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(RESEARCH ARTICLE)

In-vitro lethality assay and performance of Sumain nutritional supplement on the liver enzymes and lipid profile of wistar albino rats

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Abstract

Sumain Nutritional Supplement (SNS) is a processed composite flour of groundnut, soya bean, guinea corn, and maize grains, and substituted at 0, 25, 50, 75, and 100% diet levels of a Commercial feed. *In vitro* toxicity study was conducted using Brine shrimp larvae, while *in vivo* performance of these diets was assessed using wistar albino rats in a 21-day feeding period. Mean body weight difference was measured. Liver extracts were assayed for alanine, and aspartate amino transaminase (ALT, AST) concentrations. Plasma extracts were assayed for haematological, and lipid profiles using standard methodologies. Results showed that SNS extract was safer than a reference tannic acid by 83%, with LC_{50} of 550. Compared with AST/ALT ratio of 1.17 for 100% Commercial feed, those of SNS-incorporated feeds ranged from 0.92 to 1.16, indicating no apparent damage to liver tissue. At all levels of SNS substitutions, WBC and PCV concentrations were within standard acceptable ranges, while results of MCV and Hb-related parameters exhibited very good biochemical indices of health status during the period of study. 100% and 75% SNS substituted diets particularly, showed potential to reverse thrombocytopenia. Increased Triglyceride (TG) level coupled with lowered levels of Cholesterol (LDL and TC), led to the recommendation of these two SNS diets as good nutraceuticals for human subjects having symptoms of malnutrition.

Keywords: Sumain Nutritional Supplement (SNS); Brine Shrimp Larvae Assay; Alanine amino transaminase (ALT); Aspartate transaminase (AST); Lipid Profile.

1. Introduction

Composite flour of grains prepared from different legumes, cereals and nuts is considered world-wide as good sources of proteins, carbohydrate, energy and dietary fibre. It also provides essential vitamins needed by the body. Furthermore, composite flour of these grains can be utilized in the production of good quality snacks, pan cakes and savoury foods [1]. The flour, through "diet-based strategies", could also be employed in the management of protein-energy malnutrition disease and micronutrient deficiency. The strategy would be valuable to children and pregnant women especially in developing countries [2-3].

Leguminous crops, particularly soya bean (*Glycine max*) are valued locally and globally for their high protein and oil contents. Such crops are rich in essential amino acids, vitamins, calcium, and phosphorous, soyabean. They also contain isoflavone, which plays an important physiological role in the prevention of cancer and osteoporosis [4]. Similarly nuts such as, groundnut (*Arachis hypogaea*) possesses high dietary protein and oil contents enriched with minerals, vitamins

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and essential amino acids [5]. However, cereals like maize (*Zea mays*) which serve as staple food in some parts of the world are peculiarly low in tryptophan and lysine [6] though; they contain high proportions of carbohydrate, essential fatty acids, vitamins E, K, C, B-complex, potassium as well as phenolic compounds, phytosterols and carotenoids [7]. Previous studies on nutritional properties of composite flour or multigrain flour in supplementary foods have revealed the possible utilization of multigrain and its nutritional benefits when incorporated in diets [1, 8-9].

In view of these benefits, diets were composed from a formulated Sumain product (a composite flour of cereals, legumes, and nuts) and substituted in a commercial feed at varied percentages. This study, therefore, investigated the nutritional effects on the enzymatic activities, haematological, and lipid profiles of experimental animals fed on these diets.

2. Materials and methods

2.1. Sample collection and processing

Commercially available dried yellow Soybean (*Glycine max*), red-skin variety of Groundnut (*Arachis hypogaea*), white Guinea Corn (*Sorghum bicolor*), yellow and white varieties of Maize (*Zea mays*) seeds were procured separately from Anyigba market, Kogi State, Nigeria.The experiments were conducted at the Biochemistry Laboratory of Kogi State University, Anyigba, Nigeria between March and October 2019.

Processing steps to effectively remove most anti-nutritional factors, as described by International Institute of Tropical Agriculture (IITA) [10] were adopted: The seeds were cleaned and sorted for wholesomeness. They were further subjected, where necessary, to soaking, draining, and air-drying e.g. Guinea Corn and Soya bean, before dehulling, toasting and grinding into powdery form called "Sumain Nutritional Supplement (SNS)". Details of the treatments given are contained in Table 1.

2.2. Cytotoxicity bioassay using brine shrimp larvae

The assay was conducted using hatched brine shrimp (*Artemia salina*) larvae as described by Arogba and Matanmisi [11]. Steps involved are described thus:

2.2.1. Preparation of sample extracts

With each diet sample type, 1 g was dissolved in distilled water (50 ml) to form the stock solution from which serial dilutions were made.

2.2.2. Hatching brine shrimp eggs

Equivalent sea water was prepared by dissolving 32 g of NaCl in 1000 ml of distilled water. Some 50 mg of brine shrimp eggs were added to 300ml of the artificial sea water and incubated for 24 hours under bright light while connected to an air-voltage pump that aerated the mixture.

After incubation, groups of 10 brine shrimp larvae were counted and transferred to different vials using a Pasteur pipette and volume was made up to 5 ml with the artificial sea water. Constant volume of 500 µl of each varied concentration (2000, 1000, 500, 250 and 125 µg/ml) of a diet sample type or reference potassium dichromate was added to the vials containing the shrimps. After 24 hours, the dead larvae were counted for the determination of the percentage lethality. The lethality endpoint of the bioassay was defined as the absence of controlled forward motion during 30 seconds of observation.

The percentage lethality of the shrimp for each concentration was calculated [11]:

%Lethality = (Number of morbid shrimp larvae / Number of surviving shrimp larvae in Control) x 100.

The LC_{50} of the sample's extract was calculated from linear regression curve of Percentage lethality against Log (concentration), when y = 50.

Table 1 Processing conditions for production of sumain nutritional supplement (SNS) flour mix

Sample type	Shelling	Cleaning / Sorting	Mild sprinkling of water	Soaking for 30 min / Draining	Air - drying at ambient temp.	Dehulling by milling / Winnowing	Toasting	Manual dehulling	60g eqv. Toasting time (min)	Milling into flour	Ratio in SNS mix	Weight (g) in Batch Production
Groundnut	y	Y					y	y	1	y	1	300
Guinea corn		Y	y	y	y		y		2	y	1.5	450
White maize		Y	y			Y	y		0.5	y	1	300
Yellow maize		Y	y			Y	y		0.5	y	1	300
Soya bean		Y		y	y	Y	y		1.5	y	2	600
Total												**1.95 kg**

y = Treatment given

2.3. Procurement and management of experimental animals

Male wistar rats weighing between 97 – 132 g were procured from the animal house of the Department of Veterinary Medicine, Benue State University Makurdi, Nigeria. They were acclimatized in clean rat cages at the Experimental Animal House of the Department of Biochemistry, Kogi State University, Anyigba for a period of seven (7) days at ambient temperature with 12-hour light and dark cycle. Within the period, they were fed with water *ad-libitum* and commercial "Broiler Top Feed Finisher" purchased from a supermarket at Anyigba.

2.4. Experimental design

2.4.1. Weight measurement

The weights of the rats were taken before and after twenty one (21) days feeding period. The percentage weight difference was calculated.

2.4.2. Diet substitution/Animal Grouping (Table 2)

Fifteen (15) rats was fed with commercial "Top Feed Finisher" diet and substituted with SNS at 0, 25, 50, 75 and 100% levels. Water was provided *ad-libitum*.

Table 2 Experimental design for albino rats on substituted diets

SNS	: Comm. Feed (%)	Number of rats/ group
0	: 100	3
25	: 75	3
50	: 50	3
75	: 25	3
100	: 0	3
Total		15

2.5. Blood sample collection

On the 22nd day, the animals were sacrificed by using chloroform in desiccator. The blood was obtained through cardiac puncture into heparinized (EDTA) bottles using 5ml syringe.

2.6. Assay of lipid profile

The lipid profile [Total Cholesterol, Triglycerides, and High Density Lipoprotein, and Low Density Lipoprotein] was determined by spectrophotometric technique, using enzymatic/colorimetric assay kits (Randox Laboratories, United Kingdom). However, Low Density Lipoprotein was calculated thus: LDL = TC – [HDL + TG/5].

2.7. Enzyme assay

The activities of Alanine transaminase (ALT) and Aspartate transaminase (AST) were analysed according to the method described by Reitman and Frankel [12].

2.8. Assay of haematological parameters

The packed cell volume (PCV), mean cellular volume (MCV), platelets (PLT), white blood cells (WBC), haemoglobin (Hb), and mean corpuscular haemoglobin concentration (MCHC) were all determined as described by Baker and Silverton [13], using automated haematology analyzer (Sysmex Kox1: Sysmex corporation, Kobe, Japan, Xp 300 Series, Code No AC580857)

2.9. Statistical analysis

Statistical analysis was conducted using SEM software available at *miniwebtool.com*. Results were expressed as mean ± standard error of mean (SEM), where expedient. Separation of mean was conducted for test of significance at (P = 0.05), where different alphabets denoted significant difference.

3. Results

Table 3 Lethality Rate of Brine Shrimp Larvae (expressed as LC_{50})

Conc. (µg/ml)	Sample (%)	Tannic acid (%)	K2Cr2O7 (%)
2000	73.3	93.3	100
1000	66.7	86.7	96.7
500	50.0	70.0	70.0
250	36.7	40.0	56.7
125	13.3	26.7	16.7
LC50	549.5b	300.3a	274.4a
SEM	87.70		

Values are expressed as mean of triplicate determinations. Values of LC_{50} with different superscripts on the same row are significantly different at $p < 0.05$.

The test sample of SNS was least toxic to Brine Shrimp larvae compared with the organic and inorganic reference samples employed. Their relative LC_{50} were significantly different ($p<0.05$).

Table 4 Mean weight of animals before and after feeding for 21 days (n = 3).

Sample: Comm. Feed (%)	Before (g)	After (g)	Difference (g)	Difference (%)	SEM
0 : 100	97	146	49	51^{d}	
25 : 75	100	148	48	48^{cd}	
50 : 50	108	156	48	44^{c}	4.7
75 : 25	121	160	39	32^{b}	
100 : 0	132	167	35	27^{a}	

Values with the same superscripts in the column are not significantly different at $P > 0.05$. SEM = Standard error of mean. Comm. Feed = commercial feed (Top feed Finisher).

Difference of 24% weight reduction was recorded between animals fed with 100% SNS sample and 100% commercial feed ($p<0.05$). However, there was no negative sign in loss.

Table 5 Enzyme concentrations at different diet substitutions

Sample: Comm. Feed (%)	AST (IU/L)	ALT (IU/L)	AST/ALT
0 : 100	53.1 ± 9.95b	45.5 ± 12.50c	1.17c
25 : 75	43.1 ± 6.10a	47.0 ± 5.00c	0.92a
50 : 50	42.5 ± 3.50a	39.5 ± 2.50b	1.08b
75 : 25	43.5 ± 4.50a	37.5 ± 4.50a	1.16c
100 : 0	42.5 ± 4.50a	40.0 ± 6.00b	1.06b
SEM	2.05	1.84	0.044

Values are expressed as mean ± SEM (n=3).
Values with different superscripts on the same column are significantly different at $p < 0.05$.

AST concentration decreased significantly with SNS substitution in the commercial feed ($p<0.05$). and remained unchanged irrespective of level of substitution ($p>0.05$). Unlike AST, 25% SNS substitution did not influence ALT concentration, but higher SNS substitutions did. Consequently, AST/ALT varied and was SNS concentration-dependent. The least AST/ALT was observed at 25% SNS substitution in the commercial feed. ($p<0.05$).

Table 6 Haematological analysis

Sample: Comm. Feed (%)	WBC (x10³/L)	Hb (g/dL)	PCV (%)	MCV (dL)	MCH (pg)	MCHC (g/dL)	PLT (x10³/L)
0 : 100	6.5 ± 0.30^{b}	14.6 ± 1.60^{c}	47.0 ± 7.00^{b}	71.6 ± 0.25^{a}	18.0 ± 0.75^{b}	24.3 ± 0.30^{a}	225.0 ± 18.00^{c}
25 : 75	5.5 ± 2.00^{a}	11.8 ± 0.20^{a}	44.6 ± 0.60^{a}	71.5 ± 0.05^{a}	19.0 ± 0.55^{d}	26.5 ± 0.80^{c}	92.4 ± 6.40^{a}
50 : 50	6.9 ± 0.10^{c}	14.6 ± 0.05^{c}	55.9 ± 0,05^{d}	71.9 ± 0.10^{a}	18.4 ± 0.35^{c}	25.0 ± 1.00^{b}	89.0 ± 1.00^{a}
75 : 25	6.3 ± 0.50^{b}	12.6 ± 0.30^{b}	43.8 ± 3.80^{a}	73.3 ± 1.95^{b}	17.8 ± 0.60^{a}	24.4 ± 1,45^{a}	177.5 ± 35.50^{b}
100 : 0	7.1 ±1.25^{c}	12.4 ± 0.90^{ab}	51.8 ±1.50^{c}	73.7 ± 1.65^{b}	17.7 ± 1.15^{a}	24.0 ± 1.05^{a}	111.0 ± 5.00^{a}
SEM	0.28	0.59	2.29	0.46	0.24	0.45	26.77

Values are expressed as mean ± SEM (n=3). Values with different superscripts in the same column are significantly different at p<0.05

Optimum levels of WBC, Hb, PVC, MCH, and PLT were recorded on substituting the commercial feed with 25% SNS (p<0.05). On the contrary, 100% commercial feed exhibited highest concentrations of WBC, PCV, and PLT (p<0.05) in the experimental animals.

Table 7 Lipid profile analysis

Sample: Comm. Feed (%)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
0 : 100	132.27 ± 2.52d	224.77 ± 4.34b	33.49 ± 1.31a	53.83 ± 0.34d
25 : 75	122.85 ± 4.40c	219.34 ± 6.06b	34.53 ± 1.30a	44.45 ± 1.89c
50 : 50	106.32 ± 2.52b	102.92 ± 2.02a	39.20 ± 0.78b	46.53 ± 2.14c
75 : 25	101.92 ± 3.98ab	99.50 ± 3.57a	41.47 ± 0.59c	40.56 ± 3.86b
100 : 0	96.48 ± 3.65a	93.92 ± 3.58a	43.10 ± 2.08c	34.60 ± 4.92a
SEM	6.72	30.24	1.89	3.19

Values are expressed as mean ± SEM (n=3). Values with different superscripts in the same column are significantly different at p < 0.05. SEM = standard error of mean.

There was no significant difference in the levels of TG and HDL of animals fed with 100% commercial feed, and those on 25% substitution ($p<0.05$). However, decreasing pattern of TC, TG, LDL, and in reverse, increasing HDL concentrations were observed as SNS substitution increased ($p<0.05$).

4. Discussion

Brine Shrimp larvae are considered as suitable samples for preliminary cytotoxicity assay in pharmacology [14-15].

Between 500 to 2000 µg/ml (Table 3) tannic acid and potassium dichromate were 20 to 30% more toxic respectively, to the brine shrimp larvae than with SNS extract. The reference samples had killed almost all the larvae at 2000 µg/ml. The result indicated that SNS sample was less toxic by 83% compared with tannic acid of similar organic origin used in this study. The observation was supported by the LC_{50} of 274 - 300 µg/ml for the reference samples, and 549 µg/ml SNS.

The percentage weight difference of the experimental animals in Table 4 showed that there was positive but decrease in weight gained as SNS substitution in diet increased from 25% to 100% ($P < 0.05$). However, none of the substitution levels had negative effect on weight gain which contrasts with observation from our previous similar design using 100% diet of defatted *Mangifera indica,* or *Irvingia* kernel powder [16].

Incidentally, the initial weights of the animals in this study had direct relationship with the increasing levels of substitution of SNS in the diets, which implied that age of animal and palatability were confounding factors on the percentage weight differences observed at the end of the 21-day feeding. Nevertheless, it was deduced that experimental animal with a mean weight of 115 g would have consumed SNS alone to an average weight gain of 53% relative to a diet of commercial feed alone. Furthermore, such animal would need about 40 to 43 days to attain maximum weight gain with 100% SNS diet.

The common enzymes used as indicators of hepatocellular damage or necrosis are the transaminases, particularly Aspartate Transaminase (AST) and Alanine Transaminase (ALT). Damage to the liver tissue leads to increase in serum or plasma levels of these enzymes, which also correlates with loss of functional integrity of the cellular membranes [17]. Both enzymes are quantified by similar colorimetric technique but ALT is more liver specific, and the AST/ALT ratio of greater than 1.5 is indicative of severe damage [18].

Table 5 showed that AST concentration significantly decreased with SNS substitutions, even at 25% level compared with the Control of 100% commercial feed ($p<0.05$). The decrease, however, was insignificant between the substitution levels, indicating that SNS had no adverse effect on muscle or liver tissues of the experimental animals. On the contrary, ALT levels of Control and the 25% substitution were not significantly different ($p>0.5$) but were significantly higher than those of 50, 75 and 100% SNS substitutions. Significant fluctuations were observed with the ALT concentration among these substitutions ($p<0.05$), and such ALT variations even in 24-h life-cycle of animals were reported as normal [18].

The results further showed significant variations in the ALT/AST ratios of animals fed with substituted SNS feeds ($p<0.05$) that ranged lower than 1.17 observed with the animals fed on 100% commercial feed, indicating that SNS substitution in the diets had no adverse effect on the liver tissue. Hence, its consumption could help in maintaining the integrity of liver cells and protect against non-alcoholic steatohepatitis (NASH) or hepatic necrosis such as cirrhosis, toxic or viral hepatitis, carcinoma and obstructive jaundice [19].

Table 6 showed that different levels of SNS substitution had different significant effects on the parameters analysed ($P<0.5$). However, some levels showed similarity with specific parameters of the Control, that is, the Commercial feed; examples include 75% substitution for WBC, 50% for Hb, and 25% for MCV. Furthermore, the Hb, MCH, MCHC, and MCV of both the 75 and 100% SNS diets were similar ($P>0.5$). These observations implied that SNS diets especially at high concentrations could maintain cellular integrity and osmolarity, as well as the capability to boost WBC production in case of tissue damage resulting from immune infections. The inverse mathematical relationship in the formula, MCHC= Haemoglobin concentration X 100/PCV [20] was in agreement with the results obtained. Furthermore, normal ranges of WBC and PCV as defined by Sood [21] were also obtained in this study, to affirm the positive effect of SNS-based diets in nutrition.

On the contrary, the PLT concentration increased proportionally with SNS substitution, but were significantly lower than that of the Commercial feed alone ($P<0.5$). Basal PLT concentration of 150,000 had been described as normal [21]. The results obtained, therefore, indicated that the animals purchased for the study had symptom of thrombocytopenia, which was reversed by only the 75% SNS-substituted diet and 100% Commercial feed in the period of study.

From Table 7, it was observed that TC and LDL decreased with increasing SNS sample substitution, and inversely correlated with HDL. TG concentration using the 100% Commercial feed decreased significantly ($P < 0.05$) on 25% SNS substitution but subsequent decrease from 50 to 100% SNS substitutions showed no significant difference ($P>0.05$). In similar vein, the observed increase in HDL concentration with SNS substitution was not significantly different between 75 and 100% substitutions.

Furthermore, less than 150 mg/dl of TG was classified as 'desirable', and 150 – 500 mg/dl as 'borderline high' [22]. It implied that diets of 50 to 100% SNS substitution gave desirable TG content of 94 – 103 mg/dl. The concomitant decrease in total cholesterol and LDL concentrations with the SNS substitutions compared with the 100% Commercial feed was a reflection of the more superior lipid composition of SNS product, in respect of its phytosterol and fatty acid profiles. Findings from our independent study indicated that SNS had a high ratio 7.6 : 1.0 of unsaturated : saturated fatty acids, PUFA/SFA of 3.22 against expected $\geq$ 0.4, and $\omega6/\omega3$ of 0.71 against expected $\leq$ 4.0 in conformance with FAO/WHO [23]. This study, therefore, has shown that 100% SNS or 75% SNS when complemented with micronutrients, was nutritionally acceptable; a product recommendable to human subjects with symptoms of malnutrition.

5. Conclusion

SNS sample extract had LC_{50} of 550 and was comparatively less toxic than tannic acid by 83%. *In vitro* and *in vivo* analyses of SNS-based diets, relative to a 100% commercial feed, had no adverse effect on tissue integrity of particularly the liver. It was nutritionally acceptable to the experimental animals employed, and potentially capable of reversing thrombocytopenia. Hence, 100% SNS or 75% substitution in diets complemented with micronutrients is recommended as nutraceutical for human subjects with symptoms of malnutrition.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Author's contributions

Akpala envisioned the study and participated in the conduct of the experiments with the co-authors (Amlabu, Amodu, and Arogba). In addition, Arogba designed the methodologies, supervised the experimentation, analysed the data, and scripted the article.

References

[1] Itagi HBN and Singh V. (2012). Preparation, Nutritional composition, Functional Properties and Antioxidant Activities of Multigrain composite Mixes. *Journal of Food Science and Technology,* 49(1), 74 – 81.

[2] Ezeokeke CT and Onuoha AB. (2016). Nutrient Composition of Cereal (Maize), Legume (Soybeans) and Fruit (Banana) as a Complementary food for Older Infants and their sensory Assessment. *Journal of Food Science and Engineering,* 6, 139 – 148.

[3] Alamu EO, Popoola I and Maziya-Doxin B. (2018). Effects of Soya bean (Glycine max L.) Flour inclusion on the Nutritional Properties and Consumer Fritters for Improved Household nutrition. *Food Science and Nutrition,* 6(7), 1811 – 1816.

[4] Messina MJ. (1999). Legumes and Soyabeans: Overview of their Nutritional Profile and Health Effects. *American Journal of Clinical Nutrition,* 70(3), 439 – 450.

[5] Ukwo PS, Ntukidem VE and Udoh IE. (2019). Effect of Roasting on proximate composition and anti-nutritional content of skinned and unskinned roasted groundnut (A*rachis hypogea*) varieties in Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology,* 13(5), 59-64.

[6] Awadalkareem AM, Mustafa AI and El Tinay AH. (2008). Protein, Mineral Content and Amino Acid Profile of Sorghum Flour as Influenced by Soybean Protein Concentrate Supplementation. *Pakistan Journal of Nutrition,* 7(3), 475 – 479.

[7] Shah TR, Prasad K and Kuman P. (2016). Maize, a potential Source of Human nutrition and Health: A Review. *Cogent Food and Agriculture,* 2(1).

[8] Ndife J, Abdulraheem LO and Zakari UM. (2011). Evaluation of the Nutritional and Sensory Quality of Functional breads Produced from Whole Wheat and soya bean flour Blends. *African Journal of Food Science,* 5(8), 466 – 472.

[9] Ajanaku KO, CO Ajanaku, A Edobor and Nwinyi OC. (2012). Nutritive value of Sorghum Ogi fortified with Groundnut seed (*Arachis hypogaea* L.). *American Journal of Food Technology,* 7(2), 82 – 88.

[10] IITA. (1990). *Removal of Anti-nutritional Husk from Flour Preparation*. Bulletin of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

[11] Arogba SS and Matanmisi JA. (2014). Proximate Biological Value and Protein Efficiency Ratio of processed Bush mango (*Irvingia gabonensis*) kernel. *Journal of Pharmacy and Biological Sciences,* 2(9), 18 – 20.

[12] Reitman S and Frankel S. (1957). A colourimetric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvic transaminase. *American Journal Clinical Pathology,* 28, 56-61.

[13] Baker FJ and Silverton E. (1985). Introduction to Medical Laboratory Technology, Butterworth and Company Ltd, (6th ed.) 107 - 115.

[14] Quazi SS, Fatema CA and Mir M. (2017). Brine Shrimp Bioassay. *Bangladesh Journal of Pharmacology,* 12, 186 – 189.

[15] Pimentel Montanher AB, Pizzolatti MG and Costa Brighente IM. (2002). An Application of Brine Shrimp Bioassay for General Screening of Brazilian Medicinal Plants. *Acta Farm. Bonaerense,* 21(3), 175 – 800. ISSN 0326-2383.

[16] Arogba SS and Omada AA. (2020). Effects of diet substitution with defatted kernels of mango (*Mangifera indica*) and wild mango varieties(*Irvingia gabonensis* and *Irvingia wombolu*) on weight and plasma lipid profile of Wistar rats. *African Journal of Food Science,* 14(1), 10-15.

[17] Ekan VS and Ebong PE. (2007). Serum Protein and Enzyme Levels in Rats Following Administration of Antioxidant Vitamins during Caffeinated and Non-Caffeinated Paracetamol Induced Hepatotoxicity. *Nigerian Journal of Physiological Sciences,* 22(1-2), 65 – 68.

[18] Hall P and Cash J. (2012). What is the real function of Liver 'Function' Test? *Ulster Medical Journal,* 81(1), 30 – 36.

[19] Neuschwander-Tetri BA. (2012). Fatty liver and non-alcoholic steatohepatitis. In: Friedman LS and Keeffe EB (Ed.), Handbook of Liver Disease, 3rd Edn., Elsevier. ISBN 978-1-4377-1725-9.

[20] Khan Z, Nawaz M, Khan A and Bacha U. (2013). Haemoglobin, Red Blood Cell count, Haematocrit and Derived Parameters for Diagnosing Anaemia in Elderly Males. *Proceedings of the Pakistan Academy of Sciences,* 50 (3), 217- 226.

[21] Sood R. (2003). Haematology for Students and Practitioners, 5th Ed., Jaypee Brothers Medical Publishers Ltd., New Delhi, India.

[22] Ma H and Shieh K. (2006). Cholesterol and Human Health. *Journal of American Science,* 2(1), 46 – 50.

[23] Nile SH and Park SW. (2013). Fatty acid composition and antioxidant activity of groundnut (*Arachis hypogaea* L.) products. *Food Science and Technology Research,* 19(6), 957 – 962.

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