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The Hematological Profile, Hepatic Marker, and Histomorphology Effects on Canned Tomato Consumption Using Adult Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The study investigates the effects of canned tomato consumption on the hematological profile, hepatic markers and histology. **Methods:** The study involved 20 male Wistar rats divided into two groups, each fed Gino tomato

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Asian Hematol. Res. J., vol. 7, no. 1, pp. 41-50, 2024

sachets for 21 days. The rats were anesthetized, and liver and blood were extracted. Standard laboratory analysis, light microscope, GraphPad Prism, and ANOVA were used for descriptive and statistical analysis, with a significance level of p<0.05.

Results: Findings show the presence of Carbohydrates, Alkaloids, Flavonoids, reducing sugar and Resins were present, Results for PCV were 7.00 ± 4.15 and $53.00\pm3.69\%$, Hb levels were 11.70 ± 1.03 and 17.68 ± 1.22 g/L. (WBC) are 9.44 ± 1.32 and 7.16 ± 0.50 g, with results significant at (p<0.05), in table 2. The results for Liver function are 12.80 ± 2.78 and 4.80 ± 0.58 U/l for AST, 8.60 ± 1.33 and 5.40 ± 0.68 U/L for ALT, Total protein 62.40 ± 6.52 and 68.60 ± 8.41 (g/L), and was significant at p<0.05 in table 4. The weights of the rats for the first 21 days were 81.70 ± 3.20 and 84.50 ± 3.46 g, 102.00 ± 3.45 and 114.90 ± 3.84 g, and 103.30 ± 5.19 and 107.70 ± 5.47 g, with significance at week 2 in table 4, the weight after 21 days were 173.20 ± 7.45 and 183.20 ± 6.69 g, 177.00 ± 6.64 and 198.20 ± 12.46 g, and 189.60 ± 8.01 and 205.40 ± 9.90 g.

Conclusion: Solanum lycopersicum had no significant effect on the hematological, histological, or hepatic markers of rats fed tomato pastes, implying no effect on their weight.

Keywords: Consumption; hematological parameters; anesthetizing; standard error of mean.

1. INTRODUCTION

Tomatoes, based on several studies, are one of the most important fruits commonly consumed by people, as well as providing a healthy heart and other parts of the body and systems when consumed in moderation [1,2,3,4]. The effects of certain foods or fruits on a person are defined as either useful or dangerous [5], like in the case of cancer and other life-threatening health conditions such as heart disease when taken in large quantities [6].

Tomatoes (Solanum lycopersicum) are rich in phytochemicals and nutrients like lycopene, potassium, iron, folate, and vitamin C [7]. Aside from lycopene and vitamin C, tomatoes include other antioxidants such as beta-carotene and phenolic substances such as flavonoids. hydroxycinnamic acid, chlorogenic, homovanillic acid, and ferulic acid [8]. Tomatoes can contribute significantly to a healthy diet and can be consumed fresh and cooked while retaining their nutritional value [1,9,10]. Over 80% of commercially farmed tomatoes are consumed in processed forms such as juice, soup, and ketchup [9].

Food processing procedures have been shown to influence and alter nutrients in fruits and vegetables [11]. However, according to Sadler et al. [12], processed foods are not always nutritionally inferior or unhealthy. Processed food can have favorable impacts such as better digestibility, nutritional bioavailability, and food safety [13]. Recent works and studies have revealed that tomatoes are largely composed of many constituents and elements, necessitating an analysis of the various elements and their composition [9]. Other researchers have highlighted the various benefits of tomatoes; nevertheless, in addition to these health benefits, tomatoes have some undesirable effects on the body when ingested in big quantities or under abnormal body conditions. These adverse effects have been linked to a variety of medical disorders, including kidney difficulties, allergies, arthritis, heartburn, and migraines [14].

Lycopene, one of the active ingredients of tomatoes and an antioxidant, is substantially higher in cooked and processed (canned) tomatoes than in fresh tomatoes [9,15]. Canned tomatoes have substantially higher lycopene bioavailability than fresh tomatoes because heating opens up the plant's cell walls, allowing lycopene to be absorbed in the body [9,16]. Based on these findings, this study was designed to explore the effects of canned tomato consumption on hematological profile, liver markers, and hepatic histology.

2. MATERIALS AND METHODS

2.1 Animal Procurement and Acclimatization

20 mature male Wistar rats (*Rattus norvegicus*) weighing between 80g and 130g were purchased from the University of Port Harcourt Animal House Choba and transported to the Department of Animal and Environmental Biology Laboratory in a well-constructed wooden cage. They were allowed to acclimate to the environment for two weeks and were fed a standard basal diet with water twice daily. The animals were weighed with a weighing balance upon arrival to determine

their respective weights, and they were divided into two groups of ten male Wistar rats each and categorized as Cage A "Control" and Cage B "Experimental" for the research purpose to effectively examine the hematological and histological from the Gino tomato pastes treatment given.

2.2 Procurement of Tomatoes Paste

Fifteen (15) Gino tomato paste, which is thought to be the most often used, was purchased from the Choba market. The Gino tomato paste purchased was thoroughly examined for its NAFDAC registration number, expiration dates, and tomato paste ingredients.

2.3 Study Design

The study design used in the research was an experimental design that lasted approximately three months and was carried out in two phases, after the two-week acclimatization period seen in the research. The animals utilized in the investigation were male albino Wistar rats purchased, acclimatized in a serene setting, and fed throughout this research.

Phase one: This phase was classified as the acute phase of the study and it lasted for 21 days (3 weeks). The Wistar rats used in the study were fed with a 1:1 ratio and mixture of feed which was composed of 100% Gino tomato paste that was used in the treatment. Also, they were fed with clean water, while the control group of the experimental animals was only fed with 50g top feed for 21 days.

Phase two: This second phase of treatment was referred to as the study's recuperation phase, and it lasted the same 21 days as the first phase. The control and experimental groups were fed 50g of top feed twice daily for 21 days.

2.4 Sample Collection

After both phases of the trial, samples were taken from the two groups, control and treatment, for hematological and biological analysis, as well as the sacrifice of rats. The Wister rats were sedated with chloroform by placing them individually on a table, and organs were extracted by dissecting the rats for their liver contents utilizing liver extraction techniques. The removed liver organ was fixed in 10% formal saline, thus 2ml of blood samples were obtained and preserved in an anticoagulant bottle (EDTA bottle), after which histological tests were performed on them.

2.5 Hematological Analysis of the Organs

The properly prepared and conserved blood sample from the used Wistar rats was taken and placed in a centrifuge and spun to separate the red blood cells from the white blood cells, platelets, and plasma. Following separation, the red blood cells were placed on a slide and covered with a coverslip, and a microscopic examination was performed at magnifications of x10 and x40, with an oil immersion microscope for a better view and exams.

2.6 Histological Analysis of the Organs

The liver tissue was fixed with formalin, then dried in alcohol and embedded in paraffin. The slide was cut into five-micrometer slices, deparaffinized, and stained with hematoxylin and eosin. Before being photographed, the dyed slides were viewed using a light microscope and magnified 400 times. The resultina photomicrograph then was examined for changes to the histoarchitecture.

2.7 Liver Biomarker

The study examined the liver function on serum biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glutamyl transpeptidase (GGT), total bilirubin (TBIL). Total Protein, Albumin, and conjugated bilirubin.

2.8 Statistical Analysis

The data recorded from both the control group of animals and the ones that were treated with Gino tomato paste were entered into Micro Soft Excel 2013 to determine the Mean and standard error of the mean (SEM) as well. One Way Analysis of Variance (ANOVA) was carried out to determine the variation of the different parameters by using the International Business Machine of Statistical Package for Social Sciences (IBM SPSS version 25) to carry out the analysis, and a statistical value of (P < 0.05) was set to be statistically significant.

3. RESULTS

Table 2 shows, that the Mean and Standard Error of Mean (SEM) for PCV are 37.00 ± 4.15 and $53.00\pm3.69\%$, for the control and the groups respectively. The Hb levels are 11.70 ± 1.03 and $17.68\pm1.22g/L$. The White Blood Cells (WBC) are 9.44 ± 1.32 and $7.16\pm0.50g$. The Red Blood Cells (RBC) are 4.30 ± 0.19 and $5.98\pm0.42g/L$ in the

groups respectively. The control and concentrations of the Platelets are 224.80±20.52 several 273.60±50.05g/L. There are and variations in the concentrations and groups respectively at different times and within the control and group given to the rats. There was an increase in the mean and standard error of the mean (SEM) for the different parameters being examined. The highest mean was seen in the groups, and this had adverse effects on the liver of the rats. The results for PCV. Hb. and RBCs show significant effects at (P<0.05), between the groups control and experimental on hematological profile for the first phase of 21 while WBC, platelets, neutrophil, davs lymphocyte, monocytes, and Eosinophil display no statistical difference between the control and the experimental group for the first 21 days.

The hematological profile for the second phase of the 21 days shows the Mean and Standard Error of Mean (SEM) for PCV range from 53.40 ± 2.46 - $59.60\pm3.23\%$, for the control and the treatment respectively, and the Hb levels range from 17.00 ± 0.56 - $17.46\pm0.83g/L$, the White Blood Cells (WBC) in the study ranges from $6.82\pm0.98 - 5.44\pm0.69g/L$ from the control to the treatment in the study. The Red Blood Cells (RBC) in the study are 6.02±0.39-7.30±0.47g/L in the control and in the treatment respectively. The concentration of the Platelets ranges from (253.40±28.73 to 357.00±59.99g/L). The result also shows no statistically significant difference for PCV, Hb, WBC, RBC, platelets, neutrophils, lymphocytes, monocytes, and Eosuphil between the control and experimental groups (Table 3).

The Liver function test of different groups in the study had a mean value of 12.80±2.78 -4.80±0.58 U/I, for AST before treatment, and 8.60±1.33 - 5.40±0.68 U/L in the ALT U/L, also ALP U/L had 46.20±3.57 - 11.60±1.08 U/L, the result for Total protein (g/L) was 62.40±6.52 -68.60±8.41 after they were treated to with tomato extracts. The result further shows that AST and bilirubin were statistically significant Total between the control and the experimental group while ALT, Total protein, Albumin. and conjugated bilirubin (Table 4) In Table 5, the liver function test for the second phase of the 21 days shows no statistical significance in AST, ALP, Total protein, Albumin, Total bilirubin, and conjugated bilirubin between the control and experimental groups.

S/N	Constituents	Indication	Test carried out
1	Carbohydrates	+	Molish
2	Tannins	_	Ferric chloride test
3	Proteins	_	Protein electrophoresuis
4	Alkaloids	+++	Mayer
5	Flavonoids	++	Pew's test
6	Oils	_	Sudan III
7	Terpenoids	_	Salkowski test
8	Glycosides	_	Kedde test
9	Saponins	_	Foam
10	Reducing Sugar	+++	Benedict
11	Resins	+	HCL TEST
12	Steroids	_	GC-MS
13	Acidic Compounds	_	Litmus paper test

NB: (-) Absent, (+) Present in Little amount, (++) Present in moderate amounts, while (+++) is heavily present

Groups							
Parameter	Control	Experimental	t	P-value	Inference		
	Mean± SEM	Mean± SEM					
PCV (%)	37.00±4.15	53.00±3.69	-2.88	0.02	Significant *		
Hb (g/dl)	11.70±1.03	17.68±1.22	-3.74	0.01	Significant *		
WBC g/L	9.44±1.32	7.16±0.50	1.61	0.15	Not significant		
RBC g/L	4.30±0.19	5.98±0.42	-3.62	0.01	Significant *		
Platelets g/L	224.80±20.52	273.60±50.05	-1.13	0.29	Not significant		
Neutrophil (%)	26.20±2.65	38.60±5.19	-2.13	0.07	Not significant		
Lymphocytes (%)	60.80±3.67	53.80±4.74	1.17	0.28	Not significant		

Edibamode et al.; Asian Hematol. Res. J., vol. 7, no. 1, pp. 41-50, 2024; Article no.AHRJ.115251

Groups							
Parameter Control		Experimental t		P-value	Inference		
	Mean± SEM	Mean± SEM					
Monocytes (%)	9.80±1.39	5.60±1.63	1.96	0.09	Not significant		
Eosinophil (%)	2.80±1.16	1.00±0.63	1.37	0.21	Not significant		

Values are presented as Mean±SE. P: statistical level of significance was determined using Independent sample T-test. P<0.05 means there is significance between mean values. SE=Standard Error; n=sample size.RBC=red blood cells, WBC= white blood cells, hb= hemoglobin, PCV= Pack cell volume

Table 3. Hematological profile in both groups for the second phase of the 21 day	S
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Groups							
Parameter Control		Experimental	Т	P value	Inference		
	Mean± SEM	Mean± SEM					
PCV (%)	53.40±2.46	59.60±3.23	-1.53	0.17	Not significant		
Hb (g/dl)	17.00±0.56	17.46±0.83	-0.46	0.66	Not significant		
WBC*109/L	6.82±0.98	5.44±0.69	1.15	0.28	Not significant		
RBC*10'12/L	6.02±0.39	7.30±0.47	-2.11	0.07	Not significant		
Platelets*10'9/I	253.40±28.73	357.00±59.99	-1.56	0.16	Not significant		
Neutrophil (%)	30.20±4.61	26.60±1.86	0.72	0.49	Not significant		
Lymphocyte %	61.00±6.84	66.20±2.56	-0.71	0.50	Not significant		
Monocyte %	3.40±0.68	4.60±1.21	-0.87	0.41	Not significant		
Eosinophils %	1.20±0.20	2.60±1.25	-1.11	0.30	Not significant		

Values are presented as Mean±SE. P: statistical level of significance was determined using Independent sample T-test. P<0.05 means there is significance between mean values. SE=Standard Error; N=sample size. RBC=red blood cells, WBC= white blood cells, hb= hemoglobin, PCV= Pack cell volume

Table 4. Liver function	test for both grou	ups for the first	phase of 21 days

Groups							
Parameter Control Experimental			t	P- value	Inference		
	Mean± SEM	Mean± SEM					
AST (U/L)	12.80±2.78	4.80±0.58	5.84	0.00	Significant *		
ALT (U/L)	8.60±1.33	5.40±0.68	2.15	0.06	Not significant		
ALP (U/L)	46.20±3.57	11.60±1.08	9.28	0.00	Significant *		
Total protein (g/L)	62.40±6.52	68.60±8.41	-0.58	0.58	Not significant		
Albumin (U/L)	38.40±1.29	46.20±3.73	-1.98	0.08	Not significant		
Total bilirubin (mmol/L)	17.80±1.59	9.60±1.03	4.32	0.00	Significant *		
Conjugate bilirubin (mmol/L)	9.80±1.20	7.00±0.84	1.91	0.09	Not significant		

Values are presented as Mean±SE. P: The statistical level of significance was determined using the Independent sample T-test. P<0.05 means there is significance between mean values. SE=Standard Error; n=sample size. AST= Aspartate aminotransferase, ALT= Alanine aminotransferase, ALP= Alkaline phosphatase

Table 5. Liver function test for the second phase of 21 days

Groups							
Parameter	Control	Experimental	t	P value	Inference		
	Mean± SEM	Mean± SEM					
AST (U/L)	10.20±1.32	15.40±2.42	-1.89	0.10	Not significant		
ALT (U/L)	7.40±1.40	4.40±0.51	2.01	0.08	Not significant		
ALP (U/L)	43.00±8.47	61.20±9.89	-1.40	0.20	Not significant		
Total protein (g/L)	62.00±5.17	72.60±4.86	-1.49	0.17	Not significant		
Albumin (U/L)	43.40±0.68	41.46±0.93	1.57	0.16	Not significant		
Total bilirubin (mmol/L)	6.00±0.95	6.80±0.58	-0.72	0.49	Not significant		
Conjugate bilirubin (mmol/L)	3.00±0.89	3.20±0.74	-0.17	0.87	Not significant		

Values are presented as Mean±SE. P: statistical level of significance was determined using Independent sample T-test. P<0.05 means mean values are significant. SE=Standard Error; n=sample size. AST= Aspartate aminotransferase, ALT= Alanine aminotransferase, ALP= Alkaline phosphatase Fig. 1, observed an increase in the weights of the rats as they were fed and treated with tomatoes and the feed allotted to them as well. The increase was seen in their mean weight and increased exponentially as the number of weeks of treatment increased, and more weight as they gained against the previous or initial weights of the rats recorded before treatment was given to them. The results of the study showed an increase in the weights of the rats after treatment was administrated to 84.50±3.46g, them from 81.70±3.20 102.00±3.45 - 114.90±3.84g, and 103.30±5.19 -

107.70±5.47g respectively in the three weeks of sampling shown in as Fig. Table 4 and 4. results The for week 1 and 3 was not significant as (p>0.05), but a significance level was seen in week 2 at (p<0.05).

Fig. 2 shows that the mean ranges from $173.20\pm7.45 - 183.20\pm6.69g$, $177.00\pm6.64 - 198.20\pm12.46g$, and $189.60\pm8.01 - 205.40\pm9.90g$ respectively in the three weeks of sampling and the results for weeks 1, 2, and 3 were insignificant (p>0.05).



Fig. 1. Weight measurements in both groups for the first 21 days



Fig. 2. Weights measurements in both groups for the second 21 days

Edibamode et al.; Asian Hematol. Res. J., vol. 7, no. 1, pp. 41-50, 2024; Article no.AHRJ.115251



Fig. 3. Photomicrograph of first phase control group of rat liver using h&e @ x400 magnification fed with standard fed for 21 days. Photomicrograph displays Normal Hepatocytes (h) with normal radial arrangements, sinusoids(s) & centtrilobular vein (cv)



Fig. 4. Photomicrograph of first phase experimental group of rat liver using H&E @ x400 magnification fed with canned tomato paste for 21 days. Photomicrograph displays normal hepatocytes (h) with normal radial arrangements, Sinusoids(s), Single nucleus (n) & centtrilobular vein (CV)

4. DISCUSSION

The phytochemical analysis of tomatoes in the study shows the presence and availability of

some elements and constituents, in varying quantities which are Carbohydrates, Alkaloids, Reducing sugar, and Flavonoids as seen in Table 1, with them having anti-oxidative functions. This result was similar to (D'Introno, et al. [17], and Dominic et al., [18] who in their different studies, identified the same constituents. Tomatoes from the study are known to provide antioxidants (Lycopene) found in tomatoes, which act as free radicals, thus preventing the occurrence of harmful impacts, and are mostly active against prostate cancer which are all products of their phytochemical contents.

The results for the weight composition of the rats, show an increase in the weight of the rats from the control and till the introduction of the Gino tomato paste for the duration of the research. The results show an exponential increase in the weights of the Wistar rats used in the study. This increase in weight is 81.7±10.9 to 84.5±12.1g in the first week for control and experimented respectively to 205.4±22.1g, at the end of 6 weeks as shown in Figs. 1 and 2, these findings were similar to the study of Buabeida et al., [19], who recorded 172±5.2 g to 183±3.7 g, and the results in the study was not significant as (p>0.05), hence the findings suggest that tomatoes paste has no impact on the weight of the Wistar rats

The Results for the Haematological parameters Wistar rats' treatment in the of study show a higher value in the treatment of Solanum lycopersicum L. on Wistar rats as seen in PCV, Hb, and other biochemical parameters. The higher percentages of the hematological parameters and concentration in the study is an indication that tomato paste is highly effective in the treatment and regulation of blood-related issues, as seen in Table 2. The increasing number in the Mean and Standard Error of Mean (SEM) in the study also indicates the impacts of tomatoes on the hematology of Wister rats in the study, which was similar to Amaechi et al., [20]. The study shows a significant difference at (p<0.05) for PCV, RBC, and Platelets. However, the reasons for the hematological differences observed between the control and experimental groups could be attributed to nutritional composition because the high level of lycopene other antioxidants could influence or hematological parameters and also preservative or additive of the canned tomato may have a biological effect that could influence blood parameters. Thus, dosage and duration could also influence the hematologic parameters, however, the second phase of administration (second 21 days) seen in Table 3, shows no statistical changes in the hematological

parameters. This could be attributed to the variability in responses among the rats.

The study for the Liver function test as observed in the study shows the effects of different, Aspartate Aminotransferase (AST), on the rats' shows that it increased, and was higher in the control than it was in the Gino tomato paste feed provided to the rats for the 6 weeks of treatment and experiment. The result shows a significant difference at (p<0.001), and the study agrees with the findings of Buabeid, et al. [21] who reported a lower reading of the different Liver function test which was at (p<0.05).

The increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), levels were significant (P<0.05) in the different groups. The different liver tests carried out impact and show the different alterations that occurred during the Research.

Albumin is the most generous protein circulating in the plasma and is reported as the vital protein synthesized by the liver, in the study the albumin level recorded was similar to the research of Roche et al., [22], the lower level of the Albumin in the study was as a result of the induced toxicity level in the rats group, and was significant at (p<0.5). The results and findings on the liver impacts and other histological functions as stated in the research indicate that Gino tomato paste has the potential to inflict several levels of injuries on the liver of the body as also reported in other studies carried out in the area of impacts of tomatoes on the functioning of the liver of rats. Liver biomarkers are investigated, as they are important in diagnosing clinical conditions in patients especially when such are having some changes in their human forms. Biomarkers for the livers in the study (AST, ALP, and ALT) from Table 5 in the study, AST, ALP, and Total Bilirubin were significant at (p<0.05), while ALT, Total Protein, Albumin, and Conjugate bilirubin were not significant. And the biomarkers were significant in the first 21 days. However, following the recuperation study having withdrawn the tomato consumption in the Wistar rats, no particular significant levels were seen in the testing parameters.

5. CONCLUSION

The findings of the study indicate that *Solanum lycopersicum L.* has little or no impact on several hematological, histological, and also

phytochemical parameters which affected the rats fed with tomato pastes. The findings of the study conclude that canned tomato paste has no impact on the weight of the rat.

CONSENT

It is not applicable.

ETHICAL CONSIDERATION

This study was approved by the ethical research committee of the University of Port Harcourt, Nigeria.

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COMPETING INTERESTS

Authors have declared that there was no competing interest.

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