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BIO-IMPLICATION OF ENVIRONMENTALLY EXPOSED MALTED PRODUCTS (CANNED AND PLASTIC MALTINA) ON BIOCHEMICAL INDICES OF ALBINO-RATS

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Abstract
Environmentally exposed carbonated drinks have no biological value; rather, they have, a long-term damaging effect when accumulated in tissues, as discovered in this work. This research was conducted to investigate the biochemical effect of exposing non-alcoholic drinks to direct sunlight or adverse environmental conditions. The experimental set up was made up of malting products consisting of the exposed (Canned and plastic) sample to sunlight for a period of 15 days alongside the unexposed sample as a control. Proximate analysis was performed on the malting samples. The effect of environmental exposure on the product was investigated in an animal model experiment. Twenty-four (24) male rats of wistar strain weighing 120-140g divided into four (4) groups of six rats each, Assessment of liver marker enzymatic; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkali phosphatase (ALP); and non-enzymatic (albumin and bilirubin) parameters were investigated. The proximate analysis and vitamin, and mineral evaluation showed a decrease in the protein, vitamins and calcium content upon exposure. Assessment of liver function in rat's serum of the experimental groups showed a significant increase ($p < 0.05$) in ALP, AST, ALT, and bilirubin and decrease in albumin levels compared with the control unexposed malting sample groups. The findings showed that environmentally exposed malting elicits adverse biochemical and physical changes and therefore portends greater long-term toxicological effects with deleterious action.

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Introduction

Malt drink is a non-alcoholic beverage obtained from unfermented wort, while maltina is a brand of malt drink which is produced by mashing grains to get sugar that is blended with other essential ingredients that provide taste, color, flavor, multivitamins and minerals. It is enriched with vitamins A, B_1, B_2, B_3, B_5, B_6, C, and calcium, which makes it a drink that can deliver superior nourishment for an active, vibrant life for all (Ristovska, 2012).

The raw materials used for the production of malting include malt, mated sorghum, raw sorghum and maize grist, hops, and water with additives such as amylex, calcium chloride (CaCl), malting complex (vitamin A, B complex and C), caramelized dextrose maltase (CDM), and lactic acid (Chaudhari, 2010).

The effect of sunlight on bottled or canned drinks is that Polyethylene terephthalate (PET), the material commonly used to make the plastic bottles in which bottled drinks are sold, can leach into the content at high temperature. The contents of the PET bottle and, the temperature at which it is stored appear, to influence the rate and magnitude of leaching of organic and inorganic compounds from PET bottles (Peter, 2008). Several studies have shown compounds in canned or plastic content at, non-negligible concentrations, thereby raising concerns about possible health implications (Mutsuga, 2006). Many laboratory animal and human studies have linked exposure to bisphenol A in Cans Polyethylene terephthalate (PET) and to adverse health effects, including altered behavior and obesity in children, reproductive abnormalities, cardiovascular changes, and various cancers (Stump *et al.*, 2010). In the food industry, food (chemical) additives are added purposely to enhance food quality (Abdulmumeen et al., 2012). While some are intentionally added, others become part of food unintentionally occurring only in trace amounts due to food packaging, storage and other handlings (Cavanaugh, 2002).

Moreso, Undernutrition is an aspect of malnutrition that is currently a global public health concern (W.H.O. 2018). Healthy eating is currently being advocated as a strategy to combat deficiency diseases associated with insufficient nutrient intake (Fraser, 2012). Maltina is a good source of fat and contains high-quality proteins in addition to carbohydrates, vitamins, and minerals, particularly calcium and magnesium. Consumers expect that the malted drinks they buy contain the nutritional qualities promised on the label; however, exposure to sunlight during the distribution/storage process can significantly erode the nutritional content of malted drinks (Noluma Light Protection. (2019).

The reasons that prompted us to investigate the nutritional composition and the bio-implication of environmentally exposed malted products (maltina brand) on some biochemical parameters in albino-rats are centered on the facts that maltina as a malt drink which is produced by mashing of grains to get sugar that is blended together with other essential ingredients which provides taste, color, flavor, multivitamins and minerals, on exposure to sunlight, these essential ingredients could be broken down or altered to some by products alongside the leaching of polyethylene terephthalate (PET) from the bottle into the malting content, which may be hazardous to health.

The liver and kidneys are considered the primary targets for toxicopathological manifestations, and there are reports of biochemical alterations indicative of hepatic and renal system involvement.

The liver and kidneys are considered the primary targets of toxicopathological manifestations that might arise from environmental exposure. Maltina is a popular beverage drink consumed, globally, especially in tropical regions with harsh environmental conditions.

The nutritional content and biochemical properties of maltina can be affected by environmental exposure, potentially affecting consumer health. Nevertheless, the impact of environmental exposure on the nutritional and biochemical properties of malting remains unclear. As a result, this study becomes necessary to investigate.

Materials and methods

Sample collection: Both canned and plastic malted products were obtained from Nigeria Breweries Limited, Markurdi plant in Benue State and transported on an ice bath to a cold room in Anyigba until required for use.

Treatment of samples: The products were exposed to adequate sunlight between 11:00 am and 4:00 pm for 15 days, with the corresponding daily average temperature taken for each day as follow; 31 ^{o}C, 32 ^{o}C, 31 ^{o}C, 32 ^{o}C, 30 ^{o}C, 32 ^{o}C, 30 ^{o}C, 30^{o}C, 29 ^{o}C, 33^{o}C, 31^{o}C, 29^{o}C, 30^{o}C, 31^{o}C, 30^{o}C; for 15 days, the average daily temperature was 30.7^{o}C. Proximate analysis was performed before and after exposure to sunlight for 15 days to, determines the effects of sunlight on the available nutrients in the malted products.

Experimental Animals: Twenty-four **(**24) adult male albino rats were purchased from the experimental animal house in the Biochemistry Department, Faculty of Natural Sciences, Prince Abubakar Audu, Anyigba, Kogi State, Nigeria. The animals were housed in standard environmental conditions in the same animal facility. The experimental animals were fed standard rodent diets and clean water within the period of administration.

Weight measurement: The weights of the albino rats were measured before the commencement of the administration of the samples, on day 7, prior to the first phase of animal sacrifice and repeatedly measured at the end of week two on day fifteen (15).

Experimental design

Animal Grouping: Twenty-four adult male albino rats divided into 4 groups with 6 rats in each group were used in this Experiment. Group 1- rats were fed with normal feed and distilled water ad libitum (control group); group 2- rats were given oral administration of malt unexposed to the environment (unexposed group) 3ml; 1ml at a time in three separate administration/day + normal feed and water ad libitum. Group 3 rats were administered oral administration of 3ml total of exposed canned malting (3ml; 1ml at a time in three separate administration /day) + normal feed and water ad libitum. Group 4: rats were given oral administration of 3ml exposed Plastic malting (3ml; 1ml at a time in three separate administration /day) + normal feed and water ad libitum (Plastic maltina).The twenty-four adult male albino rats were divided into 4 groups with 6 rats in each group.

Group 1- rats were fed with normal feed and distilled water *ad-libitum* (control group); group 2- rats were given oral administration of malt unexposed to the environment (unexposed group) 3ml; 1ml at a time in three separate administration/day + normal feed and water ad libitum.

Group 3 rats were administered oral administration of 3ml total of exposed canned malting (3ml; 1ml at a time in three separate administration /day) + normal feed and water ad libitum. Group 4: rats were given oral administration of 3ml exposed plastic malting (3ml; 1ml at a time in three separate administration /day) + normal feed and water *ad-libitum* (Plastic maltina).

Administration of Products: The malt product was administered orally via oral gavage. Administration of the product lasted for 15 days. Once daily at 24 hours interval. Three (3) rats were sacrificed from each group 24 hours after the 7th and 15th doses.

Animal sacrifice and preparation of serum: After appropriate dose and completion of the experiment, the rats were anesthetized with diethyl ether and sacrificed via cardiac puncture. The collected blood samples were placed into capped non-EDTA bottles and; centrifuged using a Heraus Christ GMBH Osterode refrigerated centrifuge at 4000 rpm for 30 minutes and the serum was collected using a Pasteur's pipette. This was stored in a refrigerator for biochemical analysis.

Proximate composition and energy value: The proximate compositions of the maltina samples were determined using the Official Methods of Analysis of AOAC (2000). However, the carbohydrate content was

estimated by the difference. The energy value of the sample type was calculated using the formula reported by Sanchez-Pena *et al.* (2016). Total energy value (Kcal/100g) = (4 x % protein) + (4 x % carbohydrate) + (9 x % fat).

Determination of biochemical parameters: The biochemical parameters (AST, ALT, ALP, Direct Bilirubin and Total Bilirubin) were measured using the colorimetric method as described by (Reitman and Frankel, 1957; Haussament, 1977; Rutkowski and Debaare, 1966) and, outlined in Randox Laboratory test kits.

Data Analysis: This was conducted using One-way ANOVA, SPSS version 20.0 software. Results were expressed as mean ± standard deviation of triplicate values. Separation of Mean was conducted for test of significance at ($p>0.05$).

Results

Table 1: Proximate compositions and energy values of unexposed and exposed canned and plastic malting samples.

Sample	Protein Content (%)	Fat Content (%)	Carbohydrate Content (%)	Energy value (kcal)
Unexposed Maltina	0.20 ± 0.01^{b}	0.10 ± 0.01^{b}	14.40 ± 0.30^{a}	61.47 ± 2.05^{a}
Exposed (Canned) Maltina	0.11 ± 0.01^{a}	0.08 ± 0.01^{a}	14.20 ± 0.30^{a}	59.60 ± 3.30^{a}
Exposed (Plastic) Maltina	0.13 ± 0.02^{a}	0.10 ± 0.01^{b}	14.13 ± 0.20^{a}	60.94 ± 2.11^{a}

Values are expressed as mean ± SEM (where n=3). Values with the same letter in the same column were not significantly different at (p > 0.05).

The proximate composition and energy value of unexposed and exposed and Plastic Maltina Samples (Table 1) show that there was a significant decrease ($p < 0.05$) in protein content between the unexposed and exposed malting samples group. Fat content; there was significant difference ($P < 0.05$) between the unexposed and exposed canned malting sample group. No significant deference was observed in carbohydrate and energy content between the groups ($P > 0.05$).

Table 2: Vitamin composition (mg/100ml) of unexposed, exposed can and plastic malting samples

Sample	Vit. A	Vit. B_1	Vit. B_2	Vit. B_3	Vit. C
Unexposed Maltina	0.20 ± 0.03^{b}	0.10 ± 0.02^{b}	0.20 ± 0.03^{b}	2.00 ± 0.02^{c}	5.00 ± 0.01^{c}
Exposed (Canned) Maltina	0.14 ± 0.01^{a}	0.07 ± 0.01^{a}	0.18 ± 0.03^{a}	1.91 ± 0.01^{a}	3.04 ± 0.22^{a}
Exposed (Plastic) Maltina	0.18 ± 0.01^{a}	0.08 ± 0.02^{a}	0.20 ± 0.02^{b}	1.95 ± 0.02^{b}	4.04 ± 0.06^{b}

Values are expressed as mean ± SEM (where n=3). Values with the same letter in the same column were not significantly different at (p > 0.05).

Vitamin composition of the samples in Table 2 shows that Vitamin A was significant difference ($p < 0.05$) higher in group administered unexposed malting than those given exposed malting groups. Vitamin B_1, B_2 and B_3showed significantly different values in all groups. Vitamin C showed significantly low value in the exposed malting sample group compared to the unexposed.

Table 3: Mineral composition (mg/100ml) of unexposed, exposed canned and plastic malting samples.

Sample	Ca	Na
Unexposed Maltina	45.0±0.01[b]	0.20 ±0.01[a]
Exposed (Canned) Maltina	38.27±0.25[a]	0.20 ± 0.01[a]
Exposed (Plastic) Maltina	43.07±2.4[b]	0.20 ± 0.01[b]

Values are expressed as mean ± SEM (where n=3). Values with the same letter in the same column were not significantly different at (p > 0.05).

The calcium content of the malting samples in Table 3 shows that exposed canned and plastic malting samples had a significantly lower calcium content ($p < 0.05$) than the unexposed group. There were no significant differences in sodium content between the groups.

Table 4: Percentage weight-gain of rats in each group during fifteen (15) days of feeding (n = 6)

Sample	Average Initial Weight (g) Day 0	Weight increase in % Day 7	Day 15
Control	126	5 (6.3g)	9 (11.34g)
Unexposed Maltina	119	7 (8.3g)	11 (13.09g)
Exposed (Canned) Maltina	142	5 (7.1g)	9 (12.78g)
Exposed (Plastic) Maltina	123	6 (7.4g)	9 (11.07g)

Values are expressed as mean ± SEM (where n=3).

Percentage (%) weight increase of animals in each group after the 7th and 15thday of feeding is as shown in Table 4 above; control (naive), 5% ,9% ; positive control, 7%, 11%; canned malting, 5%, 9% and plastic, 6%, 9%

As shown in Table 1, animals in the unexposed malting group gained more weight than those in the other groups. This variation in %weight gain by animals shows that the unexposed malting sample has all its nutrients active, which in turn gives the animals enough nourishment to gain such weight, while the environmentally exposed drinks might have been affected by environmental factors resulting in reduction of its nutrient.

Table 5: Effects of oral administration of unexposed, exposed canned, and plastic malting on serum levels of AST, ALT and ALP

	AST (U/ L)		ALT (U/L)		ALP (U/L)	
Group/Day	7	15	7	15	7	15
Control	70.47 ± 2.08[a]	70.59 ± 2.87[a]	19.67± 2.67[a]	21.33±1.67[b]	42.16± 1.30[a]	42.01±1.16[a]
Unexposed	71.28 ± 1.32[a]	71.42 ± 2.27[a]	20.00 ± 0.01[a]	44.10 ±2.85[b]	44.10 ±2.85[b]	45.49±1.41
Exposed Can	75.42 ± 1.68[c]	88.25 ± 3.01[c]	22.03 ± 0.01[b]	47.06± 2.05[c]	47.06± 2.05[c]	54.88±0.10[c]
Exposed Plastic	73.32 ± 1.81[b]	75.87± 1.73[b]	21.33±1.67[b]	21.33±1.67[b]	46.22 ±1.5[c]	56.70±1.93[c]

Values are expressed as mean ± SEM (where n=3). Values with the same letter in the same column were not significantly different at (p > 0.05).

Enzyme assay of serum samples of albino rats: From the enzyme assay of the serum samples of albino rats in Table 5, the concentration of Aspartate aminotransferase (AST) was significantly difference ($p<0.05$) between the exposed and unexposed groups after days 7 and 15.

The concentration of Alanine aminotransferase (ALT) showed a significant difference ($p<0.05$) between the unexposed and exposed groups after days 7 and 15. The concentration of alkaline phosphatase (ALP) showed significant difference ($p<0.05$) between the control, unexposed, and exposed groups at day 7 and day 15.

Table 5: Effects of oral administration of unexposed, exposed canned, and plastic malting on serum albumin, total bilirubin, and direct bilirubin levels

	Albumin (g/dL)		Total bilirubin level (mg/dL)		Direct bilirubin level (mg/dL)	
Group/Day	**7**	**15**	**7**	**15**	**7**	**15**
Control	4.17 ± 0.12^{b}	4.57 ± 0.41^{b}	0.63 ± 0.05^{a}	0.68 ± 0.08^{a}	0.19 ± 0.02^{a}	0.21 ± 0.02^{a}
Unexposed	4.27 ± 0.08^{c}	4.58 ± 0.36^{b}	0.64 ± 0.18^{a}	0.64 ± 0.03^{b}	0.19 ± 0.05^{a}	0.20 ± 0.01^{a}
Exposed Can	3.23 ± 0.06^{a}	3.17 ± 0.33^{a}	1.67 ± 0.16^{b}	1.74 ± 0.08^{c}	0.32 ± 0.05^{b}	0.33 ± 0.03^{b}
Exposed Plastic	3.10 ± 0.18^{a}	3.30 ± 0.17^{a}	1.64 ± 0.21^{b}	1.70 ± 0.11^{c}	0.41 ± 0.06^{b}	0.44 ± 0.04^{b}

Values are expressed as mean ± SEM (where n=3). Values with the same letter in the same column were not significantly different at (p > 0.05).

Liver function test presents the following: From Table 6 above; at day 7 and 15, albumin values for exposed canned and exposed plastic groups were significantly lower (p < 0 05) result compared to the unexposed and control groups. This hypoalbuminemia result indicates liver and kidney damage in the affected groups.

Total bilirubin: the value for exposed canned malting had a significant difference ($p<0.05$) from the other groups at day 7, whereas at day 15, both the exposed canned and exposed plastic are significantly high ($p<0.05$) from other groups.

Direct bilirubin level: at day 7, the values showed no significant difference was observed in all groups. On day 15, both the exposed canned and plastic malting groups had significantly different values from the other groups. This implies that liver damage occurred.

Discussion

Light energy in the ultraviolet and visible light regions plays a critical role in overall food quality, leading to various degradation and oxidation reactions (Duncan and Chang, 2012). Food degradation and oxidation result in the destruction of nutrients and bioactive compounds, as seen in the proximate analysis result, which is verifiable in the body weight gain of the animals in their respective groups. Therefore, any loss in the nutrient quality of the maltina drink used in this research work could be because of display of the product in direct sunlight and other unfavorable environmental conditions. Vitamins are some minerals that are highly sensitive and can be, affected by a variety of factors, such as temperature, light, oxygen, pH, reducing agents, oxidizing agents, metal ions etc. Yenny., *et al* (2022). Thus, in line with the work of Yenny, *et al* (2022), results from the proximate composition, vitamins, and minerals of environmentally exposed malted products showed some level of reduction compared to unexposed malting drinks.

ALT and AST are cytosolic enzymes that are commonly used as complementary indicators of liver damage. The observed significant increase in serum as observed during this research implies, that the integrity of the membrane of liver cells has been compromised. This is particularly evident in the high level of activities of ALT in the serum of the exposed group maltina.

Conclusion

Proximate composition and energy, Vitamins composition, mineral composition, and liver function tests both enzymatic and non-enzymatic have shown that environmentally exposed carbonated drinks have no biological value, rather a long-term damaging effect when accumulated in the tissues as discovered in this work.

Conflicts of interest: The authors declare no conflict of interest.

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