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Effects of chronic administration of some bouillon cubes on oxidative stress and liver function markers in female albino rats

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Abstract

Aim: This study evaluated the effects of chronic administration of some bouillon cubes on oxidative stress and liver function markers in female albino rats.

Methodology: A total of thirty-five (35) female albino rats, weighing between 120 and 150 grams, were used for the study. The bouillon cubes, Star Maggi and Knorr were administered daily to the rats, using an oral gavage tube for 90days. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) level were determined using a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated quantitatively using the Reitman and Frankel Method. Total protein (TP) was estimated quantitatively using the Biuret colorimetric method. Albumin (ALB) was estimated quantitatively following the Modified bromocresol green colorimetric method. Liver sections were stained using haematoxylin and eosin (H&E) staining technique. Quantitative analysis of monosodium glutamate (MSG) content of the bouillon cubes was analyzed using ultraviolet (UV) spectroscopy while the sodium content was analyzed using atomic absorption spectrophotometry according to the method of the American Public Health Association.

Results: There were no significant differences ($p>.05$) in SOD and GPx levels in all the treatment groups, compared to the negative control. Group D (High Dose Maggi) had lower SOD and GPx levels than the negative control, though not significant. ALT level (5.01 ± 0.53 iu/L) in group E (High Dose Knorr) was significantly higher ($p<.05$) when compared to the negative control. There were no significant differences ($p>.05$) in AST levels in all groups, even though group E had the highest AST level. There were no significant differences ($p>.05$) in TP and ALB levels, except for group E (High Dose Knorr), which was significantly lower ($p<.05$), compared to the negative control. Liver sections of the negative control showed normal histoarchitecture. The treated groups showed histological changes in the architecture of the hepatocytes indicating moderate dilation of the central vein, moderate disruption of the hepatic lobules, and mild sinusoidal dilation, with associated inflammatory cell infiltration.

Conclusion: Chronic administration of bouillon cubes did not have significant impact on the levels of the oxidative stress markers, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the rats. Chronic administration of Knorr cubes impacted the liver, with elevated alanine aminotransferase (ALT) levels, with a reduced total protein and albumin level. There were changes in the architecture of the hepatocytes of the treated rats, indicating moderate dilation of the central vein, moderate disruption of the hepatic lobules, and mild sinusoidal dilation. Bouillon cubes should be used in moderation, taking into account their potential effects on overall health and wellbeing when consumed for long periods.

Keywords: Bouillon cubes; Star Maggi, Knorr cubes; Oxidative stress; Liver function

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1. Introduction

Bouillon cubes are ingredients that are added to food to enhance the flavor and aroma. Enriching the natural flavor and aroma of food is part of the art of cooking, because everyone expects delicious dishes. In many countries on the African continent, bouillon cubes have become an integral part of many daily dishes. According to the Nestlé Company, more than 100 million bouillon cubes are sold daily in the West Central Africa region [1,2].

Various brands of bouillon cubes are available in the markets. These include Star Maggi, Knorr Cubes, Royco, Onga, Aji-no-Moto, etc. The bouillon cubes Maggi and Knorr refer to a broth and/or a mixture of dehydrated ingredients in the form of a cube or bouillon. The same mixtures can also be marketed in the form of powders. Bouillon cubes are widely used throughout Africa to enhance the flavors of numerous traditional African recipes. These cubes with less nutritive value have grossly taken the place of many fermented seeds and products that once added much variety, flavor, and nutritive value to our African dishes. Experts refer to bouillon cubes as "well-packaged salts", and studies also show that chronic exposure to these cubes could have adverse health effects due to the main ingredients in these products [3, 4].

With growing health awareness, the demand for products that support better health has increased. As a result, bouillon cubes have been the subject of close investigation, being an inseparable part of the food humans consume, especially in our country Nigeria [5]. For this reason, this study looks at the effects of chronic exposure of Star Maggi and Knorr Cubes on oxidative stress and liver function markers, in female albino rats. This research may contribute in understanding the health impact of bouillon cubes, and promote responsible consumption and production by informing decisions related to food safety and regulation, in line with the Sustainable Development Goals (SDGs).

2. Material and methods

2.1. Experimental Animals

A total of thirty-five (35) female albino rats, weighing between 120 and 150 grams, was used for the study. The rats were kept in standard cages and provided with unrestricted access to feed and water. A period of 14 days was allotted for the animals to acclimatize before the study officially began.

2.2. Treatments

Two (2) commonly used bouillon cubes (Star Maggi and Knorr cubes) purchased from the local market in Mile 3, Diobu, Port Harcourt was used for the study. Maggi cube is manufactured by Nestle Nigeria PLC, Industrial Avenue 22/24 Ilupeju, Lagos Nigeria. Knorr cube is manufactured by Unilever PLC, RC 113 Agbara Industrial Estate, Agbara, Ogun State, Nigeria

2.3. Acute Toxicity Study

This was done using the fixed dose procedure [6]. Eighteen (18) rats were divided into six (6) groups of three (3) rats each – 3 groups for Maggi and 3 groups for Knorr. 2000 mg/kg, 3000 mg/kg, and 5000 mg/kg of star Maggi cube was administered to the rats in groups 1, 2, and 3 respectively while 2000 mg/kg, 3000 mg/kg, and 5000 mg/kg of Knorr cube was administered to the rats in groups 4, 5, and 6 respectively. The rats were observed for signs of toxicity for 48 hours. After observation for 48 hours, there were no signs of toxicity, hence the bouillon cubes were considered safe up to a dose of 5000 mg/kg. For the study, 1500 mg/kg and 3000 mg/kg were adopted and used as low and high doses respectively.

2.4. Dose Calculation

2.4.1. Low Dose Maggi (1500 mg/kg)

1500 mg/kg of Maggi was administered to the rats daily. That is, a 1 kg would take 1500 mg of Maggi. Therefore, a 150 g rat took 150 g /1000 g x 1500 mg = 225 mg of Maggi as daily dose. A 1 kg rat would take as vehicle 10 ml of fluid orally. Therefore, a 150 g rat took 150 g / 1000 g x 10 ml = 1.5 ml. Hence, 225 mg of Maggi was dissolved in 1.5 ml of water and administered daily. That is, 150 mg/ml. A stock solution was therefore prepared and administered according to the weight of the rats daily [6, 7].

2.4.2. High Dose Maggi (3000 mg/kg)

3000 mg/kg of Maggi was administered to the rats daily. That is, a 1 kg would take 3000 mg of Maggi. Therefore, a 150 g rat took 150 g /1000 g x 3000 mg = 450 mg of Maggi as daily dose. A 1 kg rat would take as vehicle 10 ml of fluid orally. Therefore, a 150 g rat took 150 g / 1000 g x 10 ml = 1.5 ml. Hence, 450 mg of Maggi was dissolved in 1.5 ml of water and administered daily. That is, 300 mg/ml. A stock solution was therefore prepared and administered according to the weight of the rats daily [6, 7].

2.4.3. Low Dose Knorr (1500 mg/kg)

1500 mg/kg of Knorr was administered to the rats daily. That is, a 1 kg would take 1500 mg of Knorr. Therefore, a 150 g rat took 150 g /1000 g x 1500 mg = 225 mg of Knorr as daily dose. A 1 kg rat would take as vehicle 10 ml of fluid orally. Therefore, a 150 g rat took 150 g / 1000 g x 10 ml = 1.5 ml. Hence, 225 mg of Knorr was dissolved in 1.5 ml of water and administered daily. That is, 150 mg/ml. A stock solution was therefore prepared and administered according to the weight of the rats daily [6, 7].

2.4.4. High Dose Knorr (3000 mg/kg)

3000 mg/kg of Knorr was administered to the rats daily. That is, a 1 kg would take 3000mg of Knorr. Therefore, a 150 g rat took 150 g /1000 g x 3000 mg = 450 mg of Knorr as daily dose. A 1 kg rat would take as vehicle 10 ml of fluid orally. Therefore, a 150 g rat took 150 g / 1000 g x 10 ml = 1.5 ml. Hence, 450 mg of Knorr was dissolved in 1.5 ml of water and administered daily. That is, 300 mg/ml. A stock solution was therefore prepared and administered according to the weight of the rats daily [6, 7].

2.5. Experimental Design

The rats were weighed and divided into five (5) experimental groups (7 rats each) consisting of the negative control group (Group A) and 4 treatment groups (Groups B-E). The bouillon cubes were prepared into suspension form of 150 mg/ml of bouillon cubes (Star Maggi and Knorr cubes) respectively for Low Doses and 300 mg/ml of bouillon cubes (Star Maggi and Knorr cubes) for High Doses. The suspension was given daily using an oral gavage tube for 90days. Treatments were performed according to the grouping below;

- Group A (Negative control group): Received no treatment.
- Group B (Low dose Maggi): Received daily oral dose of 1500 mg/kg Star Maggi cubes.
- Group C (Low dose Knorr): Received daily oral dose of 1500 mg/kg Knorr cubes.
- Group D (High dose Maggi): Received daily oral dose of 3000 mg/kg Star Maggi cubes.
- Group E (High dose Knorr): Received daily oral dose of 3000 mg/kg Knorr cubes.

On the 91st day, the animals were fasted for 6 hours anaesthetized and later sacrificed. Blood was collected from each rat by means of cardiac puncture. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

2.6. Reagents and Biochemical Analyses

All reagents were purchased commercially and the standard operating procedures provided by the manufacturers were meticulously adhered to. Quality control (QC) samples were analyzed alongside the biochemical tests. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) were determined quantitatively using a rat-specific Sandwich-enzyme linked immunosorbent assay (ELISA) method [8,9], as described by Elabscience biotechnology company limited (China). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated quantitatively using the Reitman and Frankel Method [10], as described by Spectrum Diagnostics (Egypt). Total Protein was estimated quantitatively using the Biuret method [11], as described by Spectrum Diagnostics (Egypt). Albumin was estimated quantitatively using the Modified Bromocresol green colorimetric method [12], as described by Spectrum Diagnostics (Egypt). Quantitative analysis of monosodium glutamate (MSG) content of the bouillon cubes was analyzed using ultraviolet (UV) spectroscopy while the sodium content was analyzed using atomic absorption spectrophotometry according to the method of the American Public Health Association (APHA) [13]. Liver specimens were harvested and fixed in 10% formal saline for histological analysis using Haematoxylin and Eosin (H&E) stain, viewed and photomicrographs of the liver were captured with X40 objective lens using the ScopeTekTM device and software v1.3.

2.7. Statistical Analysis

The data generated was analyzed with GraphPad Prism version 8.0.2. Analysis of variance (ANOVA) and Tukey's post hoc test were performed to compare differences between groups. The results were considered statistically significant at the 95% confidence interval ($p \leq 0.05$). Results are expressed as mean ± SD.

3. Results and Discussion

Table 1 Quantitative Analysis of MSG and Sodium Content in the Bouillon Cubes

Samples	MSG Conc. (mg/g)	Sodium Conc. (ppm)
Star Maggi	119.636 (11.96%)	38.282 (0.00383 %)
Knorr Cube	111.455 (11.15%)	32.892 (0.00329 %)

Table 1 shows the results of Monosodium Glutamate (MSG) and sodium content of bouillon cubes. It shows that star Maggi had the highest contents of Monosodium Glutamate (MSG) with a concentration of 119.96 mg/g (11.96 %) while that of Beef Knorr cubes is 111.5 mg/g (11.15 %). The results also reveal that star Maggi had the highest sodium content with a concentration of 38 ppm (0.00383 %) while Knorr cube sodium content concentration is 32 ppm (0.00329 %).

These values were compared to the maximum allowable limits set by the National Agency for Food and Drug Administration and Control (NAFDAC), which are 1.5% max for MSG and 12.5% max for sodium in bouillon cubes. From the results above, the sodium content in both the Star Maggi and Beef Knorr cubes falls well within the permissible limit of 12.5%. However, the MSG content in both products exceeds the maximum allowable limit of 1.5% specified by NAFDAC. Even though the sodium content in the Star Maggi and Beef Knorr cubes is within the NAFDAC limit, it is still crucial to consider the cumulative sodium intake from other food items to stay within the recommended daily limits of less than 2g per day [14]. This is in consonance with the works of Apkanyung [15], and Alonge *et al*. [16], in which they found variable amounts of MSG, sodium, iron and zinc in bouillon cubes produced in Nigeria.

Table 2 Levels of the Oxidative Stress Markers, SOD and GPx of the Rats after Treatment

Groups (N=7)	SOD (ng/ml)	GPx (pg/ml)
Group A (Neg. Cont)	0.75 ± 0.06	74.25 ± 7.05
Group B (Low Dose Maggi)	1.20 ± 0.18	85.20 ± 2.02
Group C (Low Dose Knorr)	0.78 ± 0.18	75.00 ± 5.29
Group D (High Dose Maggi)	0.72 ± 0.11	69.70 ± 4.31
Group E (High Dose Knorr)	0.90 ± 0.14	89.00 ± 3.72
P-value	0.2498	0.0532
F-value	1.472	2.838
Summary	NS	NS

N= number of rats, NS= not significant

Table 2 shows the results of the oxidative stress biomarkers, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the rats after treatment. It shows there were no significant differences ($P>.05$) in SOD and GPx levels in all the treatment groups compared to the negative control. Group D (High Dose Maggi) had lower SOD and GPx levels than the negative control, though not significant. This implies the administration of Star Maggi and Knorr cubes did not alter the levels of the oxidative stress markers, SOD and GPx. This does not agree with the study conducted by Salah *et al*. [17], which found elevated oxidative stress parameters in rats administered food additives. The results are in consonance with the works of Ifemeje *et al*. [18], in which they stated that there was a non-significant decrease in glutathione peroxidase (GPx) levels in rats, after administration of food additives.

SOD (superoxide dismutase) and GPx (glutathione peroxidase) are antioxidant enzymes that play important roles in protecting cells from oxidative stress. SOD catalyzes the dismutation of superoxide (O^{2-}) radical to hydrogen peroxide

(H_2O_2). It is considered the first line of defense against the deleterious effects of oxygen radicals in the cells and it scavenges reactive oxygen radical species [19]. Glutathione peroxidase (GPx) catalyzes the reduction of H_2O_2 using Glutathione (GSH) as a substrate, thereby protecting the mammalian cells against oxidative stress. Some other studies have suggested that high levels of MSG consumption may increase oxidative stress and reduce the activity of the antioxidant enzymes SOD and GPx [20]. As a protective mechanism, antioxidant enzyme levels increase in response to oxidative stress [21], however, these responses may vary depending on the intensity and duration of oxidative stress induction.

Table 3 Liver Function Parameters of the Rats after Treatment

Groups (N=7)	**ALT (iu/L)**	**AST (iu/L)**	**TP (g/L)**	**ALB (g/L)**
Group A (Neg. Cont)	3.35 ± 0.65	6.80 ± 0.29	72.50 ± 3.52	35.75 ± 1.71
Group B (Low Dose Maggi)	3.80 ± 0.52	6.73 ± 0.55	67.33 ± 2.19	34.67 ± 2.87
Group C (Low Dose Knorr)	4.20 ± 0.64	7.60 ± 0.60	68.00 ± 2.82	33.50 ± 0.58
Group D (High Dose Maggi)	4.15 ± 0.60	6.95 ± 0.34	63.50 ± 2.29	33.50 ± 1.10
Group E (High Dose Knorr)	5.01 ± 0.53 [ae, be]	8.1 ± 0.71	59.33 ± 4.80 [ae]	31.33 ± 1.86[ae]
P-value	0.0114	0.0524	0.0039	0.0209
F-value	4.359	2.852	5.552	3.738
Summary	S	NS	S	S

N= number of rats, [ae] =significant vs Neg. control, [be] = Group E significant Vs B, S= Significant, NS= not significant

Table 3 shows the results of the liver function parameters, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total protein (TP) and albumin in the rats, after treatment. The results show the mean ALT level (5.01 ± 0.53 iu/L) in group E (High Dose Knorr) was significantly higher (P<.05) when compared to the negative control. It was also significantly higher (P<.05), when compared to group B (Low Dose Maggi) having a mean value of (3.80 ± 0.52 iu/L). There were no significant differences (P>.05) in AST levels in all groups, even though group E had the highest AST level. There were no significant differences (P>.05) in TP and ALB levels, except for group E (High Dose Knorr), which was significantly lower (P<.05), compared to the negative control.

The significantly higher value of ALT in group E (High Dose Knorr) is indicative of liver damage. ALT is an enzyme that is primarily found in liver cells, and its levels are elevated in the presence of liver damage or disease [22]. MSG is a flavor enhancer that has been shown to cause liver damage, acting as toxins to hepatocytes, thus affecting cellular integrity and causing defect in membrane permeability [23]. The results agree with the work of Eweka *et al.* [24], in which they found significantly elevated ALT and AST levels in food additive administered wistar rats. The low total protein and albumin levels in group E (High Dose Knorr) indicate decreased production of serum proteins by the liver. Decreased albumin levels could be due to the reduced synthetic ability of the liver and could also indicate the chronicity of treatment or severity of liver damage. This result agrees with the work of Augustine *et al*. [25], in which they discovered hepatotoxicities in MSG administered rats. The cytoplasmic enzymes alanine aminotransferase and aspartate aminotransferase, total protein, and albumin are employed in the assessment of hepatic disorders, and alterations in these parameters reflect liver damage and inflammatory hepatocellular disorders [26,27].

Histologic analysis of the treated groups showed histological changes in the architecture of the hepatocytes, indicating moderate dilation of the central vein, moderate disruption of the hepatic lobules, and mild sinusoidal dilation compared with the negative control group which shows normal liver architecture. Group B (low dose Maggi) indicates moderate congestive hepatocyte (moderate dilation of the central vein). The hepatocyte of Group C (low dose Knorr) indicates moderate disruption of the hepatic lobules while Group D (high dose Maggi) showed mild centrilobular sinusoidal dilation with associated inflammatory cell infiltration (mild sinusoidal dilation). Group E (high dose Knorr) on the other hand, also showed mild blood deposit in the central vein. This study agrees with the works of Eweka *et al*. [24] and AL-Khatawi *et al*. [28], which reported degeneration of hepatocytes and necrosis in the liver of the treated wistar rats.

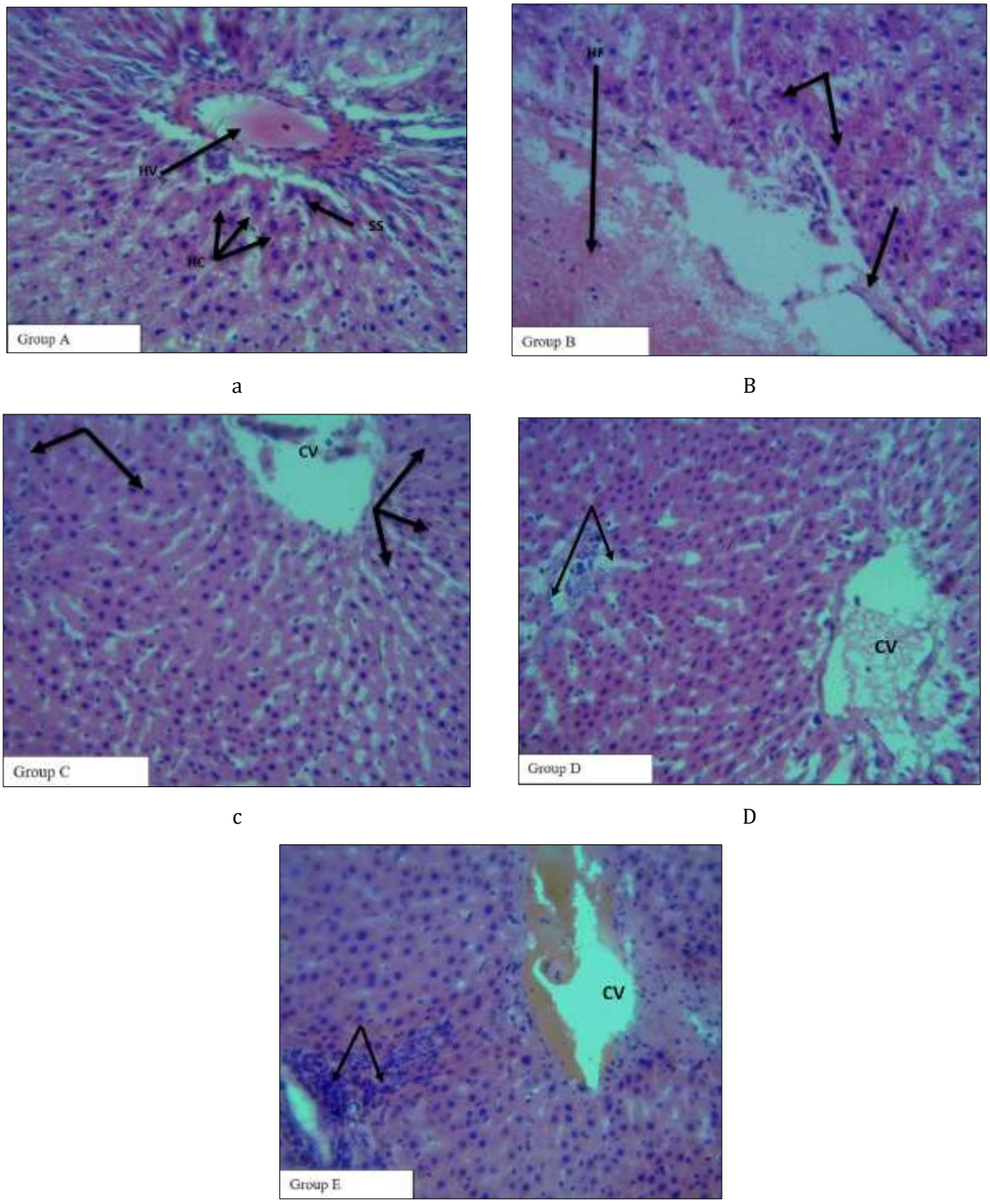

Figure 1 (a), (b), (c), (d) and (e). Shows photomicrograph (X 400) of H&E-stained histologic sections of the liver of the rats. The negative control (a) showed a normal structure of the periportal area alongside the hepatocyte (HC), with mild blood deposit in the hepatic vein (HV), blood deposit in sinusoids (SS). The low dose Maggi group (b) showed moderate congestive hepatocyte: hemorrhagic foci (HF) with diffuse vascular dilation of central vein (arrows). The low dose knorr group (c) showed diffuse radiation of the hepatocyte towards the central vein associated with vacuolation and moderate hepatic lobules disruption (arrows). The high dose Maggi group (d) had mild centrilobular sinusoidal dilation with associated inflammatory cell infiltration (arrows). The High dose Knorr group (e) showed regeneration of hepatocytes (HC) with regressive change in sinusoidal appearance, mild blood deposit in the central vein (CV) (arrows)

4. Conclusion

Chronic exposure to bouillon cubes did not have significant impact on the levels of the oxidative stress markers, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the rats. Chronic administration of Knorr cubes impacted the liver, with elevated alanine aminotransferase (ALT) levels, with a reduced total protein and albumin level. There were changes in the architecture of the hepatocytes of the treated rats, indicating moderate dilation of the central vein, moderate disruption of the hepatic lobules, and mild sinusoidal dilation. Bouillon cubes should be used in moderation, taking into account their potential effects on overall health and wellbeing when consumed for long periods. Local authorities should make sure to ascertain the constituents of bouillon cubes, to ensure the safety of consumers.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have affirmed the absence of any competing interests.

Statement of ethical approval

All animal experiments were carried out following ethical norms approved by the Institutional Ethical Committee.

Authors' contributions

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

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