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Evaluation of the protective effects of co-administered *Zingiber officinale* and *Allium sativum* ethanol extracts on hepatic and renal functions using female Wister rat models

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Abstract

Hepatic and renal function tests are done to indicate how well the liver and kidneys are working. Parameters been tested include serum concentration of alanine transferase, aspartate transferase, alkaline phosphatase, blood urea nitrogen and creatinine among others. This study was aimed at evaluating the effect of singly and combined administration of *Zingiber officinale* and *Allium sativum* on hepatic and renal functions. The herbs were extracted with ethanol. A total of 40 female Wister rats were grouped into eight (n=5). Group 1 was treated with distilled water and served as control; group 2 was treated with 530 mg/kg body weight of *Zingiber officinale* and group 3 treated with the same dose of *Alliun sativum*. Groups 4-8 were treated with different ratios of Z;A (*Zingiber officinale: Allium sativum*). On the 91st day of treatment, blood samples were collected from all the rats and analyzed for the parameters. *Zingiber officinale* decreased all the tested parameters significantly ($P < 0.05$) when compared with group 1. *Allium sativum* on the contrary increased the parameters except for creatinine where it showed no activity. The combination of the two herbs reduced the concentration of the parameters but the reduction was only significant ($P < 0.05$) when the proportion of *Zingiber officinale* was greater. In conclusion, only monotherapy of *Zingiber officinale* protected the liver and kidneys from damage; and if these herbs must be used together for enhanced liver and kidney health, the ration Z:A = 8:2 in which the proportion of *Zingiber officinale* is more should be recommended.

Keywords: *Allium sativum*; Blood urea nitrogen; Hepatic and renal function; *Zingiber officinale*.

1. Introduction

Liver function tests which is also known as a liver panel use blood sample to measure some substances produced by the liver such as albumin, total protein, ALP (alkaline phosphatase), ALT (alanine transaminase), AST (aspartate aminotransferase), among others. The essence of these tests is to indicate how well the liver is working or whether the liver may be damaged by liver disease or injury. In a certain study, the liver was noted to have a significant role in metabolism, digestion, detoxification, and elimination of substances from the body. The liver function tests typically include alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, prothrombin time (PT), the international normalized ratio (INR), total protein and albumin. According to the researchers, these tests can help determine an area of the liver where damage may be taking place and, depending on the pattern of elevation, can help organize a differential diagnosis. For instance, elevations in ALT and AST disproportion to elevations in alkaline phosphatase and bilirubin denote hepatocellular disease. An

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elevation in alkaline phosphatase and bilirubin in disproportion to ALT and AST would characterize a cholestatic pattern [1]. On the other hand, the renal system is made up of the kidney, ureters, and the urethra. Its functions include elimination of toxins, metabolic waste products, and excess ion from the body among other functions. Tests of renal function is important in diagnosis of renal disease, monitoring the response of kidneys to treatment, and determining the progression of renal disease. The most commonly used endogenous marker for the assessment of glomerular function is creatinine. The calculated clearance of creatinine is used to provide an indicator of glomerular filtration rate (GFR). Urea or blood urea nitrogen (BUN) is a nitrogen-containing compound formed in the liver as the end product of protein metabolism and the urea cycle. About 85% of urea is eliminated via kidneys; the rest is excreted via the gastrointestinal (GI) tract. Serum urea levels increase in conditions where renal clearance decreases (in acute and chronic renal failure/impairment). The ratio of BUN: creatinine can be useful to differentiate pre-renal from renal causes when the BUN is increased. In pre-renal disease, the ratio is close to 20:1, while in intrinsic renal disease, it is closer to 10:1. Upper GI bleeding can be associated with a very high BUN to creatinine ratio (sometimes >30:1) [2]. Herbs such as turmeric, milk thistle, artichoke, and dandelion root among others have been traditionally used to support liver function. Herbal remedies have been used to relieve disorders that are related to liver and other internal organs for many centuries, and have currently become a favorable therapy globally for pathological liver conditions. The increasing use of herbal medicines is based on their supposed effectiveness, and the belief that they are safe. In this study therefore, the protective effects of *Zingiber officinale* and *Allium sativum* ethanol extracts administered separately and in combination were evaluated on hepatic and renal functions using female Wister rat models

2. Materials and methods

2.1. Materials

2.1.1. Animals

Female Wister rats (140.6 – 145.4 g) were used for the study. All the animals were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria. The animals were housed in standard laboratory conditions of 12 hours light, room temperature, and 40 - 60% relative humidity and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to food and water. Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes; (Approval number is NAU/AREC/2023/00021).

2.1.2. Chemicals and Reagents

Hydrochloric acid (Prime laboratories, India); Dragendoff reagent (Sigma Aldrich, United States of America); Ammonia (Shackti Industrial Gases, India), sodium hydroxide (Treveni Chemical Pvt., India); Ferric chloride (AkashPurochem. Pvt., India); Fehling's solution (Lab care Diagnostics, India); Million reagent (Interlab Chemical Pvt., India): Ethanol (TAJ Pharmaceutical Ltd., India); Acetic anhydride (Ashok Organics Industries, India); Concentrated sulfuric acid (Navin Chemical Pvt., India), Acetic acid (Kayla Africa Suppliers, South Africa); Molisch reagent (Interlab Chemical Pvt., India); alcoholic alpha naphatol (Prat Industry Corcopation, India), L-alanine (200 mmol/l), α-oxoglutarate (2.0 mmol/l) and phosphate buffer (100 mmol/l), 2, 4- dinitrophenylhydrazine,

2.1.3. Equipment

Glass column, flasks, beakers, test tubes, measuring cylinders, surgical blade, forceps, scissors, graph paper, white transparent paper, rotary evaporator, Analytical Weighing Balance (Metler H30, Switzerland), Electric Oven (Gallenkamp, England), Water Bath (Techmel & Techmel, Texas, USA), National Blender (Japan), Micropippete (Finnipipette® Labsystems, Finland), Plethysmometer (Biodevices, New Delhi, India) and Intubation tubes. Precision pipettes (25, 50, 100 and 300 µl, 1,000 µL) (Labcompare USA); Disposable pipette tips (Labcompare USA); Distilled or deionized water (SnowPure Water Technologies USA); Stop watch (Avi Scientific India); disposable hand gloves (Supermax Malaysia), ALT test kit (Span Diagnostics Ltd., India). UV-VIS spectrophotometer (Model 752, China), AST test kit (Span Diagnostics Ltd., India). ALP test kit (Span Diagnostics Ltd., India), BUN and creatinine test kits (Teco Diagnostics, USA).

2.1.4. Plant materials

Fresh *Zingiber officinale* and *Allium sativum* were procured from the market in Enugu State of Nigeria.

Extraction of plant material

The *Zingiber officinale* and *Allium sativum* were scraped, sliced, washed and dried away from sunlight at room temperature for 48 hours. The dried materials were pulverized to powder using an electronic blender and kept in clean airtight amber colored bottles separately. Then, 750 g of each of the powdered plant material was cold macerated in 95% ethanol. The mixture was allowed to stand for two days (48 hours) with intermittent agitation. It was filtered and the filtrate concentrated to dryness using water bath at 40 °C for 72 hours. The extract was stored in a refrigerator until used.

2.2. Methods

2.3. Phytochemical analysis of *Zingiber officinale* and *Allun sativum* separately

The qualitative phytochemical analysis of the extract and fractions were carried out using standard methods described by Peter *et al*., 2023 [3].

2.3.1. Acute toxicity studies

The actual median lethal dose (LD50) estimation of the *Zingiber officinale, Allium sativum* and combined *Zingiber officinale* and *Allium sativum* ethanol extracts was conducted with the method described by Lorke 1983 [4] and modified by Peter *et al*., 2023 [3].

2.3.2. Biochemical assay of serum liver marker enzymes

Quantitative determination of alanine aminotransferase (ALT)

Serum alanine transaminase was estimated by the method described by Colville (2002) [5] using ALT test kit (Span Diagnostics Ltd., India). A 0.25 ml of mixture of L-alanine (200 mmol/l), α-oxoglutarate (2.0 mmol/l) and phosphate buffer (100 mmol/l) was added to 0.5 ml of each sample and blank (containing distilled water). They were mixed and incubated at 37 °C for exactly 30 minutes in a water bath. A 0.25 ml of 2, 4- dinitrophenylhydrazine was added into the sample and blank test tubes and incubated again at room temperature for 20 minutes. A 2.5 ml of sodium hydroxide (0.4 mol) was then added to all the test tubes and the absorbance of the sample was read against the blank at 546 nm using a UV-VIS spectrophotometer (Model 752, China). The ALT concentration was extrapolated from a graph of concentration against wavelength absorbance of known ALT concentrations.

Quantitative determination of aspartate aminotransferase (AST)

Serum aspartate transaminase was estimated by the method described by Colville (2002) [5] using AST test kit (Span Diagnostics Ltd., India). A 0.25 ml of mixture of L-aspartate (100 mmol/l), α-oxoglutarate (2 mmol/l) and phosphate buffer (100 mmol/l) was added to 0.5 ml of each sample and blank (containing distilled water). They were mixed and incubated at 37 ^{0}C for exactly 30 minutes in a water bath. A 0.25 ml of 2, 4- dinitrophenylhydrazine was added into the sample and blank test tubes and incubated at room temperature for 20 minutes. A 2.5 ml of sodium hydroxide (0.4 mol) was then added to all the test tubes and the absorbance of the sample was read against the blank at 546 nm using a UV-VIS spectrophotometer (Model 752, China). The AST concentration was extrapolated from a graph of concentration against wavelength absorbance of known AST concentrations.

Quantitative determination of alkaline phosphatase (ALP)

Alkaline phosphatase was estimated by the method described by Colville (2002) [5] using ALP test kit (Span Diagnostics Ltd., India). A 0.5 ml of Alkaline Phosphatase substrate was placed in sample and blank labeled test tubes and equilibrated to 37 ^{0}C for 3 minutes. At timed interval, 0.05 ml each of standard, control (deionized water), and sample were added to its respective test tubes. The mixture was incubated for 10 minutes at 37 ^{0}C. A 2.5 ml of alkaline phosphatase color developer (0.1 M Sodium Hydroxide and 0.1 M sodium Carbonate) was added and properly mixed. The absorbance of the samples were read at 590 nm using a UV-VIS spectrophotometer (Model 752, China) and recorded. ALP concentration was calculated using the equation below;

Calculation of ALP concentration

ALP = Abs of samples x value of standard (IU/L)/Abs of standard

Where Standard value = 50 IU/L

2.3.3. Biochemical assay of kidney parameters

Serum creatinine and blood urea nitrogen (BUN) were estimated by the method described by Tietz, (1976) [6] and Heinegard and Tiderstrom, (1973) [7] respectively using BUN and creatinine test kits (Teco Diagnostics, USA).

Quantitative determination of creatinine

Creatinine working reagent was prepared by combining equal volumes of 10 mM picric acid and Creatinine buffer reagent (10 mM sodium borate, 240 nM sodium hydroxide). Then 3.0 ml of this reagent was added to labelled tubes (test, blank and standard) to which 100 µl of serum (test), 5 mg/dl of Creatinine (Standard), and distilled water (blank) were added and mixed in their designated test tubes. The tubes were incubated at 37 ^{0}C for 15 minutes and the absorbance measured spectrophotometrically at 520 nm against test blank. The concentration of Creatinine (mg/dl) were calculated thus:

$$\text{Creatinine} = \frac{\text{Abs of Test}}{\text{Abs of Std}} \times \text{Conc. of Std}$$

Where Abs = Absorbance, Std = Standard

Quantitative determination of blood urea nitrogen

A 1.5 ml of BUN Enzyme reagent (containing 10,000 µ/l Urease, 6.0 mmol/l sodium salicylate, 3.2 mmol/l sodium nitroprusside) was added to 10 µl of Test (serum), Standard (20 mg/dl) and Blank (distilled water) followed by incubation for 5 minutes at 37 ^{0}C. At timed interval, 1.5 ml of BUN color developer (6 mmol/L of sodium Hypochlorite and 130 mmol/l sodium hydroxide) was added to each of the labelled tubes and were incubated for another 5 minutes at 37 ^{0}C. The absorbance of the tests and standard were measured spectrophotometrically at 630 nm against blank. Urea nitrogen concentration (mg/dl) were calculated thus:

$$\text{BUN} = \frac{\text{Abs of Test}}{\text{Abs of Std}} \times \text{Conc. of Std}$$

Where Abs = Absorbance, Std = Standard

3. Results

3.1. Results of phytochemical analysis of *Zingiber officinale* and *Allium sativum* ethanol leaf extracts

Phytocompounds in *Zingiber officinale* were: Alkaloids, Tannins, Flavonoids, Steroids and terpenoids while those in *Allium sativum* were Alkaloids, Saponins, Flavonoids, and Glycosides [3].

3.2. Results of acute toxicity studies

The actual lethal doses of *Zingiber officinale, Allium sativum* and combination of the two were 8,660, 4,472, and 5,477 mg/kg body weight respectively [3].

Table 1 Results of ALT serum concentration assay

Groups	Treatments given/kg body weight	Mean ALT ± SEM (IU/L)	P-Value
1	Distilled water 10 ml	33.65 ± 0.49	-
2	*Zingiber officinale* 530 mg	28.85 ± 0.43	0.008114
3	*Allium sativum* 530 mg	42.40 ± 0.22	4.27E-05
4	Ratio of *Z.:A.* = 2:8 (106 mg:424 mg)	40.90 ± 0.40	0.000747
5	Ratio of Z.:A = 4:6 (212 mg:318 mg)	37.4 ± 0.59	0.069828
6	Ratio of Z.:A = 5:5 (265 mg:265 mg)	34.40 ± 0.55	0.712465
7	Ratio of Z.:A = 6:4 (318 mg:212 mg)	31.50 ± 0.56	0.16301
8	Ratio of Z.:A = 8:2 (424 mg:106 mg)	30.5 ± 0.55	0.097992

Table 2 Results of AST serum concentration assay

Groups	Treatments given/kg body weight	Mean AST ± SEM (IU/L)	P-Value
1	Distilled water 10 ml	61.62 ± 0.40	-
2	*Zingiber officinale* 530 mg	55.20 ± 0.32	0.0028
3	*Allium sativum* 530 mg	75.32 ± 0.63	0.0004
4	Ratio of Z.:A = 2:8 (106 mg:424 mg)	70.25 ± 0.50	0.0026
5	Ratio of Z.:A = 4:6 (212 mg:318 mg)	66.45 ± 0.69	0.101
6	Ratio of Z.:A = 5:5 (265 mg:265 mg)	58.48 ± 0.35	0.097
7	Ratio of Z.:A = 6:4 (318 mg:212 mg)	55.42 ± 0.58	0.016
8	Ratio of Z.:A = 8:2 (424 mg:106 mg)	56.20 ± 0.32	0.008

Table 3 Results of ALP serum concentration assay

Groups	Treatments given/kg body weight	Mean ALP ± SEM (IU/L)	P-Value
1	Distilled water 10 ml	116.47 ± 0.32	-
2	*Zingiber officinale* 530 mg	87.80 ± 0.68	1.74E-06
3	*Allium sativum* 530 mg	136.45 ± 0.28	1.28E-08
4	Ratio of Z.:A = 2:8 (106 mg:424 mg)	129.40 ± 0.44	4.16E-04
5	Ratio of Z.:A = 4:6 (212 mg:318 mg)	121.65 ± 0.54	0.096432
6	Ratio of Z.:A = 5:5 (265 mg:265 mg)	112.60 ± 0.40	0.116
7	Ratio of Z.:A = 6:4 (318 mg:212 mg)	102.60 ± 0.21	8.64E-06
8	Ratio of Z.:A = 8:2 (424 mg:106 mg)	95.08 ± 0.94	2.24E-04

3.3. Results of kidney function tests

Table 4 Results of 91st day BUN serum concentration assay

Groups	Treatments given/kg body weight	Mean BUN ± SEM (mg/dL)	P-Value
1	Distilled water 10 ml	55.17 ± 0.57	-
2	*Zingiber officinale* 530 mg	46.00 ± 0.57	0.00291
3	*Allium sativum* 530 mg	54.00 ± 0.41	0.59947
4	Ratio of Z.:A = 2:8 (106 mg:424 mg)	52.50 ± 0.76	0.386628
5	Ratio of Z.:A = 4:6 (212 mg:318 mg)	52.00 ± 0.54	0.198783
6	Ratio of Z.:A = 5:5 (265 mg:265 mg)	49.00 ± 0.51	0.022426
7	Ratio of Z.:A = 6:4 (318 mg:212 mg)	51.83 ± 0.68	0.22967
8	Ratio of Z.:A = 8:2 (424 mg:106 mg)	48.50 ± 0.62	0.019939

Table 5 Results of 91st day creatinine plasma concentration assay

Groups	Treatments given/kg body weight	Mean creatinine ± SEM (mg/dl)	P-Value
1	Distilled water 10 ml	1.12 ± 0.04	-
2	*Zingiber officinale* 530 mg	0.95 ± 0.04	2.55E-05
3	*Allium sativum* 530 mg	1.12 ± 0.05	0
4	Ratio of Z.:A = 2:8 (106 mg:424 mg)	0.99 ± 0.65	0.000492
5	Ratio of Z.:A = 4:6 (212 mg:318 mg)	0.96 ± 0.02	1.18E--05
6	Ratio of Z.:A = 5:5 (265 mg:265 mg)	0.96 ± 0.02	1.49E-05
7	Ratio of Z.:A = 6:4 (318 mg:212 mg)	0.96 ± 0.05	1.04E-04
8	Ratio of Z.:A = 8:2 (424 mg:106 mg)	0.96 ± 0.03	1.78E-05

4. Discussion

In the ALT assay, all the groups were compared with group 1 which served as the control group and were treated with distilled water. *Zingiber officinale* at 530 mg/kg body weight reduced the plasma ALT significantly (P = 0.008114) from 33.65 ± 0.49 IU/L of group 1 to 28.85 ± 0.43 IU/L while *Allium sativum* at the same dose increased ALT significantly (P = 4.27E-05) to 42.40 ± 0.22 IU/L. When the combination of the two herbs was administered in different ratios, group 4 which has higher proportion of *Allium sativum* (Z:A = 2:8) increased plasma ALT significantly (P = 0.000747) to 40.90 ± 0.40 IU/L whereas the group 8 fraction with higher proportion of *Zingiber officinale* (Z:A = 8:2) decreased ALT though not significantly (0.097992) to 30.5 ± 0.55 IU/L. Alanine aminotransferase is elevated in serum under conditions of significant cellular necrosis and is used as a measure of liver function. Levels of ALT may be elevated in cases of hepatitis, congestive heart failure, liver or biliary duct damage, or myopathy. According to a certain study, the activity of ALT in hepatocytes is approximately 3,000 times higher than that of serum ALT activity. Therefore, in patients with acute or chronic hepatocellular injury, the release of ALT from dying or damaged hepatocytes results in increased serum ALT levels [8]. This implies that *Zingiber officinale* when administered alone at the dose of 530 mg/kg body weight, had a greater hepato-protective effect than when given in combination with *Allium sativum*. Similarly, *Zingiber officinale* administered alone at the dose of 530 mg/kg body weight (group 2) had the greatest and significant (P = 0.0028) reduction of AST (55.20 ± 0.32) when compared with the control (61.62 ± 0.40). Groups 7 and 8 which received combinations of *Zingiber officinale* and *Allium sativum* (Z:A = 6:4 and 8:2 respectively) also showed significant reduction in AST (55.42 ± 0.58 and 56.20 ± 0.32 respectively) (P = 0.016 and 0.008 respectively); though not as much reduction as obtained in group 2. Since high level of AST in the blood may be a sign of liver disease or damage to the bile ducts, it suggests that *Zingiber officinale* by virtue of it decreasing AST has a greater hepatic protector effect. The same thing applies to alkaline phosphatase (ALP) where the control group 1 had a serum concentration of 116.47 ± 0.32 IU/L as compared to *Zingiber officinale* and *Allium sativum* which at 530 mg/kg body weight each resulted in ALP concentration of 87.80 ± 0.68 IU/L and 136.45 ± 0.28 IU/L respectively. The combination could only reduce ALP concentration at a higher proportion of *Z. officinale.* (8:2). The two main sources of ALP in the blood are liver and bones. High levels of ALP may indicate liver disease or certain bone disorders. Consequently, because of its ability to reduce ALP concentration, *Z. officinale* has again been shown to have a reputable hepato protective effect greater than the tested combinations.

In the renal function tests, both BUM and creatinine were analyzed. *Z. officinale* and *A. sativum* at the dose of 530 mg/kg body weight decreased the serum concentration of BUM (46.00 ± 0.57 and 54.00 ± 0.41 mg/dL respectively) when compared with the control (55.17 ± 0.57 mg/dL) But while the reduction from *Z. officinale* was significant (P = 0.00291), that of *A. sativum* was not significant (P = 0.59947). Generally, a high BUN level means that the kidneys are not working well. But elevated BUN can also be due to other causes such as dehydration, resulting from not drinking enough fluids or for other reasons, urinary tract obstruction among others. These indicated that both herbs contributed to a healthier kidney. Expectedly, the combinations in all proportions reduced BUM and these are significant at the combinations containing equal proportions of the herbs (P = 0.022426) as well as those containing higher proportion of *Z. officimale;* example (Z:A = 8:2) reduced BUM to 48.50 ± 0.62 mg/dL (P =0.019939). Creatinine is a waste product produced by the muscles and it is filtered out by the kidneys. A buildup of creatinine in the blood can be a sign of impaired kidney function. *Z. officinale* alone at the dose of 530 mg/kg body weight, reduced creatinine level from 1.12 ± 0.04 mg/dL (control group) to 0.95 ± 0.04 mg/dL. (P = 2.55E-05). On the other hand, *A. sativum* did not affect the creatinine level but its combination at all proportions decreased the creatinine levels significantly, but not as much as *Z. officinale* given

alone. According to an earlier study, even a minimal elevation in creatinine can reflect significantly decreased rate of glomerular filtration [9].

5. Conclusion

In conclusion, *Zingiber officinale* has the greatest potentials with respect to hepatic and renal protection than either of *Allium sativum* alone or combination of *Zingiber* offcinale and *Allium sativum* given concomitantly in all tested proportions.

Compliance with ethical standard

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Disclosure of conflict of interest

All authors declared no conflict of interest

Statement of ethical approval

Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes; (Approval number is NAU/AREC/2023/00021).

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