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Hepatoprotective Effect of Corn Silk Extract on Carbonated Alcoholic Herbal Beverages Induced Hepatoxicity in Adult Wistar Rats

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ABSTRACT: Corn silk is the long shiny fibers at the top of an ordinary ear of corn (Zea mays). Corn silk contains phytochemicals of medical benefit such as flavonoids compounds which act as antioxidant agents and has been widely reported possess hepatoprotective effect. The objective of this paper is to evaluate the hepatoprotective effect of corn silk extract on carbonated alcoholic herbal beverages (CAHB) induced hepatoxicity in adult Wistar rats. A total of forty five adult Wistar rats weighing between 200g and 250g were randomly assigned into nine groups of five rats each in a group. Group A was the normal control group (no CAHB administration). Groups B, C, D, E, F, G, H and I were the CAHB intoxicated groups and treated with corn silk extract at different doses. After administering a carbonated alcoholic herbal beverage, there was a substantial increase ($p \leq 0.05$) in the mean concentration of liver enzymes (ALP, ALT, ALT), total bilirubin, and direct bilirubin. While those characteristics reversed to values similar to the control after being treated with varying doses of corn silk extract The oxidative stress parameter (SOD, GPX, Catalase, MDA) shown significant increase in group. Histological examination of the liver revealed infiltrate of inflammatory cells, vascular ulceration, periportal infiltration of inflammatory cell, and vascular congestion in a group treated with carbonated alcoholic herbal beverages (CAHB). The only group exhibiting normal hepatocyte morphology and kupffer cell activity was the one administered 200 mg/kg of maize silk extract. Group D showed a similar and more powerful hepatic architecture after receiving only 600 mg/kg of maize silk extract. The hepatoprotective and anti- oxidative effects of corn silk extract were confirmed by the treatment groups (200 mg/kg and 600 mg/kg), which displayed normal hepatocytes with minor vascular congestion, respectively. Corn silk administration lowers a number of the harmful effects of in vivo carbonated alcoholic herbal beverage administration in the liver of Wister rats, according to the findings in this study.

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The liver is the hub of the body's metabolism and performs a variety of intricate tasks. However, because the liver sustains injuries the most frequently, liver damage frequently results in liver failure (Sudoyo *et al.*, 2009). Global liver damage prevalence indicates a significant number to be aware of (Poli and Parola, 1997). There are over 2 billion cases of hepatitis worldwide, and the disease claims the lives of over

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350,000 people annually. According to WHO (2011), cirrhosis and liver cancer are two more serious liver function disorders that patients with hepatitis are at a high risk of developing. According to Podolsky and Isselbacher (2002), liver illness is ranked third after infectious and pulmonary diseases. The use of hepatotoxic medications is one of the causes. Hepatotoxic effects have the potential to cause very serious liver damage. Alcohol has many different harmful effects. According to Abubakar *et al.* (2015), alcohol addiction is acknowledged globally as a leading cause of morbidity and mortality, with substantial health and financial ramifications. The most often abused substance among young people in Nigeria is reportedly herbal alcoholic drinks (Makinde, 2014). Due to the consumer belief that these herbal alcoholic drinks contain body purifiers, antimalarial components, and ingredients that strengthen men's virility, they are consumed by both young and old at higher rates than their counterparts (Badmus, 2014). Carbonated alcoholic herbal beverages can have both negative and positive effects. Acute and sub-chronic use of carbonated alcoholic herbal beverages has been shown in prior studies (Eze *et al.,* 2016) to have modest to severe effects on the cerebellum and dose-dependent behavioral patterns. Abuse of carbonated alcoholic herbal beverages, often known as ethanol, can lead to end-stage alcoholic liver disease (Longstreth *et al*., 2009) or liver scarring. Herbs are a crucial part of the care of liver illnesses because there are no effective liver-protecting medications in allopathic medical procedures (Azam *et al.*, 2018). It is crucial to present scientific evidence for the different medical applications of plants in the modern era. Due to their effectiveness, low cost, and lack of adverse effects, herbal medications are frequently recommended even in cases where their physiologically active ingredients are unknown (Comert and Gokmen, 2018). Corn (Zea mays) is the most productive plant that can be produced in tropical and subtropical climates. One part of the corn plant is corn silk. Corn silk is considered a waste whose value has not been maximized, e.g., when it is thrown away or used as animal feed, despite its promise as a treatment. Liu *et al*. (2011) state that maize silk is rich in phenolic compounds, especially flavonoids. According to Hasanudin *et al.* (2012), corn silk has a wide range of therapeutic uses, including the treatment of kidney stones, nephritis, cystitis, inflammation, prostatitis, kaliuretics, urinary tract infections, nephrotoxicity, depression, hypertension, hyperglycemia, hyperlipidemia, hypokalemia, gout, hyperthyroidism, and gonorrhea.According to research by Sarepoua *et al*. (2013), phenolic chemicals like flavonoids, which have potent antioxidant properties, are the active ingredients in maize silk. In male wistar rats, the hepatoprotective effect of infusion of maize silk was studied by Ramadani et al. in 2020. The results showed that treatment reduced the levels of the enzyme Alkaline Phosphatases (ALP) and increased glutathione (GSH). Another study by Karami *et al*. (2013) used an isolated rat liver perfusion system to test the hepatoprotective effect of corn silk against ecstasy dose-induced injury (MDMA). The findings demonstrated histopathological improvement of the liver cells and liver protection by raising glutathione (GSH) levels.

The objective of this paper was therefore designed to evaluate the hepatoprotective effect of corn silk extract on carbonated alcoholic herbal beverages induced hepatoxicity in Wistar rats

MATERIALS AND METHOD

Plant Material: Corn silk was collected in Egor Local Government Area of Benin City, Edo State, Nigeria. They were identified in the herbarium of the Department of plant Biology and Biotechnology, Faculty of life Science, University of Benin, Nigeria. The collected corn silk were air dried and grounded into powder, the powder was soaked and concentrated at the Department of Pharmacognosy, faculty of pharmacy, University of Benin.

Preparation of extract: Corn silk were washed with water, dried and powdered in a grinding mill. The powdered corn silks were dissolved with two litters of distilled water and left in it for ten hours. Filter paper was used to filter the resultant mixture. After that, the liquid extract was put in a beaker and allowed to evaporate in a water bath at 70 degrees Celsius for six to nine hours per day for four to five days, or until a semisolid state was achieved. The resulting semisolid extract was freeze-dried in the University of Benin's Department of Anatomy after being stored in a deep freezer at -20 degrees Celsius for the whole night. The resulting extract was weighed and kept in desiccators at 22 degrees Celsius until it was needed again (Mahuya *et al.,* 2011).

Experimental Animals: forty five adult Wistar rats were obtained from the Department of Anatomy University of Benin. Animals weighing 160-220 g were used for this study. They were bred in the Animal House of the Department. The animals were acclimatized for two weeks and maintained under good environmental conditions. The animals were housed in plastic cages measuring about (29×15×12) cm, with five Wistar rats per cage. The Wistar rats had free access to feed and clean water during the study period. All procedures complied with the norms of the Institutional Animal Ethics Committee (IAEC).

Determination of Dosage: The canned beverage purchased contained 330ml, 6% alcohol by volume (ABV). A standard dose is 330ml/70kg which is also equivalent to 4.7ml/kg from which the administered dose were extrapolated from the known weight of the animal and administered following the administration protocol. If the animal weighs 150g, let D be the required dose, Therefore D = 150 x 4.7/1000 = 0.71ml/Kg/BW

Experimental design: Forty five adult Wistar rats were randomly assigned into nine groups. Each group consists of 5 animals (n=5). All the groups had access to feed and water for 60 days.

- Group A. (Control) received normal feed and water.
- Group B. Was treated with CAHB-A(neat) for sixty day
- Group C. Was treated with CAHB–B(bitters) for sixty days
- Group D. was treated with a low dose EXTRACT (200mg/kg) only for sixty days.
- Group E. Was treated with a high dose EXTRACT (600mg/kg) only for sixty days.
- Group F. CAHB-A(neat) + low dose EXTRACT(200mg/kg) for sixty days
- Group G. Was treated with CAHB–B(bitters + low dose EXTRACT(200mg/kg) for sixty days
- Group H. Was treated CAHB-A(neat) + high dose EXTRACT(600mg/kg) for sixty day
- Group I. Was treated with CAHB–B(bitters + high dose EXTRACT(600mg/kg) for sixty days

Histopathological studies: Liver samples that had been excised were washed using regular saline and left in 10% buffered neutral formalin for two days. Hematoxylin and eosin was used to stain sections that were paraffin embedded and 5 milimicron thick. The liver sections were taken and studied on a Leica DM750 research microscope that had a Leica CC50 digital camera attached. Tissue sections were digitally photomicrographed at a magnification of x400.

Biochemical Studies: Animals were weighed, anesthetized with chloroform desiccator, and blood (3.0 ml) was obtained by heart puncture using sterile disposable syringes after 24 hours had passed since the last treatment. Total serum bilirubin, total cholesterol, aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), and glutathione peroxidase were all estimated utilizing standard diagnostic kits after the serum had been separated (Ohkawa *et al.*, 1979). For additional histological examinations, the livers were immediately removed from each group and fixed in 10% formal saline.

Statistical analyses: The IBM SPSS statistics application (Statistical Package for Social Science) Version 25 (SPSS, inc., Chicago, Illinois, USA) was used to assess all of the data and provide the required statistical values. A one-way analysis of variance (ANOVA) was used to compare the values of the treatment groups to those of the control group. P-values were considered significant if they were less than 0.05. LSD was used as the post hoc test.

RESULTS AND DISCUSSION

Histological Assessments: Histological assessment of the liver sections of the control group showed normal liver tissue with distinct hepatic cells, central vein and sinusoidal spaces (Plate 1).

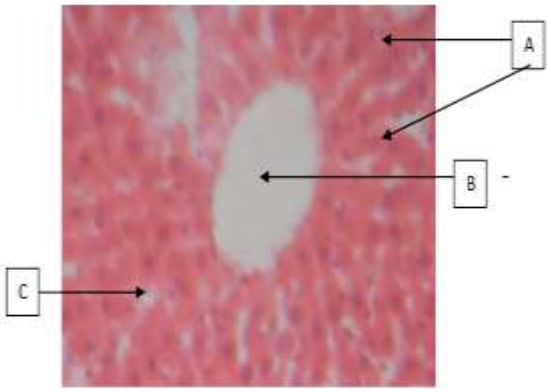

Plate 1: Control: Rat Liver composed of A: hepatocytes, B: Central Vein and C: Sinusoids (H & E x 40)

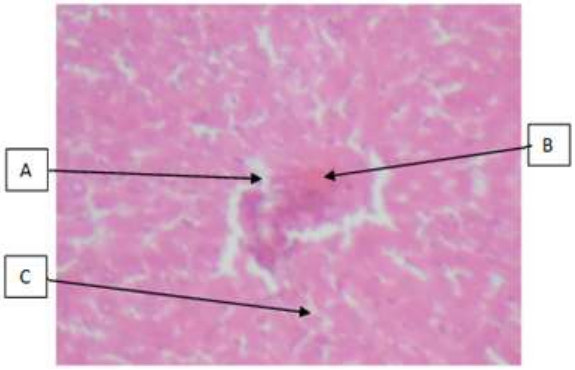

Plate 2: Rat liver given carbonated alcoholic herbal beverage (CAHB)-A(neat) showing: A, severe vascular ulceration, B, mild vascular congestion and C, mild infiltrates of inflammatory cells:Hepatitis (H&E x 40)..

The group treated with carbonated alcoholic beverages both bitters and non-bitters (CAHB) shown a severe vascular ulceration mild vascular congestion and filtrate of inflammatory cell (plate 2 and 3).

The group that were given 200mg/kg and 600mg/kg of corn silk shows normal hepatocyte architecture with mild kupffer cell activation (plate 4 and 5). Rat liver

that were treated with various grades of corn silk (200mg/kg and 600mg/kg) after CAHB damages shown a normal hepatocytes, mild vascular congestion and mild kupffer cell activation.

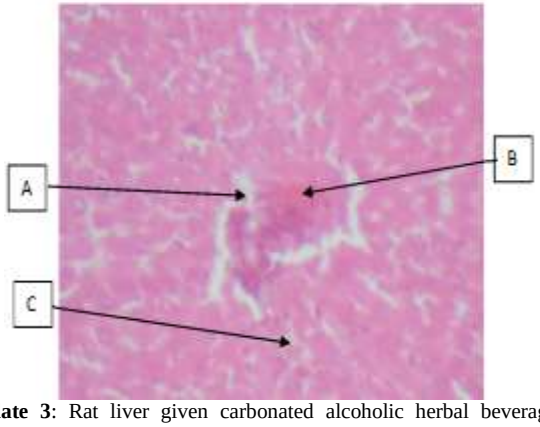

Plate 3: Rat liver given carbonated alcoholic herbal beverage (CAHB)-B(bitters) showing: A, severe vascular ulceration, B, mild vascular congestion and C, mild infiltrates of inflammatory cells:Hepatitis (H&E x 40)

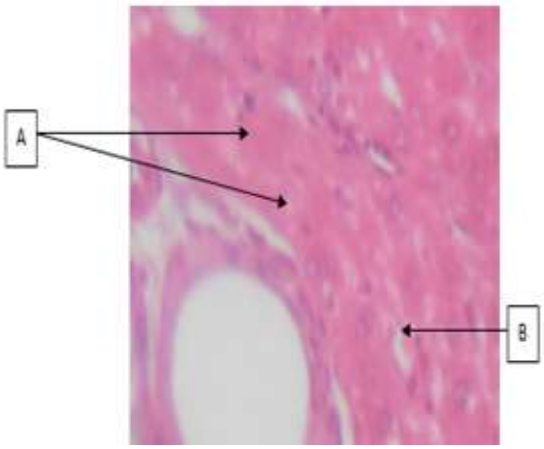

Plate 4: Rat liver given 200mg/kg Corn Silk extract only showing: A, normal hepatocyte architecture and B, mild kupffer cell activation (H&E x 100)

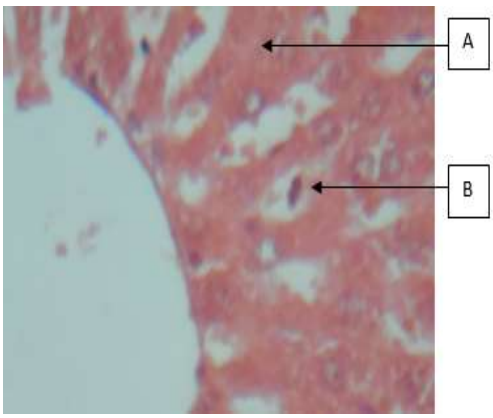

Plate 5: Rat liver given 600mg/kg Corn Silk extract only showing: A, normal hepatocytes and B, mild kupffer cell activation (H&E x 100)

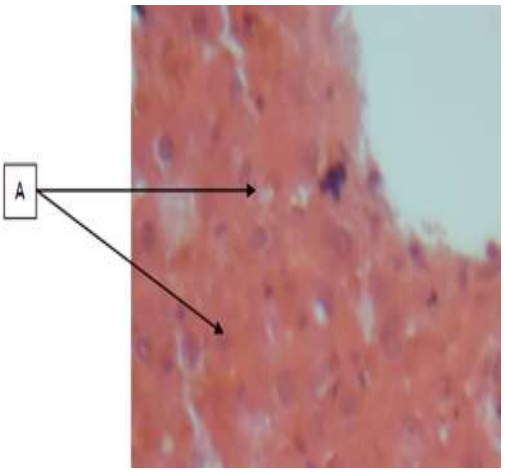

Plate 6: Rat liver given CAHB-A (Neat) + 200mg/kg Corn Silk extract showing: A, normal hepatocytes (H&E x 1O0)

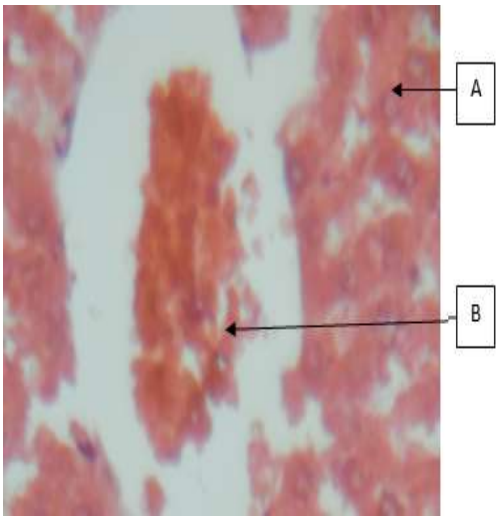

Plate 7: Rat liver given CAHB-B (Bitters) + 200mg/kg Corn Silk extract showing: A, normal hepatocytes and B, mild vascular congestion (H&E x 100)

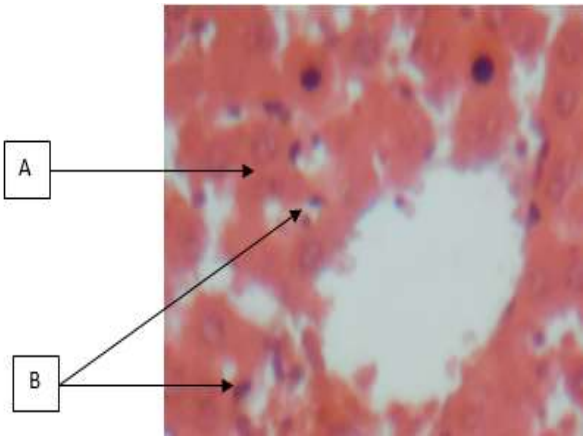

Plate 8: Rat liver given CAHB-A (Neat) + 600mg/kg Corn Silk extract showing: A, normal hepatocytes and B, moderate kupffer cell activation (H&E x 100)

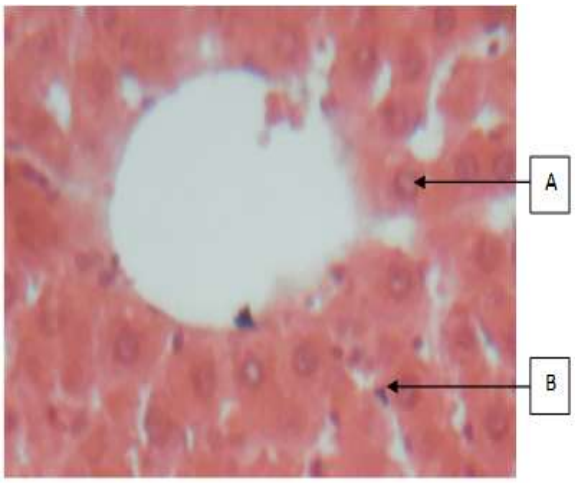

Plate 9: Rat liver given CAHB-B (Bitters) + 600mg/kg Corn Silk extract showing: A, normal hepatocytes and B, moderate kupffer cell activation (H&E x 100)

Biochemical analysis: It is believed that stress oxidative is an important element in the pathophysiology of a number of liver illnesses. Oxidative stress in the liver is linked to the production of more reactive oxygen species during the metabolism of alcohol, which results in the synthesis of acetaldehyde. Oxygen-derived free radicals played a significant role in liver damage. Numerous research on the effects of alcohol administration on oxidative stress markers, lipid profiles, liver enzymes, and experimental rats' weight have been published. Everson *et al* .(2008), reported that transaminase are sensitive indicators of liver cell injury and that they are most helpful in recognizing acute hepatocellular diseases such as hepatitis .similar findings were also observed in this work. The liver enzymes showed a significant increase ($p<0.05$) in ALT in the B and C treated groups when compare with control group. There was significant increase in AST in groups B and C compared with the control and the corn silk treated groups. Although these values were significantly reduced in group D and E when compare with CAHB -A (neat) and CAHB-B (bitter) groups. This is consistent with studies of Mohammad *et al*. (2012) and Everson *et al*. (2008). The ALP was elevated in all the groups when compared with the control group but were however lower in the groups that were treated with 200mg/kg and 600mg/kg of corn silk extracts. This correspond to the finding of Fang *et al* 2012 who also reported an elevation in ALP in carbonated alcoholic herbal beverages treated Wistar rats. The values of ALP in the groups treated with corn silk extract were however lower than CAHB -A (neat) and CAHB-B (bitter) induced groups, indicating that corn silk extract have a reducing effect on ALP. There was significant difference in the total and direct bilirubin between the treated groups and the control ($P>0.05$, respectively). The effects of the carbonated alcoholic herbal beverages on the levels of SOD and MDA in liver were showed in fig.6 and 8 respectively. In comparison with normal group, there was a significant increase in the level of MDA in group B and C, suggesting the development of peroxidation in liver tissue. Generally, the majority of these beverages did not obviously affect the levels of MDA and SOD in the liver. As can be seen in fig.5 and 8, the treatment of corn silk extract (200mg/kg and 600mg/kg) infusion significantly prevented the increase of the MDA level in liver and decreased the level of hepatic SOD. The reduction in levels of AST and ALT by the corn silk extract is an indication of repair of hepatic tissue damage caused by carbonated alcoholic herbal beverages, and the levels of MDA and SOD could reflect the extent of peroxidation damage.

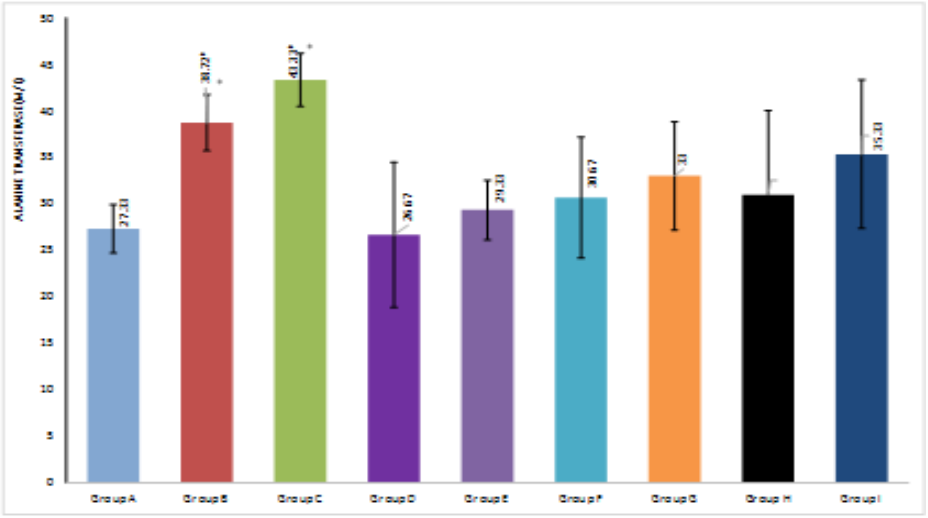

Fig 1: comparison of ALT between experimental group and control.
There were significant increases in group B and group C compared with the group A (control) ($P<0.05$, respectively).

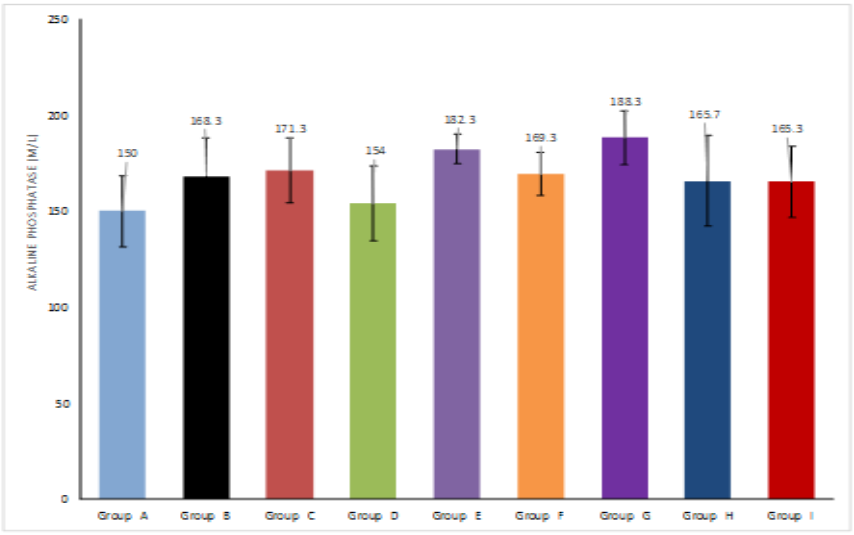

Fig 2. Comparison of ALP between the control group and treated group
There was statistical significant different in all the groups compared with the group (control) (P<0.05).

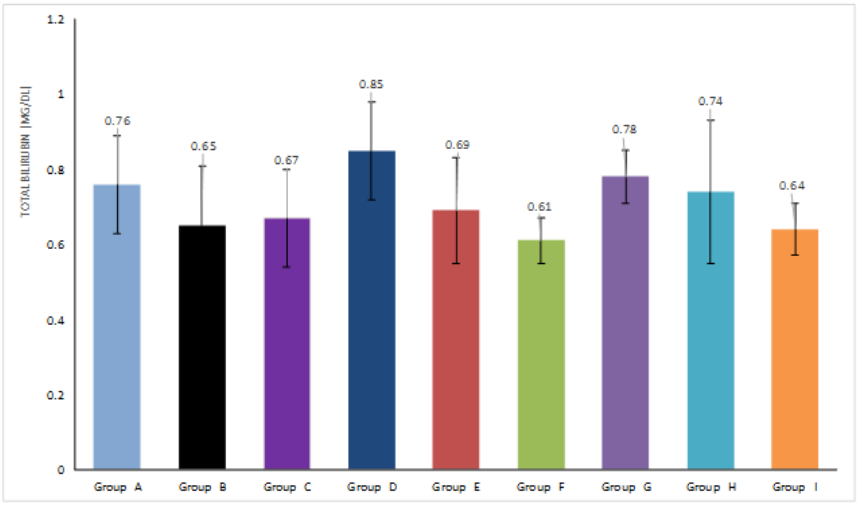

Fig 3: showing *Total bilirubin: comparison between the treated group and the control group*
There were no significant differences in all the groups compared with the group A (control) (P>0.05, respectively).

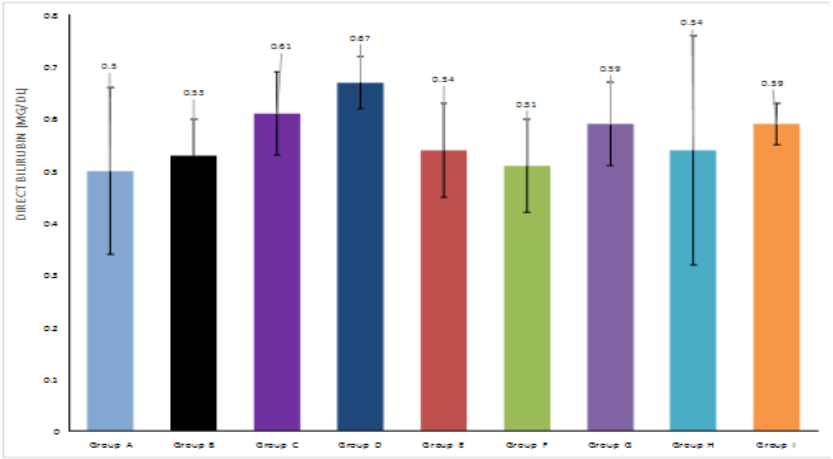

Fig 4d showing *Direct bilirubin* -comparison between the treated group and control group
There were no significant differences in all the groups compared with the group A (control) (P>0.05, respectively).

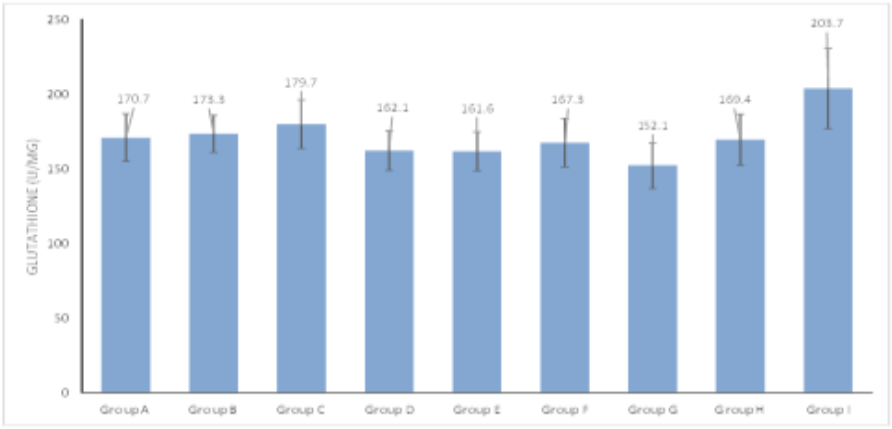

Fig 5: comparison of *Glutathione phosphatase* between the treated group and control
There were no significant differences in all the groups compared with the group A (control) (P>0.05, respectively).

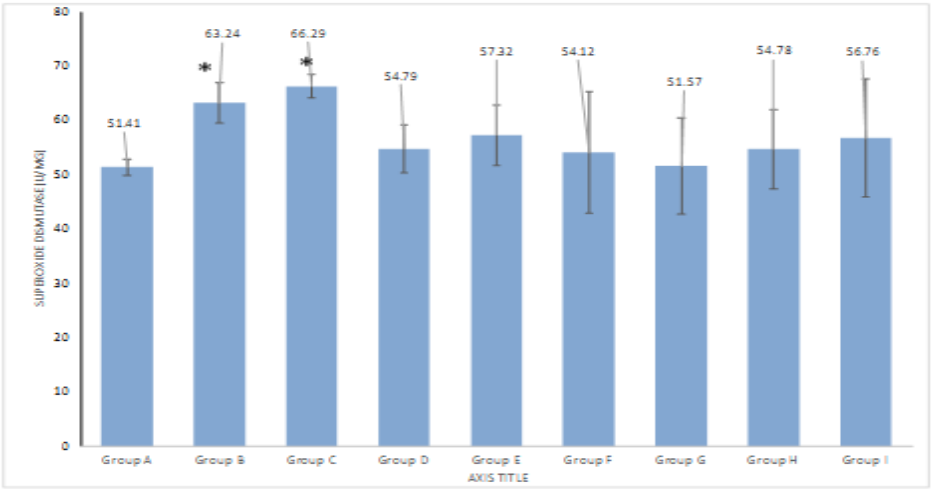

Fig 6: ***Superoxide dismutase:*** *comparison between the treated group and control*
There were significant increases in group B and group C compared with the group A (control) (P<0.05, respectively).

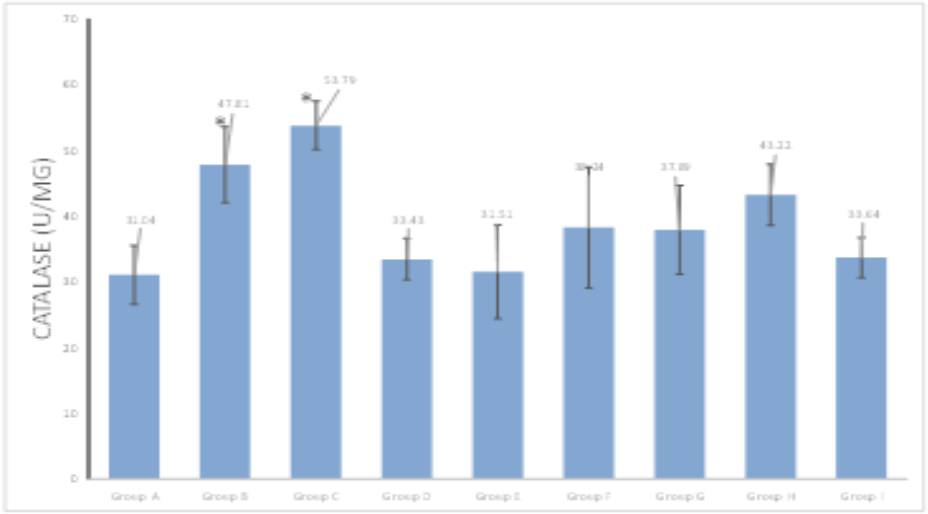

Fig 7: showing ***Catalase:*** *comparison between the treated group with the control.*
There were significant increases in group B and group C compared with the group A (control) (P<0.05, respectively).

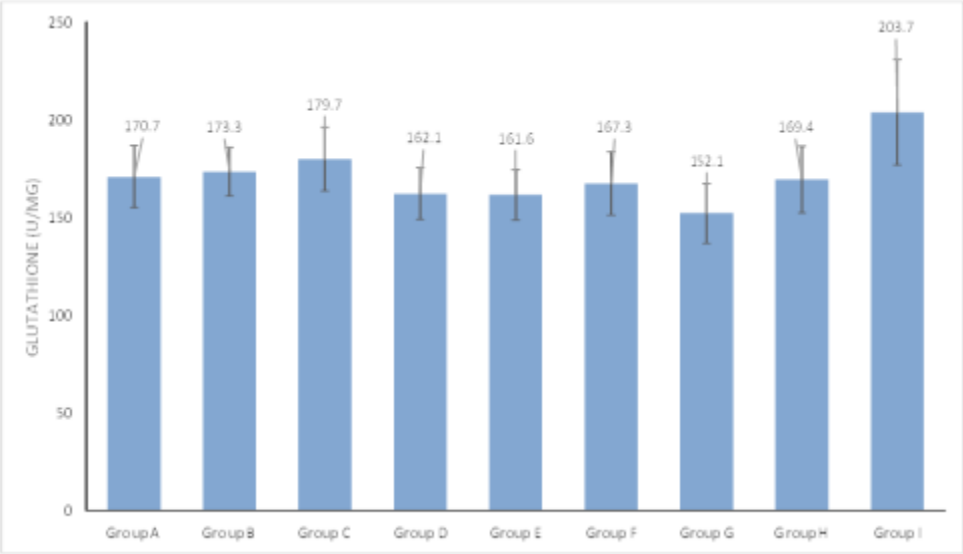

Fig 8: showing MDA: comparison between the treated group and the control
There were significant increases in group C compared with the group A (control) (P<0.05, respectively).

Table 1: Comparing the mean values of liver enzymes and some oxidative stress markers in Wistar rats treated with carbonated alcoholic herbal beverages and corn silk extract.

Group	ALT (µ/L)	ALP (µ/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	GPX (U/mg/protein)	SOD (U/mg/protein)	CAT (U/mg/protein)	MDA (U/mg/protein)
A	27.33±4.09	150±1.67	0.76±0.10	0.5± 0.6	170.7± 8.99	51.41± 8.50	31.04± 0.28	4.57± 3.33
B	38.72±17.24*	168.3±10.23*	0.65±0.35	0.53± 0.3	173.3± 34.19	63.24± 8.89*	47.81± 1.53*	4.46± 4.48
C	43.33±19.36*	171.3±12.11*	0.67±0.46	0.61± 0.7	179.7± 36.19	66.29± 9.64*	53.79± 2.01*	8.24± 5.91*
D	26.67±3.94	154±4.38	0.85±0.20	0.67± 0.6	162.16 ± 24.83	54.79± 5.40	33.43± 0.93	4.43± 7.58
E	29.33±6.14	182.8±4.72	0.69±0.11	0.54± 0.7	161.6± 21.18	57.32± 11.12	31.51± 0.48	5.42± 6.26
F	30.67±10.00	169.3±2.62	0.61±0.23	0.51± 0.5	167.3± 18.28	54.12± 4.25	38.24± 0.68	3.51± 5.09
G	33.00±12.24	188.3±7.72	0.78±0.08	0.59± 0.3	152.1± 12.68	51.57± 6.05	37.89± 0.58	4.77± 6.74
H	31.22±9.33	165.7±6.52	0.74±0.09	0.54± 0.6	169.4± 20.08	54.78± 14.17	43.22± 0.98	5.17± 4.54
I	35.33±13.50	165.3±5.24	0.64±0.045	0.59± 0.3	203.7± 6.68*	56.79± 10.14	33.64± 0.31	3.59± 3.53

*P < 0.05 indicates significant difference in treated groups compared with the control.

Over-consumption of alcohol could increase the activities of AST and ALT as well as the content of MDA. Our findings were in consonant with Eze *et al.* 2018 who reported a significant increase in MDA after carbonated alcoholic herbal beverage in adult Wistar rats. The content of malondialdehyde in liver tissues reflex the oxidative stress associated with the ingestion of alcohol, which could be used as an indirect measurement of cellular oxidative injury. The catalase shown a significant increase in group B and C when compared with control group A (p<0.005). A significant decrease also occurs in corn silk treated groups when compared with the carbonated alcoholic herbal beverages groups. The antioxidants effects of corn silk may have been responsible for this. This is consistent with a study conducted by Ebrahimzadeh *et al.*, 2008.The tissues specimen (liver) of the control animals shown' normal liver cell micrograph for group A rats (Fig.1.0).

The hepatocytes, portal tract and sinusoids were well delineated. The micrograph for group B and C (CAHB-A (neat) and CAHB-B (bitter) showed portal congestion , mild vascular congestion, vascular ulceration, haemorrhagic necrosis and mild infiltrates of inflammatory cells (hepatitis) indicating significant inflammatory response (alcoholic hepatitis) in the liver. The hallmarks of alcoholic hepatitis include extensive liver tissue damage (necrosis) and inflammation. Healthy liver tissue may start to give way to scar tissue. Even while the illness could be lethal, it might also be curable with abstinence. Up to 50% of heavy drinkers get alcohol hepatitis (National Institute on Alcohol Abuse and Alcoholism, 1993). Group D that were given 200mg/kg of corn silk extract shown normal hepatocyte architecture and mild kupffer cell activation. These findings were in agreement with Muhammad *et al.* (2012) were Corn Silk extract decreases formation of reactive oxygen species and oxidative stress, resulting in lipid peroxidation. Karami *et al*, (2001) reported that accumulation of CS extract in liver provided protection against injury. The group that was given 200mg/kg of corn silk extract showed normal hepatocyte architecture and kupffer cell activation. Similar and more potent liver architecture was seen in

group E that was given 600mg/kg of corn silk extract. These findings revealed that corn silk extract can be tolerated even at a high dose without detrimental effect. This is as a result of the virtue of its antioxidant properties.Treatment with graded doses (200mg/kg and 600mg/kg body weight respectively) of corn silk, however, attenuated the hepatitis with the higher dose achieving a more potent effect. This is in agreement with Mohammad *et al* 2012 who reported that corn silk attenuate hepatitis in adult Wister rats. The corn silk activated the local immune system of the liver with the higher dose proving to be the optimal dose which is line with (El-Ghorab *et al.*, 2007), who reported that high dose of corn silk extract activate kuppfer cell. Rat liver that were given CAHB-A (Neat) + 200mg/kg and CAHB-B (Bitters) + 200mg/kg Corn Silk extract respectively showed normal hepatocyte with mild vascular congestion in the group that were given -B (Bitters) + 200mg/kg Corn Silk extract. This might suggest that CAHB-B (Bitters) contain more hepatotoxic compound which can be affirmed based on our photochemical analysis. It could be suggested also that 200mg/kg of corn silk extract is less potent to protect the damaging effects of carbonated alcoholic herbal beverages- CAHB-B (Bitters). This is in agreement with (Eze *et al.*, 2018). Restoration of liver architecture was seen in the liver that was given CAHB-A **(**Neat) + 600mg/kg and CAHB-B (Bitters) + 600mg/kg Corn Silk extract respectively**.** There was moderate kupffer cell activation confirming the hepatoprotective efficacy of the corn silk extract by virtue of their numerous biologically active compounds, such as flavonoids

The results of this study indicate that the administration of corn silk reduces a number of negative effects of in vivo carbonated alcoholic herbal beverage administration in the liver of Wistar rats.

Conflict Of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the first author or corresponding author.

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