



Non-Heme Iron Content and Antioxidant Activities of Wistar Rats Fed Aqueous Extract of *Elaeis guineensis* (Banga Soup) Cooked Using Different Utensils

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ABSTRACT: Cooking utensils may leach toxic metals or trace elements into food. Iron is an essential nutrient and both iron deficiency and iron excess can affect optimal health. The aim of the study was to determine non-heme iron (NHI) and some antioxidant parameters of rats fed banga soup (BS) prepared using different utensils. Twenty-five Wistar albino rats were used for the study. They were allowed to acclimatize for one week before commencement of the experiment. Group 1 served as control. Rats in Group 2, 3, 4 and 5 were administered BS cooked using cast iron pot (CIP), aluminium pot (AP), blended mixture (BM) of BS and aqueous tween 80 respectively. Rats in Group 1 to 5 received tap water daily and standard laboratory diet (feed) throughout the experiment period of 28 days. There were no significant difference in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, albumin (Alb) and total protein (TP) in the serum and liver of the entire experimental Groups. Significant decrease were observed in total phenol content (TPC), total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP) and ascorbic oxidase (AO) activity in the serum and NHI content in the serum, liver and kidney of Group 2, 3 and 4 when compared with Group 1 and 5.

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Banga soup is prepared from palm fruits (*Elaeis guineensis* extract) together with spices. These includes; dried beletete leaves (*Heinsia crinita*), ataiko seeds (*Aframomum sceptrum*), fresh obenetietien leaves (*Ocimum basilicum*). The soup is consume by the people of the Niger Delta parts of Nigeria, particularly the Urhobo, Ukwuani, Izon and Itsekiri ethnic group of Delta State. Spices and herbs are known to possess a variety of antioxidant properties due to their phenol content (George *et al.*, 2011, 2012, 2013, 2015, 2019; George and Okpoghono, 2017; Okpoghono *et al.*, 2018 a, b, c; Onakurhefe *et al.*, 2020). Metal ions such as iron, aluminium and copper are likely inducers of oxidative processes that may modify electron-rich micronutrients such as phenols and carotenoids (Perron and Brumaghim, 2009). Iron is a mineral nutrient which is need for the synthesis of haemoglobin and enzymes such as heme enzymes (Oluwatoyin and Adebukola, 2018; Abbaspour *et al.*, 2014). However, study has reported that polyphenol content in food inhibits NHI bioavailability (Oluwatoyin and Adebukola, 2018). Iron deficiency causes oxidative stress and increased tissue malondialdehyde (MDA) level; a product of lipid peroxidation. Numerous antioxidants such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) maintain the cell's redox state and prevent the harmful effects of oxidative stress. In iron deficiency states, the balance of

antioxidant may be tilted to free radicals triggering oxidative stress (Tekin *et al.*, 2001). Therefore, the objective of this study is to evaluate NHI content and antioxidant activities of Wistar rats fed aqueous extract of *E. guineensis* (banga soup) cooked using different utensils.

MATERIALS AND METHODS

Soup Ingredients: The banga soup ingredients were purchased in Ughelli main market, Delta State. The ingredients were measured as described in Table 1.

Table 1: Ingredients used in preparing banga soup

Ingredients	Weight
Palm oil fruit	500 g
Fresh catfish	300 g
Ground ataiko (<i>Aframomum sceptrum</i>)	1 tablespoon
Obeletientien (<i>Ocimum basilicum</i>)	1 tablespoon
Onion bulb (<i>Allium cepa</i>) finely chopped	10 g
Ground crayfish	1 tablespoon
Maggi	2 cubes
Chili pepper powder (<i>Capsicum frutescens</i>)	1 tablespoon
Salt	2 tablespoon

Banga Soup Preparation: The palm kernel fruit was boiled (120 – 140 °C for 30 minutes) and then extracted with water. About 1.2 L of the extract was allowed to boil in AP and CIP until it thickened and oil raise to the top. Ingredients (chopped onions,

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pepper powder, ground crayfish, atiako and maggi cube) were added, and then allowed to cook for 8 minutes. Thereafter, fresh fish was added and allowed to cook. Then, obeletientien leaves and salt were added and simmered for 1 minute and the soup was ready.

Preparation of Banga Soup Extract: The BS was evaporated to dryness in an electric oven (40 °C). Fifty grams of the dried sample was homogenized in 450 ml aqueous tween 80 (5 % Tween 80) to dissolve the oil present in the soup, and then filtered with clean muslin cloth. The concentrations of the BS extracts were calculated to be 0.1 g/ml. The extracts were administered to the experimental rats at dose of 400 mg/kg b. wt. Rats in group 5 were given 1 ml/kg b. wt. of 5 % tween 80.

Toxicity Study of Banga Soup: The rats were divided into seven groups of four per group. Banga soup (BS) was administered separately to all the four rats in each group, starting with 100 mg/kg b. wt. The rats were observed for 1-3 hours to check behavioural signs, if any toxic sign and mortality. If no mortality was observed at this dose, the same procedure was repeated for dose level of 200 mg/kg b. wt, 400 mg/kg b. wt, 800 mg/kg b. wt, 1600 mg/kg b. wt and 3200 mg/kg b. wt. The protective dosage was determined, which was use for the study. The administration of the BS was observed up to 7 days.

Growers Mash Feed Composition: The growers mash feed was supplied by Grand Cereals Limited (GCL) Lagos, Nigeria. The ingredients compositions are illustrated in Table 2.

Ingredients	Quantity (kg/100kg)
Maize	45
Soya Meal	7.5
Wheat Offal	12
Limestone	5
Bone Meal	2.5
Palm Kernel Cake	12
Groundnut Cake	15
Premix	0.25
Toxin Binder	0.15
Salt	0.3
Super Liv	0.05
Methionine	0.15
Lysine	0.1
Total	100 kg

Experimental Animals: Twenty-five (25) rats were allowed to acclimatize for one week before

commencement of the experiment, and then place into five groups with five rats in each group. Rats in group 1 to 5 received tap water daily and feed throughout the experiment period of 28 days. Weights of the rats in each group were recorded at the end of every 7 days.

The feeding protocol of the experiment was as follows: m Group 1: Standard laboratory feed (SLF) only (Control); Group 2: SLF + BS cooked in CIP and extracted with 5 % Tween 80; Group 3: SLF + BS cooked in AP and extracted with 5 % Tween 80; Group 4: SLF + blended mixture (BS cooked in CIP and AP) and extracted with 5 % Tween 80; Group 5: SLF + 5 % Tween 80.

Preparation of Serum and Homogenate: The rats were sacrificed on the 29 day after an overnight fast for standardization of parameters to be measured (Jensen *et al.*, 2013). Blood was collected from the heart using syringe and needle and then allowed to clot in anticoagulant free test tube. Thereafter, the tissues (liver and kidney) were removed. About zero point five grams of the wet tissues were homogenized in 4.5 ml of normal saline. The clotted blood and tissues homogenate were centrifuged at 2,500 RPM for 15 minutes to separate the serum and supernatant which was used for biochemical analyses immediately.

Estimation of Liver Function Markers: The activities of ALT, AST and ALP in the serum and liver were assayed colorimetrically according to standard procedures using commercially available diagnostic kits (Randox Laboratories Limited, England).

Assay of Enzymatic Antioxidants (EA): EA such as SOD, CAT and AO activities were assayed.

Assay for SOD Activity: The method stated by Misra and Fridovich (1972) was used for the assay. Superoxide dismutase exhibits autoxidation of epinephrine by superoxide radical (O_2^-). The SOD assay is an indirect method that based on the inhibitory effect of SOD in the initial rate of epinephrine autoxidation that was determined at 480 nm.

Assay for CAT Activity: The method of Cohen *et al.* (1970) was adopted for the assay of CAT activity. Catalase breaks down H_2O_2 directly into water and oxygen. The decrease in H_2O_2 concentration was determined spectrophotometrically at 360 nm for 70 seconds. The disappearance of hydrogen peroxide was calculated using the molar extinction coefficient, $\epsilon = 34.9 \text{ mol}^{-1} \text{ cm}^{-1}$.

Assay for AO Activity: The activity of AO was determined by following the decrease in absorbance at 600 nm, consequent upon the oxidation of

benzaldehyde to benzoate using 2, 6-dichloroindophenol (DCIP) as the electron donor (Vines and Oberbacher, 1965).

Determination of Non-enzymatic Antioxidants (NEA): Some NEA such as GSH, TPC, TAC, FRAP, Alb and TP in the serum and liver were determined. Albumin and TP levels were estimated using the manual procedure supplied by Randox Laboratories diagnostic kits Limited, England.

Determination of GSH: The GSH levels of samples were estimated using the method of Ellman (1959). The sulphhydryl group of GSH reacted with DTNB (5,5 - dithiobisnitro benzoic acid, Elman's reagent) and produce a yellow colour, 5 - thio - 2 - nitrobenzoic acid (TNB). The rate of TNB produced is directly proportional to the concentration of GSH in the sample. The colour developed was read at 412 nm.

Determination of TPC: TPC was estimated using Folin-Ciocalteu reagent according to the method of Liu and Yao (1997). TPC results were expressed as mg of gallic acid equivalent.

Determination of TAC: The TAC of samples was evaluated by the method of Prieto *et al.* (1999). The assay is based on the formation of green phosphate/Mo (V) complex at acid pH on the reduction of Mo (VI) to Mo (V). The TAC was read at 765 nm and results were expressed as tannic acid equivalents.

Estimation of FRAP: The level of FRAP in the samples were estimated according to the method described by Oyaizu (1986). The reaction principle is based on the reduction of iron III (Fe^{3+}) to iron II (Fe^{2+}) by measuring the absorbance of Perl's Prussian blue complex at 700 nm.

Determination of NHI Content: The content of NHI was measured according to the method described by Ahn *et al.* (1993). Iron is liberated from transferrin under acidic conditions. Ascorbate reduces the conversion of Fe^{3+} to Fe^{2+} which then reacts with Ferrozine to form a coloured complex. The absorbance was measured at 562 nm. The NHI content was obtained from standard curve prepared using 1000 mg/L stock solution of $FeCl_3$ that was adjusted at 10, 25, 50, and 100 mg/L.

Determination of Lipid Peroxidation (LPO): Buege and Aust (1978) method was used for the analysis of LPO in form of MDA. Thiobarbituric acid (TBA) measure LPO after reaction with the sample. Absorbance was monitored at 532 nm. MDA in $\mu g/g$

of protein was calculated with a molar extinction coefficient of 1.56×10^5 .

Histopathology Analysis: The liver histology was carried out according to method of Drury and Wallington (1967). The tissues were allowed to fix in 10 % formylsaline for 48 hours. The tissues were cut into pieces of about 3 mm thick in pre-labelled tissue cassette. Serial sections of 5 micrometre thick were obtained from a solid block of tissue and stained using haematoxylin and eosin stains. After clearance, the tissues were oven-dried. Photomicrographs of tissues slides were taken with the aid of JVC coloured digital camera which was mounted on Olympus light microscope (Olympus UK Limited, United Kingdom).

Statistical Analysis: The data were expressed as mean \pm SD and mean bars. Statistical analyses were undertaken using the One-way ANOVA followed by LSD test. Threshold of $p < 0.05$ was accepted statistically significant. Statistical analysis was performed using SPSS for Windows version 22.0, Chicago, IL, US.

RESULTS AND DISCUSSION

A kitchen utensil is a hand held tool that is used for preparation of food. Tiny amounts of iron and aluminium can leach out of cast iron and aluminium utensils into food. The leached iron is considered a beneficial source of this nutrient (Jain, 2018). This study aimed to determine the liver function markers, some antioxidant parameters and NHI content of rats fed BS cooked using AP and CIP. Table 3 illustrated the LD₅₀ of BS in rats. No mortality was observed in rats fed BS extract from 100 - 3200 mg/kg b. wt. The LD₅₀ was greater than 3200 mg/kg b. wt. and hence has high degree of safety. However, 400 mg/kg b. wt. was chosen for the protective animal study due to the positive behavioural signs such as physical activeness and growth of new hair of the rats given this dose.

Table 3: LD₅₀ of Banga soup

Dose (mg/kg)	Oral death/survive	Behavioural signs
Control	0/4	No strange sign
100	0/4	No strange sign
200	0/4	No strange sign
400	0/4	Very active, new hair growth
800	0/4	Not active, decrease in locomotion
1600	0/4	Not active, decrease in locomotion
3200	0/4	Not active, decrease in locomotion

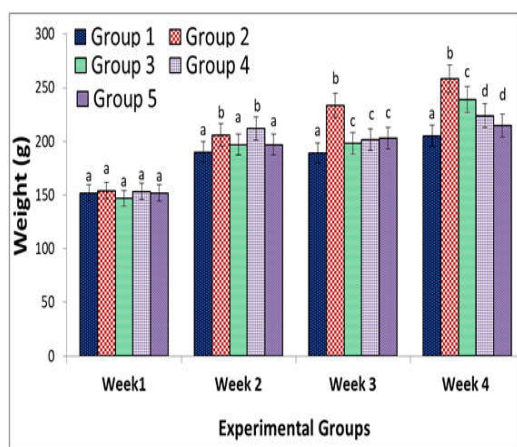


Fig. 1: Body weight of rats fed BS prepared using CIP and AP at the end of every week. Values were presented in mean bars (n=5). Bars with different superscript letter in the same column differ significantly at p < 0.05.

Changes in body weight of the experimental rats during the 28 days are presented in Fig. 1. At the end of the first week, no significant differences were observed in body weight of all the experimental Groups. However, at the second week significant (p < 0.05) increases in the body weight of Group 2 and 4 were observed when compared with Group 1 and 5. Group 2, 3 and 5 had significant increased body weight

in comparison to Group 1 at the third week. Also, at the fourth week, significant increase were indicated in body weight of Group 2, 3, 4 and 5 when compared with Group 1. The observed increase in body weight at the end of the experiment may be due to the feed and BS extracts composition efficiency. Food efficiency may contribute to increased body weight of experimental rats (Hamed *et al.*, 2010).

Changes in liver function markers of rats fed cooked BS extract using CIP and AP are illustrated in Table 4. There were no significant difference in AST, ALT and ALP activities in the serum and liver of the entire experimental Groups. The bulk of food consumed by humans is too less to provide adequate amounts of iron due polyphenols inhibitory effect on liver iron metabolism (Jain, 2018; Perron and Brumaghim, 2009). Interestingly, BS is rich with antioxidant such as polyphenols. The polyphenol content in the BS cooked using AP and CIP may chelate the NHI in the soup. However, the slight increase observed in studied liver function markers enzymes activities were in line with the study of Oloyede *et al.* (1992), who reported that iron deficiency affects enzymes in rat tissue. Iron disorder or imbalance is on one of the important cause of liver damage and this may affect liver function markers activities.

Table 4: Liver function markers of rats fed prepared BS using CIP and AP

Group	Serum AST (U/L)	Serum ALT (U/L)	Serum ALP (U/L)	Liver AST (U/L)	Liver ALT (U/L)	Liver ALP (U/L)
Group 1	40.20 ± 5.77 ^a	31.18 ± 4.51 ^a	205.43 ± 17.40 ^a	53.40 ± 20.57 ^a	42.88 ± 11.16 ^a	220.43 ± 8.33 ^a
Group 2	42.26 ± 4.58 ^a	33.10 ± 9.39 ^a	208.77 ± 17.44 ^a	56.20 ± 11.48 ^a	45.22 ± 4.83 ^a	223.40 ± 9.02 ^a
Group 3	41.26 ± 10.67 ^a	34.10 ± 4.39 ^a	207.59 ± 8.53 ^a	57.11 ± 19.43 ^a	45.10 ± 18.68 ^a	224.66 ± 7.30 ^a
Group 4	42.00 ± 12.74 ^a	33.00 ± 8.52 ^a	207.47 ± 18.68 ^a	58.10 ± 5.15 ^a	46.32 ± 22.85 ^a	223.50 ± 41.12 ^a
Group 5	40.10 ± 7.64 ^a	31.10 ± 4.53 ^a	206.56 ± 19.59 ^a	53.14 ± 2.73 ^a	43.10 ± 19.76 ^a	221.30 ± 9.66 ^a

Values were given in mean ± SD. (n=5). Values with different superscript letter in the same column differ significantly at p < 0.05.

Table 6: NEA levels in the serum and liver of rats fed BS prepared using CIP and AP

Group	Serum TPC (units/ml)	Serum TAC (units/ml)	Serum FRAP (units/ml)	Serum Alb (g/dl)	Serum TP (g/dl)	Liver Alb (g/dl)	Liver TP (g/dl)	Liver GSH (units/g wet tissue)
Group 1	30.61 ± 8.05 ^a	500.16 ± 79.59 ^a	201.42 ± 32.85 ^a	4.39 ± 1.67 ^a	7.53 ± 3.62 ^a	12.59 ± 2.47 ^a	20.33 ± 1.25 ^a	13.14 ± 4.60 ^a
Group 2	21.48 ± 3.06 ^b	396.45 ± 13.17 ^b	195.88 ± 35.70 ^b	3.37 ± 1.78 ^a	6.30 ± 1.10 ^a	8.34 ± 0.47 ^a	17.38 ± 6.89 ^a	8.44 ± 3.26 ^{a,b}
Group 3	20.41 ± 8.49 ^b	390.52 ± 34.43 ^b	190.53 ± 7.97 ^b	2.35 ± 0.88 ^a	6.49 ± 1.70 ^a	8.51 ± 3.15 ^a	17.45 ± 1.68 ^a	6.56 ± 2.20 ^b
Group 4	18.50 ± 7.33 ^b	392.33 ± 14.31 ^b	188.40 ± 17.04 ^b	3.20 ± 1.67 ^a	4.32 ± 1.64 ^a	9.55 ± 0.80 ^a	16.11 ± 3.94 ^a	5.34 ± 0.78 ^b
Group 5	31.57 ± 5.46 ^a	498.60 ± 34.83 ^a	202.56 ± 21.48 ^a	4.61 ± 2.42 ^a	7.70 ± 3.84 ^a	12.56 ± 1.67 ^a	20.26 ± 4.44 ^a	13.39 ± 2.44 ^a

Values were presented in mean ± SD. (n=5). Values with different superscript letter (a, b) in the same column differ significantly at p < 0.05.

Changes in EA activity in the liver and serum of rats fed BS prepared using CIP and AP are presented in Table 5. There were no significant (p < 0.05) differences in SOD activity in the liver of all the experimental Groups. Significant decrease were observed in CAT activity in the liver of Group 3 and 4 when compared with Group 1 and Group 5. The serum

AO of Group 2, 3 and 4 were significantly higher when compared to Group 1. The NEA levels in the serum and liver of rats given BS cooked using CIP and AP are shown in Table 6. Significant (p < 0.05) decrease were observed in liver GSH, TPC, TAC and FRAP in the serum of Group 2, 3 and 4 in comparison to Group 1 and 5. Fig. 2 illustrated the liver MDA level of rats

fed BS cooked using different pots. Significant ($p < 0.05$) increased MDA level in the liver was observed in Group 3 and 4 when compared with Group 1 and Group 5. There was no significant difference in liver MDA level of Group 1 when compared with Group 5. The observed decreased in liver EA, NEA and increased in liver MDA of rats fed BS cooked in CIP and AP could be due to functional defectiveness of the antioxidant molecules, and the balance tends to be tilted towards free radicals generation. This is in concordance with the work of Tekin *et al.* (2001), who stated that iron deficiency not only affects the production of haemoglobin but also of other iron containing proteins, viz. cytochromes, myoglobin, catalase, peroxidase etc. Since, in iron deficiency states enzymes of the antioxidant defense system are functionally defective, this may triggers oxidative damage and lipid peroxidation (Tekin *et al.*, 2001).

Table 5: Changes in EA activity in the liver and serum of rats fed BS prepared using CIP and AP

Group	Liver SOD (units/g wet tissue)	Liver CAT (units/g wet tissue)	Serum AO (units/ml)
Group 1	38.22±6.46 ^a	30.86±14.57 ^a	27.61±3.60 ^a
Group 2	36.31±10.46 ^a	27.64±1.25 ^a	17.44±3.37 ^b
Group 3	34.43±7.17 ^a	24.76±3.44 ^{a,b}	16.80±3.00 ^b
Group 4	33.52±7.66 ^a	23.38±2.53 ^{a,b}	14.43±2.58 ^b
Group 5	38.43±1.99 ^a	30.42±15.95 ^a	26.52±4.89 ^a

Values were presented in mean ± standard deviation. (n=5). Values with different superscript letter (a,b) in the same column differ significantly at $p < 0.05$.

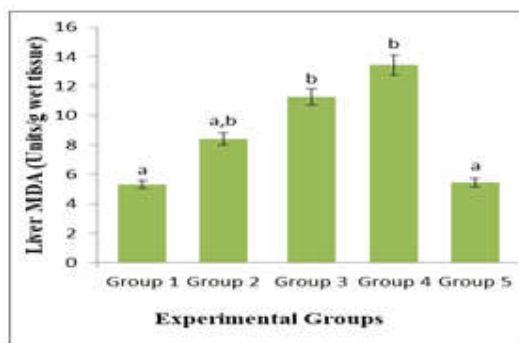


Fig. 2: Liver MDA level of rats fed BS prepared using different pots. Values were presented in mean bars (n=5). Bars with different superscript alphabet (a,b) differ significantly at $p < 0.05$.

However, the NEA (Alb and TP level) in the serum and liver were not significant in the entire experimental Groups (Table 6). This may be an indicative sign that the rats are not malnourished. The fresh fish (*Clarias gariepinus*) and crayfish used in the cooked BS may be a good source of protein. However, increase or decrease in Alb and TP levels are influenced by intake of protein, protein digestion, absorption of adequate or inadequate amount and

disease condition (Oloyede *et al.*, 1992). The NHI content in the serum and tissues of rats fed BS samples prepared using CIP and AP are shown in Table 7. Group 2, 3 and 4 had significant decrease in NHI content in the serum, liver and kidney when compared with the Group 1 and 5. The significant decrease in NHI content in the serum and tissues of rats fed BS cooked using CIP and AP could be directed to BS polyphenols inhibitory effect of NHI availability. The soups were prepared with spices such as oboletientien fresh leaves (*O. basilicum*), dried ground ataiko seeds (*A. sceptrum*) which are rich in phenolic compounds (George *et al.*, 2011, 2012, 2013, 2015, 2019; George and Okpoghono, 2017; Okpoghono *et al.*, 2018 a, b, c, d; Tonukari *et al.*, 2013). Ferroportin (FPN) is the single known iron exporter that facilitates efflux of iron to the circulation through the basolateral membrane of the enterocyte together with the coordinated action of a ferrioxidasase (Muckenthaler *et al.*, 2008). The polyphenol could firstly chemically reduce NHI and thus increase apical uptake followed by formation of the polyphenol-Fe complex inside the cell *in vivo*. Thus lower iron levels and such that when iron levels in tissues are reduced, FPN expression is decreased (Muckenthaler *et al.*, 2008). However, the decrease in NHI were more pronounced in the rats fed BS cooked in AP followed by blended mixture (BM) before CIP, but the NHI mean values were not significant. The results could portraint a clear view that, in iron deficiency (especially iron deficiency anaemic patient) CIP may be of good source of iron and should be used in cooking instead of AP.

Table 7: NHI content in the serum, liver and kidney of rats fed BS samples prepared in CIP and AP

Group	Serum NHI (µg/ml)	Liver NHI (µg/g tissue)	Kidney NHI (µg/g tissue)
Group 1	23.24±4.47 ^b	26.40±4.21 ^b	24.36±4.43 ^b
Group 2	10.30±2.41 ^a	13.30±3.54 ^a	11.30±2.56 ^a
Group 3	11.20±3.29 ^a	12.20±3.43 ^a	9.44±3.69 ^a
Group 4	7.24±2.72 ^a	10.12±3.21 ^a	7.52±3.16 ^a
Group 5	24.16±4.60 ^b	27.18±6.56 ^b	22.20±8.46 ^b

Values were presented in mean ± SD (n=5). Mean with different superscript letter in the same column differ significantly at $p < 0.05$.

The microscopic liver histology of rats fed BS prepared using CIP, AP and blended mixture are presented in Fig. 3. Histological structure of the liver showed mild sinusoidal congestion in rats given BS prepared in CIP and AP. In some cases iron deficiency are mild and do not results in symptoms which are recognized as requiring medical attention (Oloyede *et al.*, 1992). However, no hepatic damage was observed in liver histology of rats given tween 80 when compared to the rats given SLF feed only. This may prove the nontoxic effect of tween 80, this is in accordance with previous investigation of Okpoghono

et al. (2018a), who reported that, tween 80 may be regard as a stabilizing solvent, and it is not toxic.

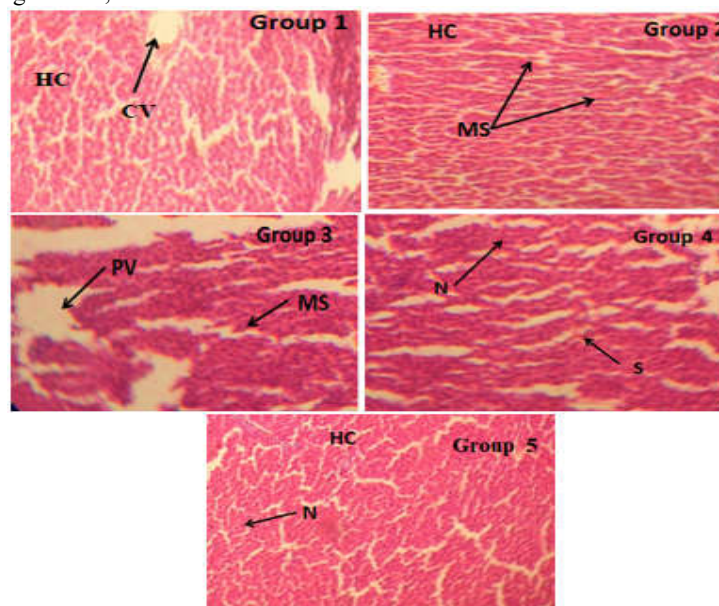


Fig.3. Microscopic representation of rats liver section fed BS prepared using CIP and AP.

Group 1: normal control, showing normal histological structure of hepatic cell (HC) and central vein (CV). *Group 2:* rat fed BS prepared using CIP, showing hepatic cell (HC) and mild sinusoidal (MS) congestion. *Group 3:* rat fed BS cooked using AP, showing portal vein (PV) and MS. *Group 4:* rat fed blended BS cooked using AP and CIP, showing normal liver architecture. The sinusoids (S) and nuclei (N) also appear to be more prominent.

Conclusion: In conclusion, the study indicated that BS cooked using AP may increase the AI content of the soup, which could be toxic resulting in a decrease in antioxidant properties. Decrease NHI in the tissues of rats fed BS cooked in AP and CIP may be due to the inhibitory property of polyphenols in the BS. However, this effect was more pronounced in BS cooked in AP. It is recommended that BS should be made available with meat or supplemented with vitamin C, that may likely keep free iron in the Fe^{2+} form.

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