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Changes in Oxidative Stress Parameters in Wistar Rats Administered Soft Drink and Menthol Candy

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ABSTRACT: The present study aimed to evaluate changes of stress oxidative enzymes and electrolytes of wistar rats administered soft drink and menthol candy. Seventy Wistar rats of weight between 125g and 175g were utilized for the study. The rats were allowed to acclimatize for seven days. The rats were divided into 10 groups of seven rats as follows: Group 1: normal control, Group 2, 3 and 4 were given 8ml, 5 and 2.5ml/kg body weight lacasara. Group 5, 6 and 7 were given 0.3g, 0.22g and 0.11g/kg body weight tom-tom candy. Group 8, 9 and 10 were administered 0.34g in 8ml, 0.22g in 5ml and 0.11g in 2.5ml/kg body weight of tom-tom mix with lacasara respectively. Treatment with tom-tom, lacasara and tom-tom mix with lacasara at various doses was carried out for 42 days. Electrolyte, creatinine and urea in the serum, superoxide dismutase (SOD), catalase (CAT) and malonyldialdehyde (MDA) in liver and kidney ware determined. The results showed significant increase in serum Na^+, K^+, Cl^- and LPO, decrease in SOD, and CAT, in the liver and kidney of groups 2, 3, 5, 6, 8, 9 and 10 compared to control. Furthermore, significant decrease was observed in LPO and increase SOD and CAT of group 7 in the liver and kidney compared to group 5 and 6. There were no significant difference in creatinine and urea in the serum of group 7 compared to group 5 and 6. In conclusion, chronic consumption of tom-tom, lacasara or tom-tom mix with lacasara induced oxidative stress. This effect was confirmed by kidney histological study, showing inflammation of proximal tubule and tubular cells.

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Soft drinks and beverages consumption had been increased among young adult (Lebda *et al.,* 2017). The ingredients of soft drink are water, sugar, phosphoric acid, fruit concentrate, preservatives, antioxidants, sweeteners, caffeine, carbon dioxide, and caramel as a colour additive (Düsman *et al.,* 2013). Lacasara is a widely known soft drink that is consumed in arguably every part of the world including Nigeria (Blasco *et al.,* 2020). Lacasera comprises of the following ingredients; apple juice (25% more), apple flavour, carbonated water, preservatives (sodium benzoate), malic acid, citric acid and sugar (Izah *et al.,* 2017). Undoubtedly, soft drinks are a major source of added sugar worldwide, and their consumption has been linked to obesity, diabetes, and metabolic syndrome (Salau *et al.,* 2013). Epidemiological and experimental evidence indicate that a greater consumption of sweet carbonated beverages is associated with overweight and obesity by virtue of the high sugar content and incomplete compensation for total energy in subsequent meals (Salau *et al.,* 2013; Tucker *et al.,* 2015; Yu *et al.,* 2013). Tom-tom is one of the Nigerian candies with strong menthol flavor. Generally, menthol (monocyclic terpene) is considered as very safe alcohol which is widely used in food industry and medicine. Kumar *et al.* (2016) stated the toxicity of excessive menthol consumption. Mustapha (2018) reported that lacasera juice and tom-tom candy has been abused by drug addict. This mainly done by mixing the two products to form solution called gigabyte. The kidneys are organs found in all vertebrates. They get rid of waste products from the body and maintain balanced electrolyte levels. Oxidative stress is considered an important player in the pathophysiology of kidney disease. Kidney disease is characterized by an abrupt loss of kidney function. Some of the antioxidants that are of veritable importance in the body for counteracting of oxidative stress assaults are catalase, superoxide dismutase, GSH and GST (Nimse *et al.,* 2011). Sucrose diet is also known for the lowering of the antioxidant system and overwhelming the body defence mechanism, consequently leading to oxidative stress (Potukuchi *et al.,* 2018). Oxidative stress, electrolyte imbalance and

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dyslipidemia are therefore, some of the major pathological pathways of sucrose-induced disorders (Tukel *et al.,* 2019). Therefore, the aim of this study was to investigate changes in stress oxidative enzymes and electrolytes of Wistar rats following chronic consumption of soft drink and menthol candy and the role of the tubular epithelial cells liberating proinflammatory mediators that could cooperate in regards to renal damage.

MATERIALS AND METHODS

Reagent and chemical: All reagent and chemicals that were used for this study were of analytical grade. The lacasera and tom-tom candy were purchased from Abraka main market, Delta State Nigeria.

Experimental Procedures: Seventy (70) male albino rats (Wistar strain) of weight between 125 g and 175 g were used for the study and were fed on standard laboratory diet (growers mash) and water *ad libitum* while the study lasted. .The rats were randomly grouped into 10 groups of seven rats as follows:

Group 1: normal control
Group 2: 8 ml/kg body weight lacasara
Group 3: 5 ml/kg body weight lacasara
Group 4: 2.5 ml/kg body weight lacasara
Group 5: 0.34 g/kg body weight tom-tom
Group 6: 0.22 g/kg body weight tom-tom
Group 7: 0.11 g/kg body weight tom-tom
Group 8: 0.34 g in 8 ml kg body weight tom-tom mix with lacasara
Group 9: 0.22 g in 5 ml kg body weight tom-tom mix with lacasara
Group 10: 0.11 g in 2.5 ml kg body weight tom-tom mix with lacasara

Rats in group 2 to 10 were given lacasara and tom-tom orally (either combined or singly) for 42 days. At the end of the study (day 42), the rats were euthanized in an airtight glass chamber saturated with chloroform and after opening up the rats surgically, blood samples, liver and kidney was collected for the biochemical analyses.

BIOCHEMICAL ANALYSES

Determination of kidney function markers: Urea, creatinine, potassium chloride and sodium in the serum were determined colorimetrically according to standard procedures using commercially available diagnostic kits (Randox Laboratories Limited, England).

Determination of superoxide dismutase (SOD) activity: The activity of superoxide dismutase (SOD) in the liver and kidney was determined by following the auto-oxidation of epinephrine as described by Misra and Fridovich (1972). A portion (0.2 mL) of sample and 2.5 mL of 0.05 M buffer (carbonate buffer pH 10.2) was placed in test tube. Thereafter, 0.3 mL freshly prepared epinephrine as the substrate to the buffer supernatant and mixed. The reference cuvette was used as blank. The absorbance at 480 nm due to the adrenochrome formed was read every 30 seconds for 120 seconds.

Determination of Catalase Activity: The activity of catalase in the liver and kidney were determined by method of Claiborne *et al.* (1984). Two millilitres (2 mL) of H_2O_2 was added to 1 mL of sample in the reaction cuvette. Sample was read at 360 nm for 70 seconds. The reference cuvette contained 2 mL H_2O_2 and 1mL of water. The disappearance of hydrogen peroxide was computed using the Molar extinction co-efficient, $\mathcal{E} = 39.4\ M^{-1}\ cm^{-1}$.

Estimation of Lipid Peroxidation: In the liver and kidney were done by the method of Buege and Aust (1978). One millilitre of the sample and 2 mL of TCA-TBA-HCl reagent [0.37% TBA, 15% TCA and 0.24 N Hydrochloric acid (HCl)] (1:1:1 ratio) was placed in test tube. The tube was then placed in boiling water (100 ^{o}C) for 15 min. The sample was cooled and centrifuged for 10 mins at 5000 g. The readings were taken at 532 nm. LPO in units/g of wet tissue was calculated with a molar extinction co-efficient of 1.56 x $105M^{-1}$

RESULTS AND DISCUSSION

Soft drink mixed with lacasara has been incriminated in the induction of oxidative stress through elevation of the free radical production, lowering the antioxidant status and elevation of free radicals. Table 1 presented serum urea and creatinine level of rats administered tom-tom mix with lacasara. The observed significant increase ($p<0.05$) in serum urea level of rats administered 8 ml/kg body weight lacasara, 5 ml/kg body weight lacasara, 0.34 g/kg body weight tom-tom, 0.34 g in 8 ml, 22 g in 5 ml and 0.11 g in 2.5 ml kg body weight tom-tom mix with lacasara (group 2, 3, 5, 8, 9 and 10) compared to the control may likely be due to kidney damage caused by tom-tom mix with lacasara toxicity. The significant decrease ($p<0.05$) illustrated in urea and creatinine of rats administered 2.5 ml/kg body weight lacasara compared to 8 ml and 5 ml/kg body weight lacasara (group 2 and 3) could be as a results of the low dose of lacasara which may cause no damage to the kidney. Studies have showed that elevated creatinine level signifies impaired kidney function (Hall et *al.,* 2014). Hence this clearly showed that lacasara drink and tom-tom at low dose do not cause any kidney damage. Interestingly, no significant difference was indicated in urea and creatinine of rats given 0.34 g in 8 ml kg body weight tom-tom mix with

lacasara (group 8) when compared to rats given 0.22 g in 5 ml and 0.11 g in 2.5 ml kg body weight tom-tom mix with lacasara (group 9 and 10). This may point to the fact that the effects of the mixture of lacasara and tom tom were not concentration dependent.

Table 1. Serum urea and creatinine level of rats administered tom-tom mix with lacasara

	Serum Urea (mg/dL)	Serum Creatinine (mg/dL)
Group 1	22.20 ± 11.05 [a]	0.38 ± 0.14 [a]
Group 2	30.00 ± 9.30 [b,c]	0.60 ± 0.1 [b]
Group 3	29.20 ± 9.90 [b]	0.58 ± 0.14 [b]
Group 4	23.00 ± 8.51 [a]	0.36 ± 0.08 [a]
Group 5	28.60 ± 9.73 [b]	0.42 ± 0.14 [a]
Group 6	26.00 ± 8.03 [a,b]	0.40 ± 0.14 [a]
Group 7	19.00 ± 4.39 [a]	0.30 ± 0.07 [a]
Group 8	36.60 ± 5.10 [c]	0.68 ± 0.13 [b]
Group 9	35.00 ± 8.36 [c]	0.58 ± 0.14 [b]
Group 10	35.80 ± 8.81 [c]	0.66 ± 0.19 [b]

Values are given in mean ± SD. Mean values (n=5) in the same column with different letter differ at p<0.05.

In this study, the serum electrolytes (Na^+, K^+ and Cl^-) of rats administered 8 ml/kg body weight lacasara, 5 ml/kg body weight lacasara, 0.34 g/kg body weight tom-tom, 0.34 g in 8 ml, 22 g in 5 ml and 0.11 g in 2.5 ml kg body weight tom-tom mix with lacasara (group 2, 3, 5, 8, 9 and 10) were significantly higher compared to the control (Table 2), this may likely be due to damage of kidney caused by tom-tom mix with lacasara at high dose of administration. According to Calvo and Uribarri (2013) elevated electrolyte level (Na^+, K^+ and Cl^-) may be main factor giving rise to kidney failure and osteoporosis. Elevated electrolyte in the serum of rats might lead to disturbed regulation of hormones giving rise to osteoporosis and cardiovascular diseases (Jiao *et al.*, 2019). Lacasara intake at low dose of 2.5 ml/kg body weight and 0.11 g/kg body weight tom-tom, the serum Na^+, K^+ and Cl^- were not significantly different compared to control. In addition, significant decrease ($p<0.05$) was observed in Na^+, K^+ and Cl^- of rats given 2.5 ml/kg body weight lacasara (group 4) compared to 8 and 5 ml/kg body weight lacasara**.** This may be due to the fact that low dose of lacasara may not compromise the serum electrolyte level. Moreover, the serum Na^+, K^+ and Cl^- of rats given 0.34 g in 8 ml kg body weight tom-tom mix with lacasara indicated no significant difference when compared to rats given 0.22 g in 5 ml and 0.11 g in 2.5 ml kg body weight tom-tom mix with lacasara. This may point to the fact that the dose were not in concentration dependent.

Table 2. Effects of tom-tom mix with lacasara consumption on serum Na^+, K^+ and Cl^- levels of rats.

	Na^+(mEq/L)	K^+(mEq/L)	Cl^-(mEq/L)
Group 1	148.60 ± 4.39 [a]	6.82 ± 1.76 [a]	111.20 ± 6.14 [a]
Group 2	170.60 ± 2.96 [b]	10.80 ± 0 .82 [b]	125.60 ± 5 .54 [b]
Group 3	168.40 ± 1.94 [b]	10.60 ± 5.58 [b]	124.40 ± 8.08 [b]
Group 4	150.60 ± 23.17 [a]	6.58 ± 1.74 [a]	113.40 ± 14.02 [a]
Group 5	160.20 ± 19.71 [c]	8.16 ± 4.55 [a]	123.60 ± 18.56 [b]
Group 6	163.20 ± 21.11 [c]	9.18 ± 0.85 [a,b]	120.80 ± 26.15 [b]
Group 7	143.40 ± 29.95 [a]	7.20 ± 2.71 [a]	110.00 ± 25.79 [a]
Group 8	188.40 ± 1.67 [d]	12.86 ± 2.28 [b,c]	136.20 ± 1.92 [c]
Group 9	187.60 ± 43.78 [d]	12.66 ± 5.43 [b,c]	135.80 ± 16.39 [c]
Group 10	188.00 ± 50.36 [d]	12.74 ± 10.43 [b,c]	136.00 ±45.30 [c]

Values are given in mean ± SD. Mean values (n=5) in the same column with different letter differ at p<0.05.

In this study, a significant decrease ($p<0.05$) in liver and kidney SOD, CAT activities and increase in MDA level of rats given lacasara at 8 ml, 5 ml/kg body weight, tom-tom at 0.34 g/kg body weight, 0.34 g in 8 ml, 22 g in 5 ml and 0.11 g in 2.5 ml/ kg body weight of tom-tom mix with lacasara in comparison to the control (Table 3 and 4), were observed. Chronic consumption of tom-tom, lacasara and tomtom mix with lacasara at high dose may induced oxidative stress which may lead to alteration of antioxidant enzymes and lipid peroxidation. This is in accordance to the work of Alkhedaide *et al.* (2016) who reported that chronic consumption of soft drink -induced oxidative stress, which may cause hepatic damage and nephrotoxicity, as indicated by the increase in MDA and the decrease in SOD and catalase levels. Soft drinks are the predominant source of sugar and are associated with obesity in children and adolescents. Soft drinks favor the incidence of insulin resistance and inflammation, and other diseases, including obesity, type-2 diabetes, osteoporosis and low nutrient level Alkhedaide *et al.* (2016). Also, significant increase was recorded in SOD, CAT activities and decrease in MDA level in the liver and kidney of rats given 2.5 ml/kg body weight lacasara compared to higher dose of 8 ml and 5ml /kg body weight lacasara.

Interestingly, low dose of tom-tom at 0.11 g/kg body weight significantly increase ($p<0.05$) in SOD, CAT activities and decrease MDA level in the liver and kidney in comparison to higher dose of 0.22g and 0.34 g/kg body weight tom-tom. This finding of this study suggests that decrease in MDA of rats given low dose of lacasara over high dose would be prudent to recommend decrease in soft drink consumption. The fact that soft drinks offer energy with little accompanying nutrition, displace other nutrient sources, and are linked to alteration of biochemical parameters and several key health conditions such as diabetes is further impetus to recommend a reduction in soft drink consumption (Huang *at al.,* 2018; Vartanian *et al.,* 2007).

Table 3. Liver SOD, CAT activities and MDA level of rats administered tom-tom mix with lacasara

	Liver SOD (Units/g wet tissue)	Liver CAT(Units/g wet tissue)	Liver MDA(Units/g wet tissue)
Group 1	15.35 ± 2.95 [a]	25.27 ± 3.83 [a]	0.18 ± 0.02 [a]
Group 2	8.21 ± 2.55 [b]	17.19 ± 7.22 [b]	1.19 ± 0.25 [b]
Group 3	11.27 ± 1.74 [b,c]	20.30 ± 2.64 [a,b]	1.01 ± 0.38 [b]
Group 4	16.19 ± 4.38 [a]	25.18 ± 7.17 [a]	0.19 ± 0.02 [a]
Group 5	10.31 ± 3.63 [b,c]	19.19 ± 2.90 [a,b]	0.83 ± 0.19 [a,b]
Group 6	11.50 ± 1.58 [b,c]	21.05 ± 5.28 [a,b]	0.29± 0.22 [a]
Group 7	15.63 ± 4.24 [a]	27.42 ± 2.11 [a]	0.15 ± 0.03 [a]
Group 8	7.33 ± 2.47 [b]	14.35 ± 3.37 [c]	2.01 ± 0.38 [b]
Group 9	7.27 ± 4.27 [b]	15.26 ± 7.97 [c]	2.01± 0.63 [b]
Group 10	7.31 ± 2.40 [b]	14.28 ± 2.26 [c]	1.98 ± 0.12 [b]

Values are given in mean ± SD. Mean values (n=5) in the same column with different letter differ at p<0.05.

Table 4. Changes in kidney SOD, CAT and MDA level of rats given tom-tom mix with lacasara

	Kidney SOD (Units/g wet tissue)	Kidney CAT(Units/g wet tissue)	Kidney MDA (Units/g wet tissue)
Group 1	10.30 ± 2.25 [a]	20.26 ± 3.65 [a]	0.16 ± 0.02 [a]
Group 2	6.17 ± 0.67 [b]	12.28 ± 1.49 [b]	0.79 ± 0.36 [b]
Group 3	8.37 ± 1.52 [b]	15.71± 2.149 [b,c]	0.61 ± 0.22 [b]
Group 4	10.32 ± 1.35 [a]	19.15 ± 1.61 [a]	0.11 ± 0.04 [a]
Group 5	8.29 ± 3.71 [b]	14.11 ± 2.84 [b]	0.43 ± 0.14 [c]
Group 6	9.50 ± 3.59 [a,b]	14.19 ± 3.189 [b]	0.21 ± 0.08 [a]
Group 7	12.53 ± 1.64 [a,c]	23.18 ± 3.57 [a]	0.15 ± 0.02 [a]
Group 8	5.01 ± 1.84 [b]	9.50 ± 3.28 [b]	0.94 ± 0.19 [d]
Group 9	5.27 ± 0.16 [b]	10.35 ± 2.45 [b]	1.00 ± 0.39 [d]
Group 10	5.29± 0.69 [b]	10.10 ± 3.48 [b]	1.01 ± 0.22 [d]

Values are given in mean ± SD. Mean values (n=5) in the same column with different letter differ at p<0.05.

The kidney histology of rats administered tom-tom mix with lacasara is presented in plates 1 to 10. . The kidney histology of the normal rats (group 1), group 4 and 7, showed normal proximal tubular cells with glomerulus attached to bowman's capsule. Interestingly, 2.5 ml/kg body weight lacasara (group 4) and 0.11 g/kg body weight tom-tom (group 7) had similar architecture (glomerulus was well attached to bowman's capsule) in comparison with the control (Plates 1 - 10). The rats in group 2, 3, 5, 8, 9 and 10 showed glomerulus detachment from bowman's capsule, inflammation of proximal tubule and inflammation of tubular cells. These effect may connect with the alteration observed in serum urea, creatinine and electrolyte in this study.

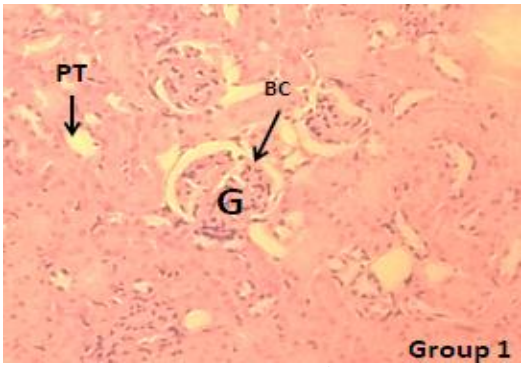

Plate 1: Group 1: Rat kidney histology of normal control: showing normal proximal tubular cells (PT) with glomerulus (G) attached to bowman's capsule (BC) (H&E stain x400).

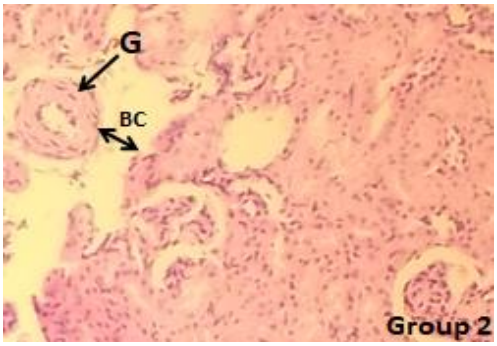

Plate 2. Group 2: Rat kidney histology given 8 ml/kg body weight lacasara, showing glomerulus (G) detachment from bowman's capsule (BC) (H&E stain x400).

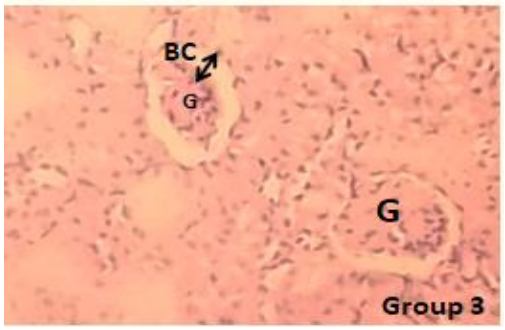

Plate 3. Group 3: 5 ml/kg body weight lacasara, showing mild detachment of glomerulus (G) from bowman's capsule (BC) (H&E stain x400).

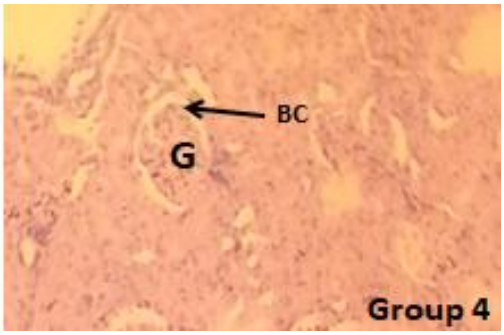

Plate 4. Group 4: 2.5 ml/kg body weight lacasara, showing glomerulus (G) attached to bowman's capsule (BC) (H&E stain x400).

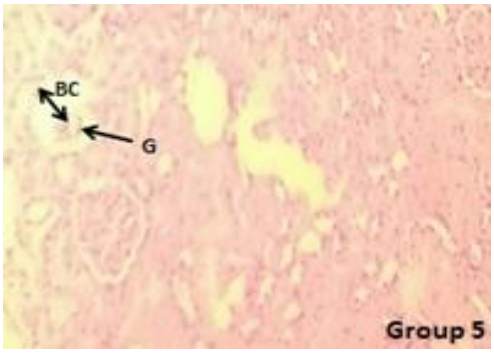

Plate 5. Group 5: 0.34 g/kg body weight tom-tom, showing mild detachment of glomerulus (G) from bowman's capsule (BC) (H&E stain x400).

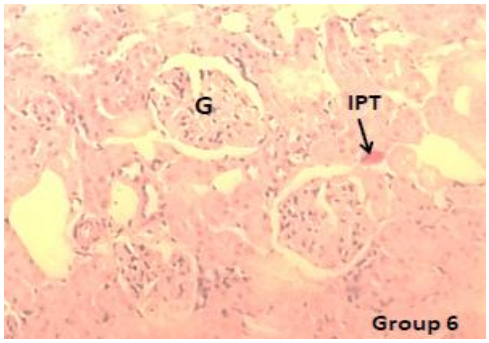

Plate 6. Group 6: 0.22 g/kg body weight tom-tom, showing inflammation of proximal tubule (IPT) (H&E stain x400).

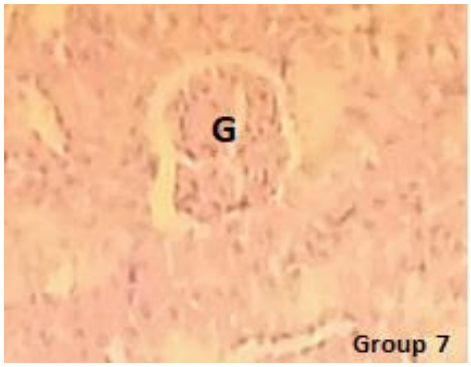

Plate 7. Group 7: 0.11 g/kg body weight tom-tom, showing glomerulus (G) attached to bowman's capsule (BC)

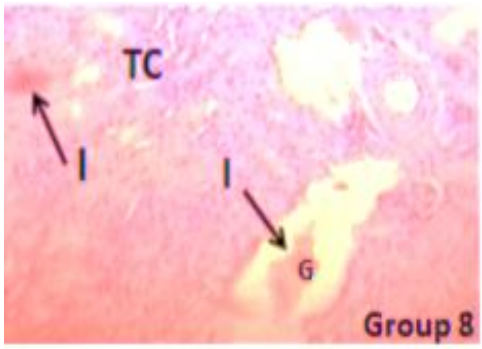

Plate 8. Group 8: 0.34 g in 8 ml kg body weight tom-tom mix with lacasara, showing inflammation of tubular cells (TC) and glomerulus (G) (H&E stain x400).

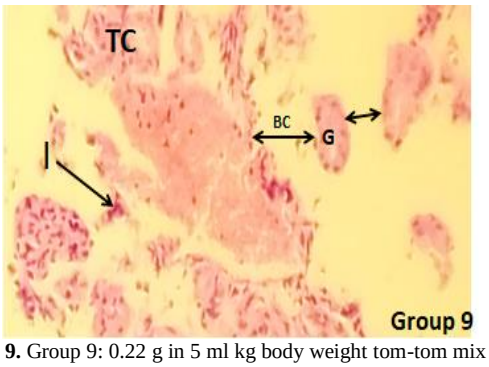

Plate 9. Group 9: 0.22 g in 5 ml kg body weight tom-tom mix with lacasara, showing inflammation (I) of tubular cells (TC) and detachment of glomerulus (G) from bowman's capsule (BC) (H&E stain x400).

Plate 10. Group 10: 0.11 g in 2.5 ml kg body weight tom-tom mix with lacasara, showing proximal tubules (PT) and detachment of glomerulus (G) from bowman's capsule (BC) (H&E stain x400).

Conclusion: This study warns about the consequences of chronic consumption of soft drink mixed with menthol candy in regards to alterations of oxidative enzymes, electrolytes and renal architecture. Additionally, the findings of this study also emphasize on the moderate intake of soft drink and menthol candy in regards to avoidance of renal damage.

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