST) of albino rats

Treatment groups / Concentrations (mg/kg)	Dose / Concentrations (mg/kg)	Weeks / Aspartate transaminase, AST (µ/L)		t	p-value
		1	2		
Normal control	0	6.00 ± 0.50^{a}	5.00 ± 0.29^{a}	3.464	0.074
Garlic	200	14.03 ± 0.52^{c}	24.00 ± 0.58^{c}	-38.283	0.001
Garlic	100	12.67 ± 0.60^{bc}	19.50 ± 0.29^{b}	-9.406	0.011
Ginger	200	13.50 ± 0.50^{c}	23.67 ± 0.67^{c}	-10.028	0.010
Ginger	100	12.00 ± 1.32^{bc}	19.00 ± 0.58^{b}	-4.355	0.049
Garlic & Ginger	100 & 100	11.167 ± 1.17^{bc}	26.67 ± 0.88^{d}	-20.294	0.002
Garlic & Ginger	50 & 50	10.00 ± 1.53^{b}	20.83 ± 0.93^{b}	-6.600	0.022

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$).
*Level of significance along rows: * = $p < 0.05$; ** = $p < 0.01$*

Table 4. Effects of *Allium sativum* and *Zingiber officinale* on the serum gamma-glutamyl transferase (GGT) of albino rats

Treatment groups / Concentrations (mg/kg)	Dose / Concentrations (mg/kg)	Weeks / Gamma-glutamyl transferase, GGT (µ/L)		t	p-value
		1	2		
Normal control	0	26.10 ± 1.04^{a}	25.77 ± 1.19^{a}	0.216	0.849
Garlic	200	62.37 ± 14.05^{bc}	76.20 ± 11.17^{c}	-1.351	0.309
Garlic	100	37.70 ± 1.21^{a}	45.57 ± 1.39^{b}	-9.532	0.011
Ginger	200	86.83 ± 10.84^{d}	94.13 ± 7.75^{c}	-2.348	0.143
Ginger	100	40.93 ± 1.43^{ab}	45.60 ± 1.08^{b}	-11.875	0.007
Garlic & Ginger	100 & 100	72.30 ± 6.94^{cd}	76.27 ± 6.24^{c}	-3.430	0.076
Garlic & Ginger	50 & 50	42.17 ± 3.76^{ab}	45.77 ± 4.84^{b}	-2.834	0.105

Mean values with different alphabets as superscripts in a column differ significantly ($p < 0.05$).
*Level of significance along rows: * = $p < 0.05$; ** = $p < 0.01$*

In the first week, the group dosed 200 mg/kg of garlic registered the highest significant ($P < 0.05$) mean serum ALP, AST, and ALT levels while the group dosed 200 mg/kg of ginger registered the highest significant ($P < 0.05$) mean serum GGT level. In the second week, the highest significant ($P < 0.05$) mean serum ALP, GGT and ALT levels were observed in the group dosed 200 mg/kg of ginger while that of mean serum AST level was observed in the group dosed combined doses of garlic (100 mg/kg) and ginger (100 mg/kg) extract.

4. CONCLUSION

This suggests that garlic extract exerts an acute effect in rats, contrary to ginger extract which exerts a chronic effect (that is to say, effect becomes more prominent with increase in duration of consumption). There is no literal or research work backing up this claim. From the acute toxicity study, ginger extract seems to be more toxic than the garlic extract. There was no significant difference between the group dosed garlic and ginger individually and those dosed garlic and ginger in combination.

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ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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