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Research Article

Comparative study of antidiarrheal potential and genotoxicity in mice exposed to corn, sorghum and millet steep liquors

Faoziyat A. Sulaiman¹, Oluyinka A. Iyiola², Muinat O. Kazeem³, Abdulfattah A. Sulaiman⁴, Amina M. Ahmed El-Imam³, Adisa M. Jimoh⁵, Olaoluwa J. Ademola², Adekunle S. Babalola¹, Elizabeth O. Sunday-Nwaso¹.

¹Department of Biochemistry, University of Ilorin, P.M.B 1515, Ilorin, Nigeria.

²Cell Biology and Genetics Unit, Department of Zoology, University of Ilorin, Ilorin, Nigeria.

³Department of Microbiology, University of Ilorin, P.M.B 1515, Ilorin, Nigeria.

⁴Federal Medical Centre, Ebute Metta, Lagos, Nigeria.

⁵Department of Chemistry, Ibrahim Badamosi Babangida University, Lapai, Niger State, Nigeria.

Correspondence: Faoziyat A. Sulaiman¹; faoziyat20022002@yahoo.com

ABSTRACT: In this study, the antidiarrheal, cytotoxic and genotoxic properties of sorghum, millet and corn steep liquors as well as mistletoe extract in wistar rats were investigated. Comparative analysis of antidiarrheal potentials of sorghum, millet and steep liquors with standard drug (loperamide) and oral rehydration solution (ORS) in a castor oil-induced diarrheal model was carried out. Blood was examined for serum biochemical parameters and genotoxic effects were investigated using micronucleus test. In the antidiarrheal test, the test agents showed action which was significant ($P < 0.05$). Mistletoe extract exerted maximum inhibition (55.56 %) against defecation, whereas 44.44 % and 59.26 % inhibitions were obtained for ORS and loperamide respectively. The genotoxicity studies of these steep liquors and mistletoe extract revealed damage to the genetic material and alteration of the activities of some marker enzymes, which is indicative of tissue damage. Although sorghum, millet and corn steep liquors, as well as mistletoe extract have anti-diarrheal activities, their indiscriminate usage, at high concentrations should be discouraged.

KEYWORDS: Antidiarrheal, micronucleus, cytotoxicity, mistletoe, cereals

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INTRODUCTION

Diarrhea is a common symptom of gastrointestinal infections caused by a wide range of pathogens, including bacteria, viruses and protozoa (UNICEF/WHO, 2009; Nyantekyi *et al.*, 2010). Nearly one in five children deaths is due to diarrhea, a loss of about 1.5 million lives each year (UNICEF/WHO, 2009). The toll is greater than that caused by AIDS, malaria and measles combined. Diarrhea that occurs due to unsafe water and lack of sanitation is the greatest cause of morbidity and mortality in under-five children in the world, especially in poor countries (UNICEF/WHO, 2009; USAID, 2010; Ahs *et al.*, 2010). Africa and South Asia are home to more than 80 per cent of child deaths due to diarrhea. Diarrhea is a leading cause of death during complex emergencies and natural disasters. The lack of adequate health services and transport reduces the likelihood of prompt and appropriate treatment of diarrhea cases in emergencies (UNICEF/WHO, 2009).

Several treatment options are available to treat diarrhea; these include oral rehydration solution (ORS), zinc tablet supplementation, antibiotics, just to mention a few. The use of anti-diarrheal drugs, vaccines and other products or methods has decreased the devastating impact of infectious diseases and improved quality of life. However, most of the etiological agents, especially bacteria have already developed resistance to most of the commonly employed antibiotics (Ashebir and Ashenati, 1999). Also, some of these antibiotics can also induce diarrhea known as “antibiotic induced diarrhea” (Chessbrough, 1994; Marteau *et al.*, 2002). Therefore, there is need to find alternative means of treatment for this disease.

Plant-derived natural products represent an attractive source of antidiarrheal agents because in spite of being natural and affordable, they are usually easy to access, especially for rural societies (Ghosh *et al.*, 2008). In the absence of ORS, homemade fluids are used in rural communities. Such fluids can be prepared at home using readily available and low-cost ingredients. Cereal-based ORS represent an attractive source of antimicrobial agents because they are natural and affordable, especially for rural societies. Fermented foods from corn, sorghum and millet have been known to exhibit health-promoting properties such as control of gastroenteritis in animals and man. This has been demonstrated by locals who drink the raw slurry when there is a case of diarrhea.

However, there is no documented work on the probiotic efficacy of sorghum steep liquor (SSL), millet steep liquor (MSL), corn steep liquor (CSL) and mistletoe in treating diarrhea. Therefore attempt have been made in this current study to investigate their efficacy. This work was carried out to assess the antidiarrheal activity of mistletoe and liquors prepared from sorghum, millet and corn as well as assess their possible genotoxicity and cytotoxicity in wistar rats.

MATERIALS AND METHODS

Steep liquor preparation from cereals

Healthy grains of corn, millet and sorghum were purchased from a local market in Ilorin, Nigeria. The grains were picked and washed thoroughly with clean water until totally free of dirt. The grains were oven-dried under hygienic condition at 37°C. Thereafter, they were grinded with sterilized blender. 200g of each cereal was measured into a separate, appropriately labeled 1L flask and soaked with distilled water. The content of each flask was then allowed to stand and ferment for five days, during which the pH was taken on a daily basis, for a period of four days.

Experimental Animals

Forty female wistar rats (*Rattus norvegicus*) averaging 183 ± 46g were used for this work. Animals were purchased from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Animals were housed in clean plastic cages, placed in a well-ventilated housing condition and were fed with rat feed (Vital Feed®, Jos, Nigeria) and tap water *ad libitum* throughout the period of the experiment. The cages were cleaned of wastes on a daily basis. The feeds and water container were washed and cleaned on daily basis throughout the period of the experiment. Animals were acclimatized for twelve days. Experimental procedure involving the use of animals was conducted following the guidelines on the care and use of laboratory animals of the ethical committee of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Animal groupings, treatments and the induction of diarrhea

Forty rats fasted for 24 hours were completely randomized into 8 groups (n = 5). Group A received normal saline and served as the positive control (no diarrhea induction). Animals in Group B received normal saline and they were not treated post-induction; they served as the negative control. Groups C, D, E, F, G and H received orally 1 ml loperamide, ORS, SSL, MSL, CSL and mistletoe respectively, post-induction.

One hour after oral administration of treatments, the animals received castor oil (1 ml) orally to induce diarrheal and they were individually placed in cages, the floor of which were lined with white nylons to collect the faecal droppings for proper observation. The weights of their faecal droppings after 4 hours were taken. The weight of the stools passed by the treated groups was compared with that of the control groups. The weight of diarrheal faeces pooled by the control group was considered as 100%. The level of inhibition (%) of defecation brought about by the treatments was calculated relative to the control using the following relationship:

Inhibition of defecation (%) = $[(WDC - WDT)/WDC] \times 100$

where WDC = weight of diarrhoeic faeces of the control group; WDT = weight of diarrhoeic faeces of the treated group.

Preparation of serum and tissue homogenates

The rats were sacrificed as previously described by Sulaiman *et al.* (2015). Diagnostic kits for aspartate aminotransferase (AST), alanine transaminase (ALT), bilirubin (BIL), creatinine (CREA), urea and albumin were purchased from Randox laboratories Ltd, UK. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities were determined according to the method of Reitman and Frankel (1957). Urea was determined according to Weatherburn (1967), while creatinine was measured according to Henry *et al.* (1974). Albumin was measured according to Doumas *et al.* (1971).

Micronucleus (MN) test

Blood samples were smeared on clean, grease free frosted glass slides. Slides were fixed in methanol for 10 minutes, left to air-dry at room temperature and finally stained with freshly prepared 5% Giemsa stain in three intervals for 20 minutes. Thereafter, slides were mounted with canada balsam. Three slides were prepared from each of the 5 specimen per concentration per liquor for scoring of MN.

The following criteria, as suggested by Carrasco *et al.* (1990), were used for identification of micronuclei: (a) the MN must be smaller than one-third of the main nuclei, (b) MN must be clearly separated from the main nuclei, and (c) MN must be on the same plane of focus and have the same color as of main nuclei. Cells with more than four MN were discarded to exclude apoptotic phenomena. From each slide, 500 cells were scored under light microscope.

The Micronucleus (MN) frequency was calculated as follows:

$$MN \% = (N_M / N_T) \times 100\%$$

Where N_M = Number of cells containing micronucleus; N_T = Total number of cells.

RESULTS

Castor oil-induced diarrhea test

This result is presented as faecal output (g) and percentage inhibition compared with the control groups in Table 1. After a 30-minutes administration of castor oil, diarrhea was clinically apparent for the next 4 hours in group B. This condition was markedly reduced by 59.26 % by loperamide. All treatments demonstrated statistically significant ($P < 0.05$) inhibition of

castor oil-induced diarrhea in the following order: Loperamide > Mistletoe > Sorghum Steep Liquor > Millet Steep Liquor > ORS > Corn Steep Liquor.

Micronucleus test

The results of the genotoxicity assay is presented in Figure 1. The frequency of micronucleated erythrocytes in SSL, MSL and CSL-treated rats increased when compared with the control. This increase was statistically significant in MSL while it was significantly lower in the group treated with loperamide and ORS.

Table 1: Percentage (%) Faecal Inhibition in the different groups

Group	Output (g)	% Inhibition
A (+ve control)	2	-
B (-ve control)	27	-
C (Loperamide)	11	59.26
D (ORS)	15	44.44
E (SSL)	14	51.85
F (MSL)	13	48.15
G (CSL)	17	37.04
H (Mistletoe)	12	55.56

ORS, Oral Rehydration Solution; SSL, sorghum steep liquor; MSL, millet steep liquor; CSL, corn steep liquor. Output values represent faecal output in a 4-hour period.

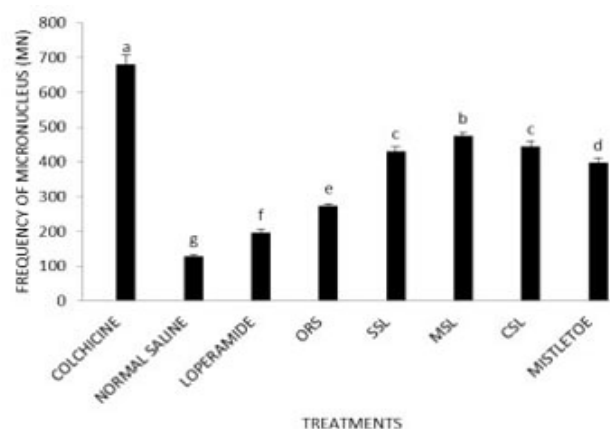


Figure 1: Frequency of micronucleus in Wistar rats treated with SSL (Sorghum), MSL (Millet), CSL (Corn) and Mistletoe.

Table 2: AST Activity in the serum, intestine and heart of treated diarrheal wistar rats

Groups	Serum (u/L)	Intestine (u/L)	Heart (u/L)
A (+ve control)	1247.8 ± 110.1 ^{a,b}	1195.7 ± 9.0 ^a	818.8 ± 12.9 ^a
B (-ve control)	1241.3 ± 219.6 ^{a,b}	1413.1 ± 17.4 ^b	752.2 ± 34.8 ^{ab}
C (Loperamide)	1554.4 ± 137.0 ^b	1213.1 ± 21.8 ^a	776.1 ± 23.9 ^{ab}
D (ORS)	1139.1 ± 84.9 ^a	1287.0 ± 96.8 ^a	804.0 ± 23.3 ^a
E (SSL)	1142.0 ± 89.8 ^a	1236.2 ± 87.0 ^a	772.5 ± 37.7 ^{ab}
F (MSL)	1245.0 ± 114.3 ^{a,b}	1223.2 ± 118.4 ^a	818.8 ± 37.3 ^a
G (CSL)	1165.2 ± 81.2 ^b	1349.3 ± 127.0 ^b	769.6 ± 19.9 ^{ab}
H (Mistletoe)	1263.8 ± 115.5 ^{a,b}	1108.7 ± 44.4 ^a	778.3 ± 15.3 ^{ab}

ORS, Oral Rehydration Solution; SSL, sorghum steep liquor; MSL, millet steep liquor; CSL, corn steep liquor. Values are expressed as mean ± S.E.M (n = 5). Values in each column with different superscript are significantly different (P<0.05)

Table 3: ALT Activity in the serum, intestine and liver of treated diarrheal wistar rats

Groups	Serum	Intestine	Liver
A (+ve control)	70.7 ± 2.0 ^a	74.7 ± 2.7 ^a	105.4 ± 11.9 ^a
B (-ve control)	68.6 ± 1.5 ^{ab}	76.2 ± 0.5 ^b	95.8 ± 16.3 ^{a,b}
C (Loperamide)	67.6 ± 3.3 ^{ab}	79.1 ± 5.2 ^c	81.3 ± 7.4 ^b
D (ORS)	75.3 ± 1.9 ^a	81.9 ± 2.9 ^a	76.7 ± 3.3 ^c
E (SSL)	71.7 ± 0.9 ^a	85.0 ± 3.1 ^e	79.1 ± 5.8 ^c
F (MSL)	72.9 ± 2.7 ^a	83.7 ± 4.7 ^a	75.4 ± 4.7 ^c
G (CSL)	69.5 ± 2.0 ^{ab}	79.5 ± 3.6 ^c	85.1 ± 5.6 ^b
H (Mistletoe)	72.0 ± 3.2 ^a	79.4 ± 2.6 ^c	87.3 ± 5.0 ^b

ORS, Oral Rehydration Solution; SSL, sorghum steep liquor; MSL, millet steep liquor; CSL, corn steep liquor. Values are expressed as mean ± S.E.M (n = 5). Values in each column with different superscript are significantly different (P<0.05).

Table 4: Bilirubin concentration in the serum and liver of treated diarrheal wistar rats

Groups	Serum (mmol/L)	Liver (mmol/L)
A (+ve control)	0.133 ± 0.019 ^{a,b}	0.150 ± 0.031 ^a
B (-ve control)	0.104 ± 0.007 ^b	0.191 ± 0.003 ^c
C (Loperamide)	0.117 ± 0.034 ^b	0.164 ± 0.015 ^b
D (ORS)	0.135 ± 0.017 ^{a,b}	0.196 ± 0.031 ^c
E (SSL)	0.091 ± 0.012 ^c	0.156 ± 0.014 ^a
F (MSL)	0.163 ± 0.009 ^d	0.225 ± 0.017 ^e
G (CSL)	0.100 ± 0.004 ^b	0.191 ± 0.003 ^c
H (Mistletoe)	0.138 ± 0.019 ^{a,b}	0.207 ± 0.026 ^d

ORS, Oral Rehydration Solution; SSL, sorghum steep liquor; MSL, millet steep liquor; CSL, corn steep liquor. Values are expressed as mean ± S.E.M (n = 5). Values in each column with different superscript are significantly different (P<0.05).

Table 5: Creatinine concentrations in the serum and kidney of treated diarrheal wistar rats

Groups	Serum (umol/L)	Kidney (umol/L)
A (+ve control)	0.108 ± 0.051 ^a	1.333 ± 0.345 ^a
B (-ve control)	0.044 ± 0.015 ^a	0.574 ± 0.182 ^a
C (Loperamide)	0.037 ± 0.008 ^{a,b}	0.560 ± 0.200 ^a
D (ORS)	0.108 ± 0.043 ^a	1.016 ± 0.179 ^c
E (SSL)	0.073 ± 0.023 ^b	0.671 ± 0.264 ^b
F (MSL)	0.137 ± 0.093 ^e	0.559 ± 0.226 ^a
G (CSL)	0.098 ± 0.026 ^c	0.923 ± 0.169 ^c
H (Mistletoe)	0.098 ± 0.034 ^c	1.119 ± 0.361 ^c

ORS, Oral Rehydration Solution; SSL, sorghum steep liquor; MSL, millet steep liquor; CSL, corn steep liquor. Values are expressed as mean ± S.E.M (n = 5). Values in each column with different superscript are significantly different (P<0.05).

Effect of treatments on enzyme activities

We used serum enzyme activities to assess the toxic effects of the experimental treatments on the animals. The results for the enzyme activities are presented in Tables 2-7.

Aspartate aminotransferase (AST)

In Table 2, group C (loperamide, the first standard) has the highest serum AST activity (1554.4) while group D (ORS, the second standard) has the lowest AST activity (1139.1) when compared with the positive control (1247.8). There is no significant difference between the AST activity of the group administered mistletoe relative to the two controls. Group B (negative control) has the highest intestinal AST activity (1413.1) while group H (mistletoe) has the lowest AST activity (1108.7) when compared with the control (1195.7). Among the four treatment groups, no significant difference was recorded except for CSL (1349.31) in which higher values were observed. The hearts of rats in group A (positive control) (818.8) and group F (MSL) (818.8) have the highest heart AST activity, which were not statistically different from each other while group B (negative control) has the least activity (752.2).

Alanine aminotransferase (ALT)

From Table 3, it was observed that group D (ORS) has the highest serum ALT activity (75.3) while group C (loperamide) has the lowest ALT activity (67.6) when compared with the control (70.7). Serum ALT activities in all the groups D, E and H are not significantly different from group A (positive control). Although, Group E (sorghum) has the highest intestinal ALT activity (85.0), nevertheless all the other treated groups have intestinal ALT activities that are higher when compared to the positive and negative controls. All treatments have liver ALT activities that are lower than that of the positive control (105.4), group A's ALT activity is also significantly higher than in other groups, since there was no liver damage caused by the liquors. Any damage to the hepatic membrane would have brought about ALT leakage from the liver into the blood.

Bilirubin

Table 4 shows that group F (millet) has the highest serum bilirubin concentration (0.163) while group E (sorghum) has the least bilirubin concentration (0.091) when compared with the positive (0.133) and negative (0.104) controls. The results from the table also show that the liver bilirubin concentration in all the test groups (B – H) are statistically higher when compared with the positive control (0.150) whereas group F (millet) has the highest bilirubin concentration (0.225) among the treatment groups.

Creatinine

From Table 5 it was observed in the serum that group F (millet) has the highest serum creatinine concentration (0.137) while group B (negative control) has the lowest concentration (0.044), however, group D is not significantly different from the positive control. All the kidney creatinine concentrations in the test groups are significantly lower than groups D (1.016) and H (1.119). Also, both groups D and H are not significantly different from the positive control.

Urea

Group G (CSL) has the highest serum urea concentration (2.280), although all the groups have elevated serum urea concentrations that are higher than that of the positive control (1.423). Group D (ORS) has the highest kidney urea concentration (13.031) while the negative control (group B) has the least concentration (1.858). The positive control (group A) has the highest liver urea concentration (12.821) (Table 6) which is not significantly different from that of group B (12.788).

Albumin

The results from Table 7 show that group C (loperamide) has the highest serum albumin concentration (11.1) compared to the control (7.0). All the groups have elevated levels of albumin in their serum than the positive control. Group H (mistletoe) has the highest liver albumin concentration (5.6) while group F (mistletoe) has the lowest concentration (2.4) compared to positive control (5.4). ORS (group D) has the highest kidney albumin concentration (3.8) with the negative control (group B) having the lowest concentration (2.3) when compared with the positive control (3.0). Groups G and H have the highest intestinal albumin concentration (2.6) while loperamide (group C) has the lowest concentration (2.1) when compared with the positive control (2.3).

DISCUSSION

The present study showed that sorghum, millet and corn steep liquors as well as the extract of mistletoe exhibited a significant inhibition of castor oil-induced diarrhea in wistar rats, that is, they possess varying degrees of anti-diarrhoeal properties. Fermented products are generally known to possess antimicrobial properties (Marugg, 1991). The most predominant microorganism involved in the fermentation of sorghum, millet or corn is *Lactobacillus plantarum* although other microorganisms may also be associated with it. *L. plantarum* is responsible for the production of lactic acid. The anti-diarrheal activity exhibited by sorghum, millet and corn steep liquors as well as the mistletoe extract, could be as a

Table 6: Urea concentrations in serum, kidney and liver of treated diarrheal wistar rats

Groups	Serum (mmol/L)	Kidney (mmol/L)	Liver (mmol/L)
A (+ve control)	1.423 ± 0.263 ^a	2.991 ± 1.100 ^a	12.821 ± 0.411 ^a
B (-ve control)	2.253 ± 0.751 ^a	1.858 ± 0.949 ^a	12.788 ± 0.020 ^a
C (Loperamide)	2.036 ± 1.127 ^a	11.810 ± 1.058 ^a	11.938 ± 1.226 ^a
D (ORS)	1.818 ± 1.121 ^c	13.031 ± 0.592 ^f	9.645 ± 1.668 ^d
E (SSL)	1.225 ± 0.237 ^b	4.950 ± 1.838 ^b	7.880 ± 0.446 ^d
F (MSL)	2.003 ± 0.244 ^a	7.590 ± 0.678 ^c	5.205 ± 1.154 ^c
G (CSL)	2.280 ± 0.533 ^a	9.434 ± 0.716 ^a	8.314 ± 0.165 ^c
H (Mistletoe)	1.529 ± 0.013 ^a	9.065 ± 1.760 ^a	6.443 ± 1.398 ^c

ORS, Oral Rehydration Solution; SSL, sorghum steep liquor; MSL, millet steep liquor; CSL, corn steep liquor. Values are expressed as mean ± S.E.M (n = 5). Values in each column with different superscript are significantly different (P<0.05).

Table 7: Albumin concentrations in the serum, liver, kidney and intestine of treated diarrheal wistar rats

Groups	Serum (g/dL)	Liver (g/dL)	Kidney (g/dL)	Intestine (g/dL)
A (+ve control)	7.0 ± 2.4 ^{ab}	5.4 ± 0.7 ^{ab}	3.0 ± 1.0 ^{ab}	2.3 ± 0.3 ^a
B (-ve control)	9.3 ± 2.6 ^c	3.1 ± 1.3 ^{abc}	2.3 ± 0.2 ^a	2.4 ± 0.6 ^a
C (Loperamide)	11.1 ± 6.8 ^a	2.8 ± 0.8 ^a	3.0 ± 0.2 ^{ab}	2.1 ± 0.1 ^a
D (ORS)	8.6 ± 0.7 ^b	3.1 ± 0.4 ^{abc}	3.8 ± 0.8 ^b	2.6 ± 0.2 ^b
E (SSL)	6.3 ± 0.7 ^a	3.9 ± 0.2 ^{abc}	3.6 ± 1.0 ^a	2.5 ± 0.2 ^b
F (MSL)	7.5 ± 1.9 ^{ab}	2.4 ± 0.8 ^c	2.8 ± 0.7 ^a	2.5 ± 0.1 ^b
G (CSL)	9.6 ± 3.2 ^c	5.5 ± 1.1 ^a	2.5 ± 0.6 ^a	2.6 ± 0.3 ^b
H (Mistletoe)	7.3 ± 1.5 ^{ab}	5.6 ± 0.8 ^b	2.9 ± 0.4 ^a	2.6 ± 0.1 ^b

ORS, Oral Rehydration Solution; SSL, sorghum steep liquor; MSL, millet steep liquor; CSL, corn steep liquor. Values are expressed as mean ± S.E.M (n = 5). Values in each column with different superscript are significantly different (P<0.05).

result of their bioactive constituents. Tannin is a constituent of these cereals and has been reported to have antidiarrheal activity (Longanga-Otshudi, 2000). Administration of the sorghum, millet and corn steep liquors and mistletoe extracts to wistar rats over forty-two days revealed some cytotoxic and genotoxic effects.

Mean body weights of the rats in other groups were slightly lower than the mean body weights of the negative control (group B). The result supported the claim by Richard (1992), where only female rats exposed to castor oil, experienced slightly lower mean body weights compared to the control. There was also a significant reduction in the weight of the rats from the second to the third week. The kidneys of the toxicology group have the largest weight compared to the diarrheal group and there was a significant increase in the weight of the positive control compared with the other concentrations in both group. This conclusion is consistent with the observed increases reported by Richard (1992). He reported that due to the presence of triacylglycerols in the castor oil, the increased kidney weights could be a reflection of elevated metabolic activity, rather than a toxic response.

The percentage inhibition of faecal discharge, which indicates the presence of anti-diarrheal properties, is shown in table 1. There was a significant increase in the anti-diarrheal properties of sorghum steep liquor and mistletoe extracts with both having the highest inhibition rates, compared to the two standards, loperamide and ORS, while there was no significant difference between loperamide and normal saline. ORS has the lowest level of inhibition. This result is consistent with the work reported by Muhammed *et al.* (2013). In their study, they reported increased anti-diarrheal property of ethanolic extract of *Ficus pumila* as compared to the conventional drug (loperamide). This may be due to the fact that the liquors increase the reabsorption of water from the intestine. Moreover, the pharmacological effect of loperamide is due to its anti-motility and anti-secretory properties (Longanga-Otshudi *et al.*, 2000).

The liquors and mistletoe extract might have exerted their antidiarrheal activity via secretory mechanism as evident from reduction in total number of wet faeces. Furthermore, this antidiarrheal activity could have resulted from the inhibitory activity of the liquors and mistletoe extract on prostaglandins synthesis, nitric oxide and platelet activating factors production, as inhibitors of prostaglandins and nitric oxide syntheses have been reported to delay diarrhea induced by castor oil (Capasso *et al.*, 2001; Adzu *et al.*, 2003; Tangpu and Yadav, 2004). From this investigation, it was likely that the SSL and mistletoe extracts mediate their anti-diarrheal effects through similar mechanisms as that of ORS and loperamide but in a more improved and specialized way.

Possible genotoxic effect of SSL on the experimental animals was assessed using micronucleus assay. The level of abnormalities in SSL treated animals was significantly different from the conventional drug groups. Comparing the micronuclei number, there was significant increase in micronuclei for SSL and the positive control. This result support the that reported by Muhammed *et al.*, (2013) with chloroform group showing the highest number of abnormalities and the most active anti-diarrheal extract, significantly higher than the conventional drug groups. Nuclear abnormalities such as notch nucleation, binucleation, lobed and blebbed nucleation were observed.

There was significant increase in the abnormalities caused by the positive group compared to other groups (B-F). The positive control group, which was administered with colchicine, has the highest aberrations due to its genotoxic property. The group treated with millet steep liquor has significant increase in the number of micronucleated cells compared to other groups. From previous studies, it was known that notch formation in the erythrocytes causes a typical nuclear abnormality that leads to genotoxic damage (Cavas and Ergene-Gozukara, 2003). It is also pertinent to note that nuclear damage in erythrocytes might be from viral infections as a non-genotoxic stressor.

The three liquors administered were able to reduce leakage of enzymes; AST and ALT into serum, implying little or no damage to the liver membrane. In deviation, increased bilirubin concentration was recorded in the serum for the liquors and mistletoe extract. This is indicative of haemolytic anaemia (Stocker *et al.*, 1987) caused by administration of the liquors and mistletoe extract

Table 5 shows the variation in creatinine concentrations in the kidney. There was a significant increase in the concentration of creatinine in the positive control compared to the negative control. However, there was no significant increase in the concentration of creatinine between the negative control, SSL and loperamide. Mistletoe and ORS also show significant increase in creatinine concentration. These results are consistent with the data reported by Richard (1992).

The level of urea concentration in the kidney of the animals in the diarrheal group (table 6) showed highest urea concentration in group E (sorghum) (6.977) which is significantly different from the positive control (1.641). The negative control, loperamide, ORS and mistletoe extract groups also have higher urea concentrations, which are significantly different from each other when compared with the positive control. This result reflects the level of abnormalities, which is similar to the result reported by Muhammed *et al.*, (2013). Group D (ORS) has the highest concentration (13.031), which could be due to the presence

of more amino-acids, and it is significantly different from the negative control, which has the least concentration (1.858). The urea level in ORS (13.031) is also significantly higher than that of the other treatments i.e. loperamide (11.810), mistletoe (9.065) and sorghum (4.950). This could be due to the presence of amino-acids like alanine, glycine and glutamine which, according to Pradip (2007), are added to its composition to improve its anti-diarrheal efficiency.

Table 7 shows the level of albumin concentration for diarrheal group with loperamide having the highest albumin concentration (5.1) which is significantly higher than the positive control rats with the lowest concentration (2.8) when compared with sorghum and mistletoe, both having the same albumin concentration (2.9). This result is consistent with work done by Gudehithlu *et al.* (2004), who also concluded that albumin is rapidly and extensively degraded by the proximal tubule cell in the kidney and absorbs it in the apical cells within. There was no significant difference in albumin concentration between loperamide (3.0), positive control (3.0) and mistletoe (2.9) among the groups. The slightly higher albumin level in sorghum (3.6) could be due to the presence of tannins, a constituent of anti-diarrheal plants. The result is consistent with work done by Havagiray *et al.* (2004), who reported that the anti-diarrheal activity of tannins could be due to their ability to denature proteins. The observed reduction in albumin concentrations in the liver of all tested groups (table 7) than the positive control indicates liver damage (Naganna, 1989), arising from the administration of the colchicine. This may be an indication of diminished synthetic function of the liver, which may consequently lead to enhanced retention of fluid in the tissues spaces (Naganna, 1989). Varying significant differences exist between all the groups (table 7). Group F (mistletoe) has the highest albumin concentration (5.6), which could be due to the presence of tannins that has the potential to denature the liver enzymes. The result is in consistence with the work done by Havagiray *et al.*, (2004). Group C (loperamide) has the lowest albumin concentration (2.8) as a standard drug compared to positive control (5.4).

Similarly, the observed increase in the cytotoxic effects of ALT, AST, bilirubin, urea and creatinine may be an indication of cytotoxic effect and diminished synthetic function of the liver, which may consequently lead to enhanced retention of fluid in the tissues spaces (Naganna, 1989). The results of the present study revealed the alterations, which appears to be more pronounced in the liver, probably because it is the organ that is mainly responsible for detoxification of foreign compounds in the body, more so since the colchicine has adversely affected the liver functional parameters investigated. This study therefore calls for caution while using this extracts to manage diarrhea, the genotoxicity studies of these steep liquors and mistletoe extract revealed damage to

the genetic material and alteration of the activities of some marker enzymes, which is indicative of possible tissue damage. Although sorghum, millet and corn steep liquors, as well as mistletoe extract have anti-diarrhoeal activities, their indiscriminate usage, at high concentrations should be discouraged.

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