



Levels of Acute Blood Indices Disarrangement and Organ Weights of Wistar Rats Fed with Flavour Enhancer- and Contraceptive-Containing Diets

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ABSTRACT

Objectives: The present study ascertained levels of blood indices disarrangement and alterations in organ weights of Wistar rats following short-term feeding with flavour enhancer- and contraceptive-containing diets. **Materials and Methods:** The rats were categorized based on hourly feeding duration and type of diet received for 33 days. At the end of the experimental feeding period, the rat groups were weighed, after which blood samples were drawn for measurement of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities as well as plasma total protein concentration (PTPC), serum total bilirubin concentration (STBC) and serum lipid profile (SLP) using standard methods. The weights of the heart, kidney, liver and spleen were measured. **Results:** Enzyme assay showed mild adjustments in serum ALT and AST activities. PTPCs of the experimental rat groups were within relatively narrow range: Generally, STBCs of the experimental rat groups were less than 1.0 mg/dL and SLP patterns were altered. Alterations in organ-to-body weight ratios of the experimental rat groups were within narrow ranges, except that of liver-to-body weight ratio. **Conclusions:** Serum ALT and AST activities were diagnostic of moderately compromised hepatocellular integrity, which did not affect hepatic functionality and devoid of hyperbilirubinemia. Alterations in SLP patterns portrayed incidences of atherogenicity in majority of the experimental rat groups. Also, the test diets neither provoked hypertrophy nor atrophy of the heart, kidneys and spleen in the experimental rat groups. However, comparative evaluation of liver-to-body weight ratios of rats fed with contraceptive-containing diets showed evidence of hepatic hypertrophy, whereas those fed with flavour enhancer-containing diets showed evidence of hepatic atrophy.

KEY WORDS: Organ weight; blood; contraceptive; flavour enhancer; Wistar rats

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INTRODUCTION

Blood is a tissue composed of suspension of corpuscles and arrays of biomolecules dissolved in plasma that are, to a large extent, confined within the vascular system. Under normal physiologic conditions, the concentrations of blood components and metabolites may oscillate within narrow range, whereas their wide variations in plasma or serum denote incidence of pathologic conditions. For instance, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are non-functional plasma enzymes that originate from hepatic, cardiac, alveolar, renal and brain tissues. In diagnostic pathology, elevated ALT and AST activities in plasma are associated with hepatic dysfunction and skeletal/cardiac muscle necrosis, which are outcomes of hepatic injuries, infections and other parenchymal tissues infarction [1-8]. Hepatic dysfunction elicits impaired capacity of the hepatocytes to conjugate bilirubin molecules prior to their excretion with attendant hyperbilirubinemia. In human, plasma bilirubin concentration greater than 1.0 mg/dL is diagnostic of hyperbilirubinemia [2,9]. Also, because of the central role of the liver in mobilization and metabolism of lipoproteins and dietary lipids as well as the biosynthesis of plasma proteins, a compromised hepatic integrity and functionality engenders blood lipid profile

disarrangement and low circulating level of plasma proteins [10].

Hyperlipidemia describes raised levels of plasma lipid components above reference range as a result of primary genetic defect or secondary causes associated with diet, drugs, or underlying pathophysiologic disorders such as diabetes mellitus [11-13]. Serum lipid profile (SLP), which is a measure of proportionate blood lipid concentrations, has now become almost a routine test in clinical diagnosis [14]. Previous reports had noted that dietary components implicated in alteration of blood lipid profile exhibited corresponding propensity to provoke atherosclerosis with ensuing cardiovascular morbidity and mortality [15-19]. The capacity of a dietary component to provoke atherogenic outcome is defined by its atherogenic index [20,21]. Therefore, evaluation of blood lipid components concentrations serves as readily available and reliable diagnostic parameter for establishing dietary causes of macro-vascular diseases. Additionally, measurement of body and organ weights is a veritable method in clinical appraisal of nutritional status and toxicity outcomes in experimental animals [10,22]. Body and organ weight indices are easy to measure and the procedures are cost-effective but could suffer low accuracy and reproducibility

[23]. Alteration in organ/body weight ratio is diagnostic of tissue swellings, atrophy or hypertrophy [24].

Monosodium glutamate (MSG) is one of the main flavour enhancers widely used as ingredient in various food products in restaurants, household kitchens, and food processing and packaging industries. The average daily consumption of MSG in industrialized countries is estimated to be within the range of 0.3-4.0 g/day [25,26] due to wide variations in individual's taste preference and quantity of MSG in foods [27]. According to Walker and Lupien [28], LD₅₀ of MSG in rats is within the range of 15-18 g/kg body weight. Commercial MSG is produced by fermentation of starch, sugar, beet sugarcane or molasses [28] and sold in various trademark names in Nigeria and other countries in America, Asia and Europe [29-31]. MSG enhances appetite by improving taste stimulation and palatability of wide variety of processed foods. Although the Food and Drug Administration (FDA) of the United States approved the use of MSG as a flavor enhancer [32-34], its safety as a food additive remains a subject of controversy by nutritional toxicologist and allied scientists as well as international committees on food and drug regulation [33,35].

Chemically synthesized estrogen and progesterone or their derivatives are effective contraceptive agents that serve for convenient family planning for women and couples worldwide. The active principles of some steroidal oral contraceptive pills are listed elsewhere [36,37]. The estrogen receptor antagonists are also notable alternatives to steroidal contraceptives. For instance, Saheli™ contains centchroman (Hindustan Latex Limited) and clinically considered as a third generation selective estrogen receptor modulator (SERM) because it acts as estrogen receptors antagonist in reproductive tissues [37,38]. Prolonged usage of oral contraceptives as an option for birth control has been linked to several side effects and pathologic conditions [38-40], whereas their short-term secondary effects on normal physiology are not often mentioned in literatures. Accordingly, the present study ascertained levels of acute blood indices disarrangement and alteration in organ weights of Wistar rats following short-term feeding with flavour enhancer- and contraceptive-containing diets. Additionally, the effects of dosage of the active principles in the diets and pattern of feeding on blood homeostatic parameters and organ weights were considered for investigation.

MATERIALS AND METHODS

Collection of Flavour Enhancer and Contraceptive Tablets

Two brands of commercially available flavour enhancer, designated as FEa and FEB, were purchased from a grocery store in Eke Onunwa Main Market, Owerri Municipal Local Government Area, Owerri, Nigeria, whereas standard steroidal oral contraceptive (CON) tablets were purchased at Cympok Pharmacy, Owerri North Local Government

Area, Owerri, Nigeria. The separate tablets of FEa, FEB and CON were ground into fine powder using ceramic mortar and pestle. Finally, samples of FEa, FEB and CON were stored in sterile vials until used for compounding the rats' diets.

Compounding of Diets

Separate diets for feeding experiments were prepared by compounding specified quantities of FEa, FEB and CON with pelletized standard guinea feed (PSGF) (United Africa Company Nigeria Plc., Jos, Nigeria). The corresponding concentrations of active principles of FEa, FEB and CON in the compounded diets are summarized in Table 1.

Table 1: Descriptive compositions of flavour enhancer- and contraceptive-containing diets

Samples	Compositions/concentrations of active principle (s) [†]
FEa	MSG = 3.41 g/100 g feed sample
FEB	MSG = 0.39 g/100 g feed sample
CON	Ethinylestradiol = 0.03 mg/100 g feed sample Ethinisteron = 29.56 mg/100 g feed sample

[†] According to manufacturer's instructions.

Animal Handling

The present study was approved by the Ethical Committee on the use of animals for research, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria (Ethics Approval Number: ODVC/REN/990/15). The female albino (Wistar) rats were obtained from the Animal House of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. The rats were housed in well-ventilated metal cages and maintained at room temperatures of $28 \pm 2^\circ\text{C}$, 30-55% of relative humidity on a 12-h light/12-h dark cycle, with access to water and PSGF *ad libitum* for 2 weeks acclimatization period. Handling of the rats was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

Design of Animal Feed Experiment

A 90-day old female Wistar rats ($n = 42$) of average weight of 102 ± 2.1 g were divided into 7 groups of 6 rats each. The rats were categorized based on hourly feeding duration and type of diet received for 33 days. The rats were fasted for 6 h prior to commencement of feeding experiment.

- Group 1 (CTRL): Control group Wistar rats received PSGF + water *ad libitum*.
- Group 2 (FEa_{24h}): Wistar rats received FEa-containing diet for 24 h + water *ad libitum*.
- Group 3 (FEa_{6h}): Wistar rats received FEa-containing diet for 6 h regular intervals + water *ad libitum*.
- Group 4 (FEB_{24h}): Wistar rats received FEB-containing

diet for 24 h + water *ad libitum*.

- Group 5 (FEb_{6h}): Wister rats received FEB-containing diet for 6 h regular intervals + water *ad libitum*.
- Group 6 (CON_{24h}): Wister rats received CON-containing diet for 24 h + water *ad libitum*.
- Group 7 (CON_{6h}): Wister rats received CON-containing diet for 6 h regular intervals + water *ad libitum*.

At the end of the experimental feeding period, the 12 h post fasted rat groups [25] were weighed and subsequently sacrificed by cervical dislocation, after which blood samples were drawn by cardiac puncture for measurement of serum AST and ALT activities, as well as plasma total protein concentration (PTPC), serum total bilirubin concentration (STBC) and serum lipid profile (SLP). Furthermore, the rats were dissected to excise the heart, kidneys, liver and spleen for measurement of internal organ weights.

Preparation of Blood Samples

Blood samples were collected into Na₂EDTA anti-coagulant test tubes and sample bottles. Blood corpuscles were separated from plasma in the anti-coagulant test tubes using centrifugation methods at 1500x *g* for 10 minutes at 4°C [25]. Serum was separated from coagulated blood in the sample bottles using simple bench centrifuge. The plasma and serum samples were harvested by aspiration using Pasteur pipette and transferred into sample bottles. Accordingly, the plasma and serum samples were used for biochemical analyses.

Biochemical Analyses

Serum ALT/AST Activities

Measurement of serum ALT and AST activities were according to the standard methods of Reitman and Frankel, [41] using Bio Merux kits.

Plasma Total Protein Concentration

PTPC was measured using the Biuret method as described by Gornall *et al.*, [42].

Serum Total Bilirubin Concentration

STBC was measured using diazotized sulphanilic acid methods as described [43].

Serum Lipid Profile/Atherogenic Index

Measurement of SLP and evaluation of atherogenic index was according to the standard methods previously described [21].

Measurement of Feed Consumed and Alterations in Organ and Body Weights

Feed Consumed and Weight Gained

Measurement of quantity of feed consumed by the experimental rats groups and their corresponding weight

gained were according to the methods previously described [21].

Organ/Body Weight Ratio

The excised organs (heart, kidney, liver and spleen) of the experimental rats groups were washed with normal saline to remove blood, dried between blotting paper and then weighed. The organ-to-body weight ratios were evaluated according to formula adopted [10].

Statistical Analysis

The data collected were analyzed by the analysis of variance procedure while treatment means were separated by the least significance difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version, (2006).

RESULTS

An overview of Figure 1 showed that serum ALT activities were greater than serum AST activities in the experimental rats groups. Furthermore, serum ALT activity of CTRL = 116.0 ± 0.81 IU/L was lower than that of FEa_{24h} = 141.2 ± 0.61 IU/L, FEa_{6h} = 135.2 ± 0.58 IU/L, CON_{24h} = 130.0 ± 0.51 IU/L and CON_{6h} = 124.4 ± 0.60 IU/L; $p < 0.05$.

Conversely, serum ALT activities of FEb_{24h} = 110.2 ± 0.91 IU/L and FEb_{6h} = 113.2 ± 0.53 IU/L were lower than that of the CTRL group; $p > 0.05$. Specifically, FEa_{24h} group exhibited the highest serum ALT activity. Serum AST activities of the experimental test rat groups (FEa_{24h}, FEa_{6h}, FEb_{24h}, FEb_{6h}, CON_{24h}, CON_{6h}) were lower than that of the CTRL group and the values were generally within the range of 25.2 ± 0.41 IU/L – 39.4 ± 0.25 IU/L. Specifically, serum AST activity of FEb_{24h} = 25.2 ± 0.41 IU/L was significantly lower ($p < 0.05$) than that of the CTRL group.

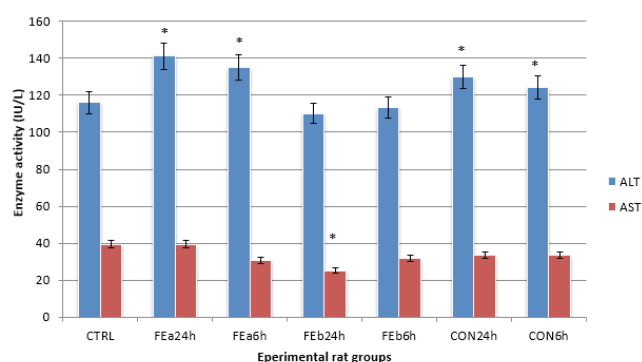


Figure 1. Serum alanine aminotransferase and aspartate aminotransferase activities of experimental rat groups
Bars without asterisk (*) are not significantly different from the control group at $p > 0.05$ according to LSD.

Diagnostic serum AST/ALT ratios (Table 2) of the experimental rat groups were less than 1.0 unit and was within the range of 0.23 ± 0.07 – 0.34 ± 0.02 . A cursory look at Table 2 showed that serum AST/ALT ratios of the

experimental test rat groups were less than that of the CTRL group, which was in the order: CTRL > FEa_{24h} > FEB_{6h} > CON_{6h} > CON_{24h} > FEa_{6h} > FEB_{24h}. Specifically, serum AST/ALT ratio of CTRL group was significantly greater ($p < 0.05$) than that of FEa_{6h} and FEB_{24h} groups.

Table 2. Serum aspartate aminotransferase and alanine aminotransferase activities ratios of experimental rat groups

Groups	AST/ALT ratios
CTRL	0.34 ± 0.02 ^a
FEa _{24h}	0.28 ± 0.07 ^{a,b,c}
FEa _{6h}	0.23 ± 0.08 ^{b,c,d,e,f,g}
FEB _{24h}	0.23 ± 0.07 ^{b,c,d,e,f}
FEB _{6h}	0.28 ± 0.04 ^{a,b}
CON _{24h}	0.26 ± 0.05 ^{a,b,c,d,e}
CON _{6h}	0.27 ± 0.04 ^{a,b,c,d}

The mean (X) ± S.D of six ($n = 6$) determinations. Means in the column with the same letter are not significantly different at $p > 0.05$ according to LSD.

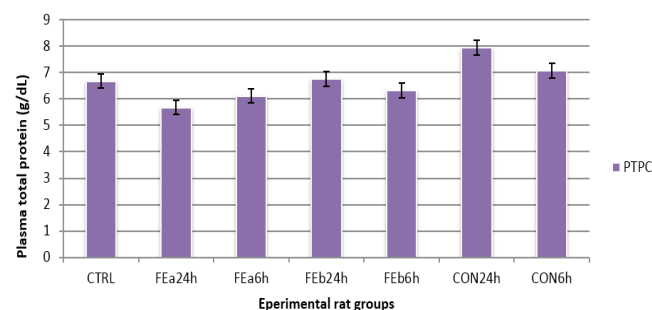


Figure 2. Plasma total protein concentrations of experimental rat groups; PTPC: Plasma total protein concentration. Bars without asterisk (*) are not significantly different from the control group at $p > 0.05$ according to LSD.

Figure 2 showed that PTPCs of the experimental rat groups were with a relatively narrow range: $5.68 \pm 0.84 - 7.94 \pm 1.02$ g/dL; $p > 0.05$. Nevertheless, CON_{24h} group gave the highest PTPC, whereas FEa_{24h} group registered the lowest PTPC value.

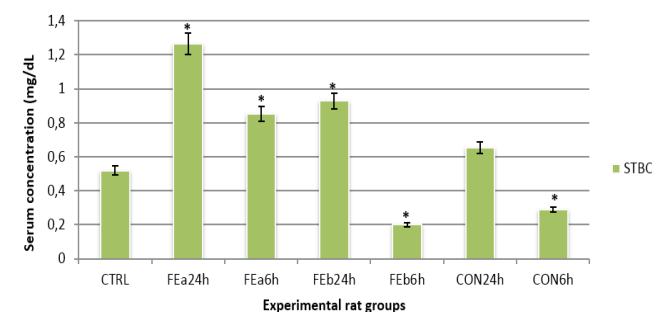


Figure 3. Serum total bilirubin concentrations of experimental rat groups; STBC: Serum total bilirubin concentration. Bars without asterisk (*) are not significantly different from the control group at $p > 0.05$ according to LSD.

In diagnostic terms, Figure 3 showed that STBC of FEa_{24h} group was greater than 1.0 mg/dL. Additionally, STBCs of FEB_{6h} = 0.20 ± 0.02 mg/dL and CON_{6h} = 0.65 ± 0.04 mg/dL were lower than that of the CTRL = 0.52 ± 0.09 mg/dL. Conversely, STBCs of FEa_{6h} = 0.85 ± 0.04 mg/dL, FEB_{24h} = 0.93 ± 0.06 mg/dL and CON_{24h} = 0.65 ± 0.04 mg/dL showed no significant difference ($p > 0.05$) from that of the CTRL group.

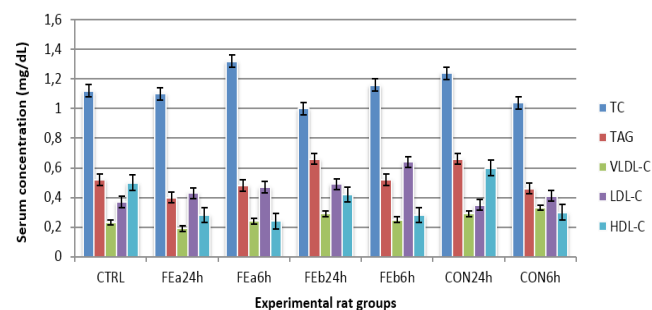


Figure 4. Serum lipid profile of experimental rat groups

An overview of Figure 4 showed that SLP patterns of the experimental test rat groups were altered when compared with that of the CTRL group. For instance, serum LDL-C concentration of FEB_{6h} was 1.7 fold greater than that of the CTRL group, whereas FEa_{6h}, CON_{24h} showed marginal elevations in their serum TC concentrations when compared with the CTRL group. Finally, CON_{24h} gave the highest serum HDL-C concentration = 0.60 ± 0.04 mg/dL.

The atherogenic indices of the experimental rat groups were in the order: TC/HDL-C > TAG/HDL-C > LDL-C/HDL-C (Table 3). Additionally, atherogenic indices of the CTRL group were lower than that of the experimental test rat groups. In terms of the TAG/HDL-C and TC/HDL-C ratios, FEa_{6h} exhibited the highest atherogenic indices, which corresponded to 5.50 ± 1.04 and 2.00 ± 0.09 , respectively. However, LDL-C/HDL-C ratio showed that FEB_{6h} exhibited the highest atherogenic index when compared with other experimental rat groups.

Table 4 showed that the quantity of feed consumed by the experimental rat groups was not significantly different ($p > 0.05$), which was within the range of 433.04 ± 12.04 g – 485.69 ± 14.14 g. However, WG of the various experimental rat groups exhibited significant ($p < 0.05$) variations. For instance, WG = 88.94 ± 0.56 g of CON_{6h} was significantly different ($p < 0.05$) from other experimental rat groups, except that of FEa_{24h} = 88.05 ± 0.26 g; $p > 0.05$. FEa_{6h} exhibited the highest FCR value of 2.01 folds greater than the CTRL group. The lowest FCR = 5.15 ± 0.31 was observed in CON_{6h} group.

Table 3. Atherogenic indices of experimental rat groups

Groups	Atherogenic indices		
	TAG/HDL-C	TC/HDL-C	LDL-C/HDL-C
CTRL	1.04 ± 0.04	2.24 ± 0.06	0.75 ± 0.03
FEa _{24h}	1.43 ± 0.06	3.93 ± 0.07	1.54 ± 0.05
FEa _{6h}	2.00 ± 0.06	5.50 ± 0.09	1.96 ± 0.06
FEb _{24h}	1.57 ± 0.05	2.38 ± 0.06	1.17 ± 0.05
FEb _{6h}	1.86 ± 0.06	4.14 ± 0.09	2.29 ± 0.07
CON _{24h}	1.10 ± 0.04	2.07 ± 0.07	0.58 ± 0.01
CON _{6h}	1.53 ± 0.05	3.47 ± 0.09	1.37 ± 0.04

The mean (X) ± S.D of six (*n* = 6) determinations. Reference values: TAG/HDL-C ratio [44,45], TC/HDL-C ratios < 1.66 and LDL-C/HDL-C ratio < 1.06 [19]. Castelli risk indices I (TC/HDL-C) and II (LDL-C/HDL-C) [46].

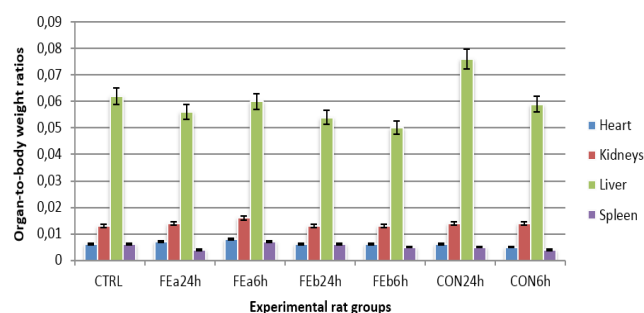
Table 4. Quantity of feed consumed, weight gained and feed conversion ratios of experimental rat groups

Groups	TFC [#] (g)	WG (g)	FCR [#]
CTRL	433.04 ± 12.04	71.52 ± 0.91 ^c	7.06 ± 3.76
FEa _{24h}	485.69 ± 14.14	88.15 ± 0.26 ^{a,b}	5.69 ± 1.17
FEa _{6h}	475.13 ± 17.63	66.72 ± 0.34 ^d	14.78 ± 9.88
FEb _{24h}	473.89 ± 17.78	56.86 ± 0.45 ^{f,g}	8.56 ± 1.06
FEb _{6h}	455.35 ± 9.35	62.00 ± 0.66 ^{d,e}	7.91 ± 2.39
CON _{24h}	444.88 ± 10.84	58.48 ± 0.99 ^f	8.44 ± 3.12
CON _{6h}	456.44 ± 18.36	88.94 ± 0.56 ^a	5.15 ± 0.31

TFC: Total feed consumed; WG: Weight gained; FCR: feed conversion ratio.

The mean (X) ± S.D of six (*n* = 6) determinations. *Means are not significantly different at *p* > 0.05, whereas means in the column with the same letter are not significantly different at *p* > 0.05 according to LSD.

An overview of Figure 5 showed that the alterations in organ-to-body weight ratios of the experimental rat groups exhibited comparatively narrow numerical variations, except that of the liver-to-body weight ratio. The heart-to-body weight ratios of the CTRL, FEB_{24h}, FE_{b6h} and CON_{24h} were numerically equivalent, whereas those of FEa_{24h} and FE_{a6h} exhibited marginal increases. Contrary, heart-to-body weight ratio of CON_{6h} was numerically lower than those of other experimental rat groups.

**Figure 5.** Organ-to-body weight ratios of experimental rat groups

Likewise, kidney-to-body weight ratios of the CTRL, FEB_{24h} and FEB_{6h} were numerically equivalent, whereas those of FEa_{24h}, FEa_{6h}, CON_{24h} and CON_{6h} were numerically greater

than that of the CTRL. The liver-to-body weight ratios of the experimental test rat groups were lower than that of the CTRL, except that of CON_{24h}. Also, spleen-to-body weight ratios of the experimental test rat groups were numerically lower than that of the CTRL, except that of FEa_{6h}, whereas that of FEB_{24h} was numerically equivalent to that of the CTRL.

DISCUSSION

The comparative levels of serum ALT and AST activities was such that ALT > AST was diagnostic of hepatic tissues injuries or inflammation [3,47-50], exemplified by AST/ALT ratios of the experimental rat groups (Table 2). Specifically, the FEa_{24h}, FEa_{6h}, CON_{24h} and CON_{6h} groups exhibited mild adjustments in serum ALT and AST activities, which were corresponding indications of moderately compromised hepatocellular integrity and in concord with previous interpretations [10,26,49,51]. Fittingly, MSG could be deleterious to the hepatocytes of adult Wistar rats when consumed at comparatively higher doses, and by extension, may affect liver functions [52]. Conversely, lower serum AST activities of FEa_{6h}, FEB_{24h}, FEB_{6h}, CON_{24h} and CON_{6h} groups when compared with that of the CTRL group apparently provided further biological information bordering on the pathophysiological

status of the heart and kidney tissues [53]. Additionally, reports according to Akanji *et al.*, [54] noted that outright inhibition or inactivation of ALT and AST activities occurred when experimental animals were treated with potassium bromate and may have accounted for low plasma activities of the two enzymes as well as other hepatic diagnostic enzymes. The serum ALT and AST activities of FEB_{6h} and FEB_{24h} groups were comparatively lower than that of FEa_{6h} and FEa_{24h} groups (Figure 2), which may not be unconnected with the concentrations of MSG in the corresponding diets; FEa = 3.41 g/100 g feed sample > FEB = 0.39 g/100 g feed sample (Table 1). Therefore, the capacity of MSG-containing diet to alter serum ALT and AST activities in the experimental rat groups was dependent on the concentration of active principle (MSG) in the diets and in corresponding order of hourly feeding durations. In contrast, the capacity of CON-containing diets to alter hepatic tissue necrosis indicators (ALT and AST) in serum was not affected by adjustments in the pattern of hourly feeding durations of the experimental rat groups. The present findings corroborated the outcome of previous investigations [55], which noted that oral contraceptives affected functional integrity of many organs in addition to those of the reproductive system. Similarly, using intravenous injected bromsulphophthalein (BSP) clearance test and levels of activities of hepatic non-functional plasma enzymes, previous investigation demonstrated that synthetic androgens are toxic to the liver by virtue of established increased BSP retention, and elevated serum AST, ALT, γ -glutamyl transpeptidase and sorbitol dehydrogenase activities in rabbits [56].

However, mild perturbation of hepatic tissues integrity did not profoundly affect the capacity of the hepatocytes to biosynthesize plasma proteins that eventually found their way into blood circulation [9], typified by the marginal differences in PTPCs of the experimental test rat groups when compared with that of the CTRL group (Figure 2), which was in conformity with previous reports [32,57]. These findings appeared to suggest that both flavour enhancer- and contraceptive-containing diets provoked mild alterations in hepatic integrity but did not precisely affect hepatic functionality. Specifically, the mild alterations in hepatic integrity did not interfere with the capacity of the hepatocytes to biosynthesize plasma proteins for export to the vascular system, irrespective of the experimental hourly feeding duration of the rats as previously described [10].

The liver is the primary organ involved in the metabolism of xenobiotics and earlier reports have associated the metabolism of MSG with the production of overwhelming levels of reactive oxygen species (ROS) and attendant tissue necrosis [58-61]. Previous related studies showed that dietary exposure of animal model to MSG promoted testicular lesion [62] and decreased pancreatic β -cell mass [35] among other several pathologic outcomes. According

to Evans *et al.*, [63], activation of cellular stress-sensitive pathways such as nuclear factor- κ B (NF- κ B), p38 mitogen-activated protein kinase, NH₂-terminal Jun kinases/stress-activated protein kinases and hexosamines are responsible for oxidative tissue damage. Therefore, the raised serum levels of ALT, in particular, and AST activities appeared to suggest ROS-mediated hepatic necrosis following the consumption of MSG-containing diets by the corresponding experimental rat groups. Likewise, metabolism of steroidal contraceptives is associated with compromised coenzyme Q₁₀ and/or α -tocopherol antioxidant systems and overwhelming cellular levels of ROS-mediated tissue damage, which may be ameliorated by antioxidant supplementation [64]. Accordingly, previous reports showed that drugs and biological derived antioxidant such as N-acetyl-cysteine and β -carotene exhibited protective effect against MSG-induced hepatic injuries [58]. Also, oral administration of ascorbic acid, α -tocopherol and quercetin were demonstrated to be effective in ameliorating MSG-induced oxidative stress associated with tissue necrosis in human and animal models [26,32,65,66].

Bilirubin and its derivatives are endogenous products of haem catabolism in the hepatocytes that are eventually excreted in bile secretion. Studies have shown that normal blood bilirubin concentration exerts beneficial antioxidant properties *in vivo* and *in vitro* [67-70]. However, elevation in blood bilirubin concentration connotes rapid haemolysis and/or hepatic dysfunction as a result of dietary, viral, parasitic and secondary metabolic causes [2,4,71,72]. By definition, flavour enhancer- and oral contraceptive-containing diets did not cause hyperbilirubinemia ([bilirubin] > 1.0 mg/dL [2,9]) in the experimental rat groups. However, the relatively higher STBC above baseline value in FEa_{24h} group (Figure 3) was a pointer to the fact that higher concentration of MSG may provoke hyperbilirubinemia. Additionally, the present study showed that concentrations of the active principles in flavour enhancer- and steroidal oral contraceptive-containing diets and corresponding order of hourly feeding durations dictated the capacities of the experimental diets to alter STBC when compared with that of the CTRL group. Marginal alterations in STBCs in the experimental rat groups was an indication of MSG-mediated mild oxidative tissue injuries as earlier described [10,26,51]. Contrary to the present findings, previous studies showed that administration of ethinylestradiol based contraceptives was responsible for the development of jaundice in women and animal models [36,73]. However, slight alterations in STBCs, precisely in CON_{24h} group, were not diagnostic of hyperbilirubinemia. These seemingly contradictions in STBCs patterns between the present study and previous ones mentioned appeared to be in connection with the disparities in concentrations of the active principles, mode and route of administration of the contraceptives, which involved oral administration via an oro-gastric tube [73] and intra-peritoneal or intravenous injections [36].

The central role of the hepatocytes in the metabolism and mobilization of lipoproteins influences blood lipid concentrations and factors that have direct bearing on the structural and functional integrity of the hepatocytes affects SLP pattern [74,75]. Furthermore, profound alteration in SLP pattern is characterized by raised levels of atherogenic lipoproteins in plasma, which are major contributors to the development of atherosclerosis and cardiovascular disease [10]. The present study showed differential perturbation in SLP with attendant incidences of atherogenicity in majority of the experimental rat groups as previously described in adult female rats [76]. By definitions [19,44,45], data of TAG/HDL-C and LDL-C/HDL-C ratios (Table 3) were obvious indications that FEa_{24h}, FEa_{6h}, FEB_{24h}, FEB_{6h} and CON_{6h} groups exhibited atherogenicity. By extension, atherogenicity is defined by Castelli risk indices I (TC/HDL-C) and II (LDL-C/HDL-C): such that < 0.11 (low risk), 0.11 – 0.21 (intermediate risk), > 0.21 (increased risk) [46]. The present findings appeared to suggest that oral ingestion of MSG promoted atherogenicity, irrespective of the experimental concentration of MSG in the diets and pattern of hourly feeding durations of the experimental rat groups. However, alterations in SLP in CON_{6h} shared similar outcome with participants receiving low dose of injectable and oral contraceptives, which may not contribute to increased risk of atherosclerosis and development of coronary heart disease over time [77,78]. These findings were at variance with the report of Asare *et al.*, [46], in which they noted that administration of hormonal contraceptives caused altered SLP among various population groups with different patterns of dyslipidemia who carried potential risks of developing cardiovascular disease in Ghanaian community. Meanwhile, the level of alteration in SLP depended on the delivery route of contraceptives [46], which may have accounted for the disparities in these previous reports.

The approximate equal quantity of feed consumed by the experimental rat groups was an indication that the flavour enhancer- and contraceptive-containing diets were of equal palatability index, and probably of equivalent organoleptic properties with that of the CTRL diet. Furthermore, the comparable gain in body weights by FEa_{24h} and CON_{6h}, which was significantly greater than that of the CTRL as well as other experimental rat groups, confirmed the propensity of MSG to promote adiposity in animal models as previously described [79-82]. Paradoxically, FEa_{24h} and CON_{6h} exhibited the relatively lowest FCR, which was an indication that the active principles in the compounded diets were intrinsic contributory factors to the gains in body weight of the experimental rats. It is likely that dietary contents of MSG, ethynylestradiol and ethisteron enhanced the digestibility of the diets, absorption of nutrients and metabolic outcomes leading to differential weight gains and FCR indices in the experimental rat

groups. The data equally showed that the capacity of MSG to cause weight gain in corresponding experimental rat groups, which conformed with previous reports elsewhere [79-82], was dependent on the concentration of MSG in the diet, duration and feeding patterns of the rats.

Results of organ-to-body weight ratios of visceral organs of the experimental rat groups signified that flavour enhancer- and contraceptive-containing diets neither provoked hypertrophy nor atrophy of the heart and kidneys. Likewise, the present study did not reveal incidence of splenomegaly in the experimental rat groups, typified by the relatively narrow range of spleen-to-body weight ratios. The non-presentation of splenomegaly in the experimental rat groups was an indication of a blood system devoid of microbial infections and haemolytic/compromised immune system diseases reminiscent of sickle cell anaemia, thalassaemias, malarial infection and thrombocytopenia as previously described [2,83-85]. However, comparative evaluation of liver-to-body weight ratios of CON_{24h} showed evidence of hepatic hypertrophy, whereas that of FEa_{24h} was indicative of hepatic atrophy that may have paralleled the marginal elevation in serum ALT and AST activities diagnostic of mild hepatic necrosis as earlier described here. Fittingly, reports according to Eweka and Om'Iniabohs, [29] noted that high dose of MSG provoked deleterious effects such as atrophic and degenerative changes on the liver of adult Wistar rats. In the same manner, previous related animal studies had demonstrated that parenteral administration of MSG caused changes in pancreatic islets such as hypertrophy, hyperplasia, and decrease in acinar cells, α -cells and somatostatin cells as well as increase in fibrosis [35,86-89] and testicular lesion [62].

CONCLUSIONS

Flavour enhancer- and contraceptive-containing diets provoked mild alterations in serum ALT and AST activities, which were diagnostic of moderately compromised hepatocellular integrity. Mild alterations in hepatic integrity did not interfere with the capacity of the hepatocytes to biosynthesize plasma proteins for export to the vascular system, irrespective of the experimental hourly feeding durations of the rats. Marginal alterations in STBCs in the experimental rat groups were indications that flavour enhancer- and contraceptive-containing diets did not provoke hyperbilirubinemia. Alterations in SLP patterns portrayed incidences of atherogenicity in majority of the experimental rat groups. Also, the test diets neither provoked hypertrophy nor atrophy of the heart, kidneys and spleen in the experimental rat groups. However, comparative evaluation of liver-to-body weight ratios of rats fed with contraceptive-containing diets showed evidence of hepatic hypertrophy, whereas those fed with flavour enhancer-containing diets showed evidence of hepatic atrophy.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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