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THE LIVER FUNCTIONS OF WISTAR RATS: THE EFFECTS OF SELECTED SPICES

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ABSTRACT: *The effects of selected spices (Aframomum danielli, rough skin plum, and country onion) on the liver functions of Wistar rats fed with feed substituted with 5%, 10%, and 15% of the individual spices were evaluated. Consequently, the total protein content and Alkaline phosphatase (ALP) decreased from 62.69% (control sample) to 55.19% and 56.49mg/dl to 55.83mg/100g, respectively, during the 4-week feeding period. The serum bilirubin and Alanine Aminotransferase (ALT) decreased in rats fed with the 5% concentration of A. danelli, increased at 10%, 15% of A. danielli, and all concentrations of the other spices. By the end of the 28 days, all the results were: Albumin - 36.33 to 37.72%; total protein – 55.19 to 56.70%; ALP - 55.90 to56.47; bilirubin- 13.08 to 13.63; AST -26.47 to 29.00U/Land ALT- 29.25 to 33.73U/L. These values were within the standard acceptable levels in the blood, indicating the positive effects of these spices on liver functions.*

KEYWORDS: Spices, Liver function, Wistar rats.

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INTRODUCTION

Spices are naturally occurring plant materials that enhance culinary products' flavour, aroma, taste, and colour. The Food and Drug Administration (FDA) in the United States defines a spice as an aromatic vegetable substance that can be whole, broken, or ground. Its primary purpose in food is to season rather than to provide nutrients, and it contains no part of any volatile oil or other flavouring ingredient (Gottardi et al., 2016). These materials include fruit, leaves, seeds, roots, bark, berries, buds, flowers, and vegetables.

Spices can be added to food for various health benefits, including increased salivary flow, improved digestion, protection against the flu and cold, and decreased nausea and vomiting (Sultana et al., 2010). In addition to this, food's physical appearance has been altered through the application of spices. For example, adding pepper and turmeric to food alters its colour, texture, and flavour while providing numerous health advantages. Ginger, nutmeg, and cinnamon also benefit the spleen and sore throats, enhancing digestion. Spices offer numerous health benefits by helping to prevent and treat various illnesses, including cancer, aging, metabolic, neurological, cardiovascular, and inflammatory diseases. They help manage convulsion, leprosy, stomach ache, cough, and loss of appetite (Valko et al., 2007). The liver is the largest organ in the body and one of the most vital organs. It is a centre for nutrient metabolism and waste metabolite excretion (Ozougwu & Eyo, 2014). As the largest organ in the body, it accounts for approximately 2% to 3% of average body weight (Sherif *et al.*, 2010).

The liver's primary function is to control the flow and safety of substances absorbed from the digestive system before distributing these substances to the systemic circulatory system (Allen, 2002). A total loss of liver function could lead to death within minutes, demonstrating the liver‘s great importance (Ozougwu, 2014). Hence, proper liver function is of paramount importance, and it can be verified by several specialized clinical studies, which measure the presence or absence of typical enzymes, metabolites, or substances associated with regular activities (Sivakrishnan, 2018).

Liver function tests (LFTs) are categories of blood tests that provide information on the health of a patient's liver. These tests include Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT), which are good indicators of liver injury in people and animals with some degree of intact liver function (Lee, 2009). Most liver illnesses initially show minor symptoms, but early diagnosis is essential. Hepatic (liver) involvement in certain disorders can be critical (Johnston, 1999).

Some tests are related to functionality (e.g., Albumin), others to cellular integrity (e.g., transaminase), and still others to biliary tract conditions (e.g., gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP)) (McClatchey, 2002). This study evaluated the effects of some selected spices on the liver functions of Wistar rats.

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MATERIALS AND METHOD

Materials Procurement

The spices *Aframonum danielli*, rough skin plum, and country onion were obtained from the Relief Market, Owerri, Imo State, Nigeria, while the adult Albino Wistar rats of comparable sizes and weights ranging from 150g-200g were purchased from the animal farm of Ceslab Global Service, Kilometre 7, Ikot Ekpene Road, Umuahia, Abia State, Nigeria.

Preparation of Samples

The purchased test spices were carefully poured into a clean, dry plastic container. From this container, they were sorted, cleaned, measured, milled, sieved, and packaged in small plastic containers, which were then stored pending usage. The test animals were allowed to acclimate for one week in a wire mesh cage.

Table 1: Formulation of Feed Indicating Proportions of the Spices in the Wistar Rat Diet

Sample	Growers mash (%)	A. danielli	Plum (%)	Onion (%)
A	100	-	-	-
B_1	95	5	-	-
B_2	90	10	-	-
B_3	85	15	-	-
C_1	95	-	5	-
C_2	90	-	10	-
C_3	85	-	15	-
D_1	95	-	-	5
D_2	90	-	-	10
D_3	85	-	-	15

Where A = Control (100% growers mash), 1 =Treatment at 5% concentration, 2 = Treatment at 10% concentration, 3 = Treatment at 15% concentration

B = *Aframonum danielli:* B_1 = 95% feed: 5% *A. danielli;* B_2 = 90% feed: 10% *A. danielli* and B_3 = 85% feed:15% *A. danielli*

C = Rough skin plum: C_1 = 95% feed: 5% plum*;* C_2 = 90% feed: 10% plum and C_3 = 85% feed: 15% plum

D = Country onion: D_1 = 95% feed: 5% C. onion*;* D_2 = 90% feed:10% C. onion and D_3 = 85% feed:15% C. onion.

Animal Grouping

The adult albino Wistar rats of comparable sizes and weights ranging from 150-200g were divided into ten (10) groups of six rats each and allowed to acclimate for one (1) week. During acclimatisation, the rats were housed in wooden-wire gauze cages, fed with growers' mash, and provided with water ad libitum. The animals were managed and utilised following the established standards for managing and utilising laboratory animals. Group A served as the control while B_1, B_2, and B_3 served as the groups fed with feed spiced with 5%, 10%, and 15%

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of *Aframomun danielli*, respectively. C_1, C_2, and C_3 served as groups fed with spiced feed of 5%, 10% and 15% of rough skin plum and D_1, D_2 and D_3 served as groups fed with spiced feed of 5%, 10% and 15% country onions, respectively. The animals were starved for 12 hours before feeding with formulated feed commenced.

The respective spices were prepared by mixing with the rat feed at 5%, 10%, and 15%, respectively. Group A received normal feed and water only. The feeding period lasted 28 days (4 weeks).

Blood Sample Collection

The blood samples (5ml) of each rat were obtained by sacrificing the rat at the end of every seven days for 28 days and dispensed into a plain container labelled appropriately - A, B_1, B_2, B_3, C_1, C_2, C_3, D_1, D_2, and D_3. The blood samples were frozen until the time of analysis. Laboratory analysis carried out were for Albumin, total protein, serum bilirubin, Alkaline phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine transaminase (ALT) of Wistar rats.

Determination of Liver Function Test

The parameters assessed were Albumin, total protein, serum bilirubin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and alkaline phosphatase (ALP) to ascertain the liver's enzymatic activity. Commercially available kits were used to measure the activity of all serum enzymes, following the manufacturer's instructions.

Statistical Analysis

The data obtained in this work were statistically analysed using SPSS version 20. Data obtained were expressed as mean ± standard deviation and subjected to analysis of variance (ANOVA) to test the difference among means. Significant values were at $P < 0.05$.

RESULTS

Table 2: Albumin Content of Wistar Rats Fed with Spiced Diet

	Days of Feeding				
Sample	0	7	14	21	28
Ad.					
C_1	$36.48^{a}\pm0.62$	$36.62^{ab}\pm0.28$	$36.59^{a}\pm0.09$	$37.05^{a}\pm0.12$	$37.72^{a}\pm0.07$
C_2	$36.48^{a}\pm0.62$	$36.49^{ab}\pm0.30$	$36.56^{a}\pm0.32$	$36.93^{a}\pm0.02$	$37.57^{a}\pm0.13$
C_3	$36.48^{a}\pm0.62$	$36.47^{ab}\pm0.33$	$36.23^{bc}\pm0.02$	$36.48^{bc}\pm0.00$	$37.27^{b}\pm0.08$
Plum:					
C_1	$36.63^{a}\pm1.33$	$37.00^{a}\pm0.91$	$36.13^{cd}\pm0.02$	$36.23^{d}\pm0.04$	$36.33^{f}\pm0.03$
C_2	$36.63^{a}\pm1.33$	$36.09^{ab}\pm0.11$	$36.27^{b}\pm0.29$	$36.31^{d}\pm0.05$	$36.53^{de}\pm0.30$
C_3	$36.63^{a}\pm1.33$	$35.93^{b}\pm0.09$	$35.95^{e}\pm0.05$	$36.34^{cd}\pm0.19$	$36.44^{ef}\pm0.05$
Onion					
C_1	$38.00^{a}\pm0.17$	$36.34^{ab}\pm0.17$	$36.50^{ab}\pm0.04$	$34.57^{e}\pm0.04$	$36.68^{c}\pm0.11$
C_2	$38.00^{a}\pm0.10$	$35.99^{b}\pm0.17$	$36.05^{de}\pm0.13$	$36.21^{d}\pm0.05$	$36.65^{cd}\pm0.36$
C_3	$38.00^{a}\pm0.17$	$35.95^{b}\pm0.17$	$36.08^{d}\pm0.09$	$36.25^{d}\pm0.07$	$36.39^{f}\pm0.14$

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Control	36.55^{a}±0.01	36.54^{ab}±0.11	36.56^{a}±0.04	36.55^{b}±0.00	36.56^{cd}±0.08
LSD	1.64	0.99	0.12	0.16	0.13

Values show means of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different (P<0.05). Typical range: 35 to 55g/L.

Table 3: Total Protein in Blood of Wistar Rats Fed with Spiced Diet

	Days of Feeding				
Sample	0	7	14	21	28
A.danielli					
C_1	58.43^{d}±0.52	58.01^{e}±1.10	57.33^{d} ±0.05	56.75^{c}±0.10	56.39^{e}±0.15
C_2	58.42^{d}±0.52	56.87^{f}±0.39	55.54^{f}±0.14	55.27^{c}±0.01	55.21^{g}±0.02
C_3	58.42^{d}±0.52	55.72^{g}±0.04	55.41^{f}±0.17	55.21^{c}±0.04	55.19^{g}±0.01
Plum					
C_1	60.57^{c}±1.41	59.39^{d}±0.74	56.48^{e}±0.04	56.87^{c}±0.12	55.67^{f}±.12
C_2	60.57^{c}±1.41	59.01^{d}±1.02	56.21^{e}±0.03	55.60^{c}±0.20	55.33^{g}±0.23
C_3	60.57^{c}±1.41	58.45^{d}±0.01	56.20^{e}±0.04	55.40^{c}±0.20	55.20^{g}±0.20
Onion					
C_1	64.21^{a}±0.08	62.53^{a}±0.23	61.41^{b}±0.12	59.73^{b}±0.93	57.17^{b}±0.25
C_2	64.21^{a}±0.08	61.96^{b}±0.25	61.61^{b}±0.18	59.9^{b}±0.17	56.70^{c}±0.17
C_3	64.21^{a}±0.08	61.03^{c}±0.23	61.00^{c}±0.35	59.40^{b}±0.20	56.43^{d}±0.06
Control	62.70^{b}±0.36	62.63^{a}±0.48	62.73^{a}±0.45	62.82^{a}±0.43	62.69^{a}±0.25
LSD	0.11	0.15	0.34	0.76	0.19

Values show means of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different (P<85). Typical range: 60 to 83g/L

Table 4: Alkaline Phosphatase Content of Blood of Wistar Rats Fed with Spiced Diet

	Days of Feeding				
Sample	0	7	14	21	28
Adanielli.					
C_1	54.63^{a}±0.35	54.63^{e}±0.15	55.00^{d}±0.17	54.93^{f}±0.12	55.90^{d}±0.17
C_2	54.63^{a}±0.46	54.96^{cd}±0.15	55.17^{cd}±0.21	55.20^{e}±0.00	56.13^{c}±0.15
C_3	54.63^{a}±0.35	53.93^{a}±2.28	55.47^{b}±0.12	55.57^{d}±0.06	56.30^{b}±0.00
Plum					
C_1	54.63^{a}±0.35	54.70^{e}±0.20	55.37^{bc}±0.12	55.73^{c}±0.12	55.83^{d}±0.06
C_2	54.63^{a}±0.53	54.87^{d}±0.23	55.97^{a}±0.16	55.97^{ab}±0.16	56.23^{bc}±0.06
C_3	54.63^{a}±0.35	54.90^{d}±0.52	55.93^{a}±0.12	55.97^{ab}±0.16	56.27^{bc}±0.12
Onion					
C_1	54.63^{a}±0.35	55.03^{bc}±0.12	55.40^{b}±0.10	55.73^{c}±0.12	56.13^{c}±0.15
C_2	54.63^{a}±0.39	55.20^{b}±0.00	55.73^{a}±0.12	55.93^{b}±0.12	56.13^{c}±0.18
C_3	54.63^{a}±0.35	55.47^{a}±0.12	55.97^{a}±0.15	56.07^{ab}±0.12	56.47^{a}±0.06

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Control	54.63[a]±0.31	55.08[bc]±0.11	55.77[a]±0.15	56.11[a]±0.24	56.49[a]±0.13
LSD	0.09	0.18	0.27	0.15	0.17

Values show means of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different (P<0.05). Typical range: 40 to 129IU/L

Table 5: Bilirubin Content of Wistar Rat Blood (MMOL/L)

Sample	Days of Feeding 0	7	14	21	28
Adanielli.					
C_1	12.96[a]±0.16	12.89[d]±0.01	13.01[f]±0.06	13.13[d]±1.03	13.08[d]±0.16
C_2	12.91[a]±0.03	13.10[b]±0.02	13.11[e]±0.01	13.14[d]±0.03	13.21[cd]±0.04
C_3	12.95[a]±0.01	13.06[b]±0.02	13.15[e]±0.01	13.17[d]±0.01	13.38[bc]±0.04
Plum					
C_1	13.10[a]±0.49	13.30[ab]±0.30	13.39[bc]±0.09	13.47[ab]±0.04	13.61[a]±0.20
C_2	13.22[a]±0.30	13.51[a]±0.03	13.46[ab]±0.11	13.54[ab]±0.02	13.67[a]±0.01
C_3	13.29[a]±0.23	13.53[a]±0.04	13.53[a]±0.02	13.55[a]±0.01	13.56[ab]±0.02
Onion					
C_1	12.94[a]±0.16	13.15[bc]±0.04	13.24[d]±0.02	13.41[c]±0.60	13.48[ab]±0.00
C_2	12.99[a]±0.11	13.16[bc]±0.00	13.24[d]±0.00	13.43[bc]±0.04	13.51[ab]±0.01
C_3	13.11[a]±0.02	13.25[b]±0.02	13.31[cd]±0.04	13.50[ab]±0.04	13.63[a]±0.15
Control	12.95[a]±0.01	12.96[cd]±0.00	13.01[f]±0.03	13.15[d]±0.02	13.16[d]±0.02
LSD	0.44	0.25	0.09	0.12	0.21

Values show means of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different (P<0.05). Normal range : 3 to 17mmol/L

Table 6: Aspartate Amino-transferase (AST) Level in Serum of Rats Fed with Spiced Feed

Sample	Days of Feeding 0	7	14	21	28
Ad.					
C_1	24.10[f]±0.27	24.27[f]±0.05	24.63[e]±0.03	25.17[h]±0.05	26.47[e]±0.12
C_2	24.67[de]±0.12	24.87[e]±0.12	25.57[c]±0.06	25.80[g]±0.00	26.90[d]±0.17
C_3	24.93[d]±0.23	25.27[bc]±0.12	25.73[c]±0.12	26.03[f]±0.06	27.07[d]±0.12
Plum					
C_1	25.33[c]±0.10	25.10[cd]±0.17	26.00[b]±0.20	26.83[e]±0.29	27.97[c]±0.40
C_2	26.20[a]±0.53	26.00[a]±0.20	26.17[ab]±0.12	27.20[d]±0.35	28.57[b]±0.06
C_3	26.53[a]±0.45	25.20[bc]±0.20	26.23[a]±0.06	27.40[bc]±0.20	28.73[ab]±0.12
Onion					
C_1	24.50[e]±0.17	24.97[de]±0.15	25.67[c]±0.31	27.33[cd]±0.12	28.50[b]±0.00
C_2	25.00[cd]±0.20	25.37[b]±0.40	26.03[b]±0.06	27.53[b]±0.25	28.53[b]±0.12
C_3	25.77[b]±0.71	25.27[bc]±0.12	26.33[a]±0.12	28.13[a]±0.15	29.00[a]±0.17

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Control	24.33^{ef}±0.45	24.37^{d}±0.31	24.99^{d}±0.27	25.19^{h}±0.24	26.11^{f}±0.10
LSD	0.38	0.19	0.18	0.18	0.31

Values below means of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different (P<0.05). Typical range: 7 to 55U/L

Table 7: Alanine Amino-transferase (ALT) Level in Serum of Rats Fed with Spiced Diet

	Days of Feeding				
Sample	0	7	14	21	28
A.danielli:					
C_1	27.25^{c}±0.90	27.08^{g}±0.30	27.85^{h}±0.66	28.75^{g}±1.02	29.25^{g}±1.44
C_2	27.25^{c}±0.35	28.15^{e}±0.35	29.50^{d}±0.00	30.30^{def}±0.28	31.35^{d}±0.21
C_3	28.33^{b}±0.15	28.50^{d}±0.00	30.20^{c}±0.35	31.93^{ab}±0.12	33.07^{a}±0.23
Plum:					
C_1	26.57^{d}±0.21	27.81^{f}±0.23	28.07^{g}±0.23	28.47^{g}±0.12	28.60^{h}±0.20
C_2	26.90^{cd}±0.17	28.47^{d}±0.31	29.03^{e}±0.06	30.80^{cd}±0.20	32.10^{c}±0.30
C_3	27.10^{c}±0.17	28.87^{c}±0.31	30.80^{b}±0.20	31.53^{bc}±0.12	32.27^{b}±0.23
Onion:					
C_1	28.37^{b}±0.23	28.50^{d}±0.00	29.07^{e}±0.38	29.93^{ef}±0.64	31.10^{e}±0.17
C_2	29.00^{ab}±0.35	29.70^{b}±0.35	30.07^{c}±0.12	30.67^{de}±0.31	33.73^{a}±0.12
C_3	29.27^{a}±0.23	30.47^{a}±0.42	31.80^{a}±0.20	32.70^{a}±0.35	32.27^{b}±0.23
Control:	28.33^{b}±0.95	28.47^{d}±0.31	28.50^{f}±0.27	29.67^{f}±1.22	30.10^{f}±0.10
LSD	0.42	0.23	0.18	0.81	0.10

Values show means of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different (P<0.05). Typical range: 30 to 130 IU/L

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DISCUSSIONS

Liver function tests, including Albumin, total protein, serum bilirubin, alkaline phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine transaminase (ALT), are groups of blood tests that provide information about a patient's liver condition. These tests were conducted on Wistar rats fed spiced feed to determine the effects of the spices on the liver. The results for each parameter analysed are presented in Tables 2 to 7.

Albumin content: The percentages of Albumin contained in the blood of Wistar rats fed with spiced feed are shown in Table 2. The results obtained showed that at the beginning of the experiment (0 weeks), the albumin content in the blood of the rats fed with spiced feed showed no significant difference (p>0.05) between the lowest (36.48%) and highest (38.00%) albumin content obtained irrespective of the spice type or concentration. The Albumin in the liver of the control rat samples and those fed with spiced feed did not show any significance (p>0.05). However, at the end of the 28-day feeding period, there were significant differences (p<0.05) in the levels of Albumin in the rat's blood. The rat fed with *A.danielli* had the highest albumin content in the range of 37.27% to 37.72% and decreased with increased concentration of the spice. For the rats fed with rough skin plum, there were decreases in the albumin content in the rat's blood, which ranged from 36.33 to 36.53% as against 36.63% at the onset of the experiment. There were significant differences (p<0.05) in the albumin content of the rat fed with the same spice (rough skin plum) at different concentrations. No particular pattern was followed, and there was no dose dependency. Similar results were obtained in the country onion spiced-fed rats, where Albumin in the blood of these rats reduced to 36.68%, 36.65%, and 36.39% from the initial 38.00%. Here, a sequential decrease with a high concentration of spices supplement indicates a reducing effect of spices with higher doses. The control of the different spice diet-fed rats had no significant changes (p>0.05) in the albumin content, which varied from 36.55% to 36.56% at the end.

Total protein content: The resulting total protein values in the blood of rats fed with a spiced diet and control diets are in Table 3. The result shows variations in the blood protein content of the rats used in other groups (treatment). At the zero-week feeding period, the total protein content for all the spices at various concentrations was 58.43% to 64.21%. From the results, the rats fed with a spiced diet experienced a sequential reduction in their blood total protein content as the feeding days progressed.

In the rat fed with *A. danielli*, there were reductions in the total protein content of their blood in the range of 58.43% to 49.72% (C_1),58.43% to 55.21% (C_2), and 58.43% to 55.19% (C_3). As the dose of rough skin plum increased, the Wistar rats' total protein decreased more significantly from the initial 60.57% to 55.67%, 55.33%, and 55.20% for Concentration C_1, C_2, and C_3, respectively. For the rats fed with country onion-supplemented diets at C_1, C2, and C_3 concentrations, there was a reduction from the initial total protein content of 64.21% to 57.17%, 56.70%, and 56.43% with dose-related effects. The control Wistar rats' blood samples did not show significant (p<0.05) variations, as the total protein in the rats' (control) blood varied from 62.63% to 62.82%. Generally, the spices effectively reduced the total blood protein of the Wistar rats, and the extent of reduction varied between the three different spices and the concentration added to the feeds.

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Alkaline phosphatase (ALP): Table 4 shows the changes in the alkaline phosphatase level in the blood of Wistar rats fed with spiced diets at different levels of spice addition. The ALP level in the rat's blood did not vary as the ALP of all the test animals was 54.63mg/dl at the onset of the experiment, with no significant differences (P<0.05).

In the *Aframomum danielli* spiced diets, there were slight but significant increases in their ALP level in a dose-dependent trend. Increases of 2.33% (54.63-55.90mg/dl), 2.75% (54.63-56.13mg/dl), and 3.06% (54.63-56.30mg/dl) in the Wistar rats fed with C_1, C_2, and C_3 added *A. danielli* spiced diets respectively were observed. There were significant differences (P<0.05) in the ALP levels in the blood of the rats fed with different concentrations of *Afromomum danielli* spiced feed.

The effect of the rough skin plum spiced diet on the blood ALP of the rats also varied in dose-dependent trend with percentage increases of 2.20% (54.63-55.83mg/dL), 2.93% (54.63-56.23mg/dL) and 2.98% (54.63-56.26mg/dL) for rats fed with C_1, C_2 and C_3 concentration of rough skin plum spiced feed respectively. Similarly, the rats fed with country onion spice also recorded slight increases of 2.51% (54.63-56.00mg/dl), 2.75% (54.63-56.13), and 1.83% (54.63mg/dL-56.46mg/dL) forC_1, C_2 and C_3 spice concentration respectively.

Generally, there was an increase in the ALP level of all the test animal blood (Test animals fed with spiced and un-spiced feed). Thus, the increased APL level could not be due to the spiced diets. The ALP detects blocked bile ducts, liver damage, or bone disorders. When liver cells are damaged, they release increased amounts of ALP into the blood. ALP levels in plasma also increase with significant bile duct obstruction, intrahepatic cholestasis, or liver infiltrative diseases. The usual range of ALP is 44 to 147 IU/L (Medline Plus, 2017), and the results obtained in this study were within the average ALP level.

Serum bilirubin content: Table 5 below shows the bilirubin contents in the blood of rats fed with a spiced feed ration. The result showed the initial bilirubin level in the blood of the rats to be in the range of 12.91mol/L to 13.29mol/L, and there were no significant differences (P>0.05) between the bilirubin content of the blood of all the rats. The bilirubin contents of the rats increased significantly in the entire test of the samples, including the controls. At the end of the feeding period of 28 days, the bilirubin content of the rat blood increased to an average range of 13.08mol/L to 13.38mol/L for rats fed with *A. danielli* supplemented diet, while the rats fed with rough skin plum supplemented diets increased to a range of 13.56 to 13.67mol/L and from 13.48mol/L to 13.63mol/L in those fed country onion supplemented diets at the same respective concentrations. The control rat blood bilirubin increased from 12.95 to 13.16mol/L. From the above figures, it means that bilirubin increased in the control rats' blood by 1.62% while the increases in the spiced diet-fed rats were between 0.93% (12.96-13.08) to3.40% (12.95-13.38) in Wistar rats fed with *A. danielli* while those fed with rough skin plum spiced diets increased by 3.9% (13.10-13.61) to 2% (13.29-13.56mol/L). The ones fed with country onions had their blood bilirubin raised by 4.17% (12.94-13.48) to 3.97% (13.11-13.63mol/L). All the values obtained showed a higher increase in the bilirubin content of rats fed with spiced feed compared to the control, and the level of increase varied significantly with the types of spices used.

Aspartate Aminotransferase (AST): Table 6 shows the changes in Aspartate Aminotransferase (AST) levels in the serum of rats fed with *Aframomum danielli*, rough skin plum, and country onion spiced feed. There were significant differences (p>0.05) in the serum

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AST level of all rats irrespective of the spice. On the first day of the 28-day feeding trial, the enzyme (AST) level in both rats fed with spiced and control feed ranged from 24.10 mg/dL to 26.53 mg/dL. The results also showed a slight but continuous increase in the serum enzyme (AST) of all the test rats during the twenty-eight days of feeding with the trial diets. The serum AST of the rats fed with *A.danielli* increased from 24.10mg/dL at the C_1 substitution level to 26.46mg/dL, translating to 1.49%increase. The increases recorded at C_2 and C_3 inclusion of the *A. danielli* spice were 9.04% (24.67-26.90) and 8.54% (24.93-27.06). A similar increase was observed in the result for plum spice supplemental diet-fed rats, which recorded increases of 8.25% (25.83-27.96), 9.01% (26.2-28.56), and 8.29% (26.53-28.73). The variation was not significant between the different concentrations of spices in the diets. The percentage increase in serum AST of the rats fed with country onion diets was 15.75% (24.5-28.5), 14.12% (25.00-28.53), and 14.17% (25.40-29.00) for the rats fed with C_1, C_2, and C_3 concentrations of the country onion spice. The country onion spice caused a significant ($p<0.05$) rise in the AST level of the Wistar rats compared to the other spices' effect on the serum AST.

Alanine Aminotransferase (ALT): Table 7 shows the changes caused by the liver enzyme (ALT) resulting from spiced and un-spiced feed given to the test animals from 1 to 28 days. The level of the enzyme Alanine amino-transferase (ALT) in the blood of rats fed with the different test spices showed significant differences ($p<0.05$) in the ALT activity of the rat's blood at the beginning of the feeding trials. The ALT activity in the rats' blood varied between 26.57u/L and 29.27u/L at the start of the experiment. At 5%, 10%, and 15% concentrations of the spices (C_1, C_2, and C_3), the ALT increased by 7.34% (27.25u/L – 29.25u/L), 15.05% (27.25u/L – 31.35u/L) and 16.7% (28.33u/L – 33.07u/L) in *A. danielli* fed rats. The increase in ALT activity in the blood of rats fed with rough skin plum feed at 5%, 10% and 15% concentration increased by 7.64%(26.57u/L – 28.60u/L), 19.3% (26.90u/L – 32.10u/L) and 19.08%(27.10u/L – 32.27u/L) while the rats fed with feed substituted with 5%, 10% and 15% country onion spice increased by 9.62% (28.37u/L – 31.10u/L), 16.31% (29.00u/L – 33.73u/L) and 10.2% (29.27u/L – 32.27u/L) throughout the 28 feeding trials. There were higher levels of ALT activities at increased levels of spice substitution in the rat's feed. The increases in the enzymes' activities in the Wistar rats' blood were significantly different ($p>0.05$) based on spice type and concentration.

As analysed in this study, the increase in the enzymatic activities of AST and ALT supports an earlier study by Singh *et al.* (2001), where the administration of plant extract resulted in increased activities of ALT and AST in rat serum. Iweka *et al*. (2016) reported that AST and ALT typically reside inside the liver cells, and any increase in their levels could suggest hepatocellular damage of the Wistar rats' liver, which indicates the possible hepatotoxic effect of *Afromomum danielli*, rough skin plum and country onion spices. However, the possibility of the spices having hepatotoxic effects is in doubt as the AST and ALT levels of the control also increased as the feeding period progressed.

Generally, on the liver function test, there was a slight dose increase in the liver function enzyme activities, and all cannot be due to the consumption of the spices by the test rats, as test rats fed with un-spiced feed also exhibited increases in their enzymatic activities. These enzymes are essential in producing amino acids, and they serve as indices for diagnosing and evaluating any disease that affects the liver organ. Alkaline Phosphatase is a hydrolase enzyme that increases a phosphate group from many molecules through phosphorylation. Alkaline Phosphatase is a liver enzyme; its variation from the standard range indicates an anomaly in the organ and, as such, is used as an index to establish the possibility of some diseases. As

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shown in the results, there were increases in the ALP activity. However, these did not amount to actual elevation as they did not exceed the normal ranges and were within acceptance criteria (20-40U/L), as Odiegwu *et al.* (2021) reported. Thus, the spices did not cause an elevation of the enzyme activities (rising beyond the accepted level).

CONCLUSION

In this study, the albumin content in the blood of the test animals fed with rough skin plum and country onion spiced diets at different concentrations and times was lower than that of the control test animals and the rats fed with *Aframomum* danielli spiced feed. Albumin values lower than the normal range indicate liver disease, while higher values would indicate dehydration, including proteinuria. Generally, the results obtained in all the parameters evaluated showed that the inclusion of the different proportions (5%, 10%, 15%) in the diet of the rats affected the liver functions positively. In conclusion, consuming these spices at these levels is safe and impacts a healthy life and good liver function.

Further research on the pharmacology of these spices' bioactive properties is recommended. This could lead to the pharmacology of these compounds being used in the treatment or management of health issues and diseases.

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