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ORIGINAL ARTICLE

Evaluation of Chronic Cola Consumption effect on the Liver and Kidney Functions in Adult Male Rats: Role of Serum 25-hydroxyvitamin D

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ABSTRACT

Background: Recently, the consumption of cola markedly increased. So, the health effects of cola drinks are an important public matter. However, there are contradictory reports on its effects on hepatic, renal function.

Aim of the study: To examine the effect of chronic Pepsi-Cola consumption on liver, kidney functions, their association to serum vitamin D (Vit.D) level and oxidative stress biomarkers in rats.

Material, Methods: 32 adult male albino rats were divided equally into two groups: Group I: control group, Group II: chronic cola consumption group which had free access to cola beverage for 3 months. At the end of the study serum alanine aminotransferase(A.L.T.), serum aspartate aminotransferase(A.S.T), total proteins, albumin, globulin, urea, creatinine, Vit.D, calcium, phosphorus, glucose, insulin, malondialdehyde(M.D.A.), total antioxidant capacity(T.A.C.), glutathione(G.S.H.), superoxide dismutase(S.O.D.) levels were determined. Livers, kidneys were processed for histopathology.

Results: There was a significant increase in serum A.S.T, A.L.T., urea, glucose, insulin, M.D.A. in cola group compared to control group. There was a significant decrease in serum Vit.D, T.A.C., G.S.H., S.O.D., calcium, accompanied by a significant increase in serum phosphorus. Additionally, there was a significant negative correlation between Vit.D and(A.S.T, A.L.T., M.D.A.) in cola group. However, there was a significant positive correlation between Vit.D and(T.A.C., G.S.H., S.O.D.) in cola group. Also, liver, renal sections showed histopathological changes.

Conclusion: cola consumption induced Vit.D deficiency which was associated with oxidant/antioxidant imbalance that could explain its hazardous impact on liver, kidney functions in rats.

Keyword: Cola beverage, renal, hepatic function, vitamin D.

INTRODUCTION

Sugar-sweetened beverages(S.S.Bs.) are defined as beverages containing added caloric sweetener(high-fructose corn syrup, sucrose, etc.). S.S.Bs., including soft drinks, fruit drinks, carbonated drinks, sport drinks, are one of the most common beverages second to coffee, tea and considered as the main source of artificially added sugar. They include non-alcoholic drinks like soda, cola, carbonated drink. Soft drinks contain predominantly water, phosphoric acid, caffeine, sugar in the form of sucrose, other chemicals in the form of preservatives, colorings, flavors[1].

As soft drinks consumption has increased worldwide in the past two to three decades, it has been shown that the intake of S.S.Bs. is a risk factor for many medical conditions such as diabetes, obesity, metabolic syndrome, inflammation, osteoporosis, cardiovascular disease, may increase the risk of mortality[2].

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Interestingly, oxidative stress has been served a vital role in the etiology, pathogenesis of various chronic diseases. High levels of free radicals or reactive oxygen species(R.O.S.) can cause direct damage to lipids inside cells, induce peroxidation. It worth mentioning that chronic soft drinking consumption induces not only metabolic changes, but also oxidative stress[3].

Additionally, Vit.D deficiency has been considered as a major public health problem with a prevalence of more than a billion people worldwide. Recently, there is a growing body of evidence supporting the importance of Vit.D in many non-skeletal biological processes such as endothelial function, renin-angiotensin-aldosterone system modulation. Also, Low levels of Vit.D have been associated with increased markers of oxidative stress, chronic kidney diseases[4].

Interestingly, **Seif, Abdelwahad.[5]** demonstrated that Vit.D supplementation ameliorated necro-inflammatory, apoptotic changes following the induction of hepatic ischemic-reperfusion(I-R) injury in rats.

Despite some authors focused on biochemical Alterations induced by soft drinks on the serum levels of hepatic, renal biomarkers, their effects on health are still unclear . So, this study was designed to examine the effect of chronic Soft drink consumption on liver, kidney function, to evaluate their association to serum Vit.D level, oxidative stress biomarkers in order to outline its effect on the health of adult male albino rats.

MATERIAL, METHODS

Material:

This study was carried out on a total number of 32adult male albino rats weighing 180-200gm (age:10-12weeks old). They were obtained from the animal house of Faculty of Veterinary Medicine of Zagazig University.

The animals were kept in steel wire cage (4animals/cages) in the animal house of Faculty of Medicine of Zagazig University under hygienic conditions.

Animal were fed standard chow and had free access to water and food, kept at room temperature (22 to 25°c) and were maintained on a natural light/dark cycles. The rats were accommodated to animal house kept under observation for one week before the onset of the experiment to exclude any infection**.**

The experimental protocol was approved by physiology department and by local medical ethics committee in Faculty of Medicine of Zagazig University *(The Institutional Animal Care and Use Committee, Zagazig University, ZU-IACUC) approval number: ZU-IACUC/3/F/60/2018*

Experiments complied with the ARRIVE guidelines and was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) .

The animals were divided into two groups:

Group I(n=16):Control group. Animals of this group were fed normal chow diet consisting of (5%fat, 52%carbohydrate, 20%protein), with total calorific values 20 kJ/kg and had free access to water**.**

Group II:(n=16):Chronic cola consumption group. Animals of this group received free access to soft drink(Pepsi Cola beverage) and were fed chow diet all through the study for three consecutive months[6]**.**

Methods:

1. **At the end of the experimental protocol duration**: the rats were anesthetized by injection of Urethane(1200mg/kg) then sacrificed by decapitation. Blood was collected into clean plastic centrifuge tubes, allowed to clot then centrifuged at 3000 rotation/min. by spin Eppendorf, the serum was separated then stored at-20ºC in a dark container until assayed for:

A. Serum aspartate aminotransferase(A.S.T), serum alanine aminotransferase(A.L.T.): Using rat ELISA kits(Sigma, Aldrich) [7]**.**
B. Total protein, albumin, globulin using spectrophotometer(Spinreact, S.A.U. ctra. Santa Coloma, 7e-17176 Sant esteve de bas(gi), Spain[7]**.**

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C. Serum urea, creatinine enzymatically by Spectrophotometer(Spinreact, S.A.U. ctra. Santa Coloma, 7e-17176 Sant esteve de bas(gi), Spain[8].
D. Serum Vit.D level by Rat Total 25OH Vit.D ELISA kit(BioVendor, BioVendor - Laboratorni medicina a.s. Karasek 1767/1621 00 Brno, Czech Republic according to manufacture instruction
E. Malondialdehyde(M.D.A.) using rat ELISA kit.(Bio diagnostic, Egypt)[9].
F. Total antioxidant capacity(T.A.C.) spectrophotometrically(OxiSelect™, Cell Biolabs, Inc.7758 Arjons Drive San Diego, CA 92126[9].
G. Reduced Glutathione(G.S.H.) using rat ELISA kit MyBioSource, Inc.P.O. Box 153308, San Diego, CA 92195-3308, USA[9].
H. Superoxide Dismutase(S.O.D.) using rat ELISA. MyBioSource, Inc.P.O. Box 153308, San Diego, CA 92195-3308, USA(Chi et al. 2002) [9].
I. Serum total Calcium, Phosphorus by Spectrophotometer. Spinreact, S.A.U. ctra. Santa Coloma, 7e-17176 Sant esteve de bas(gi), Spain [8].
J. Serum glucose levels: by using glucose enzymatic (GOD-PAP)-liquizyme Kits (Biotechnology, Egypt) [10].
K. Serum insulin levels: by using rat insulin enzyme-linked immunosorbent assay kit, (Product Number: RAB0904, Sigma-Aldrich Chemie GmbH, U.S.A). Calculation of homeostasis model assessment of insulin resistance (HOMA-IR): The following equation was used; [insulin (μU/mL) x glucose (mg/dl) /405] [10].

3) Histopathological examination

- Kidney, liver were harvested then sections were fixed in 10% formalin solution. After automated dehydration, kidney, liver slices were embedded in paraffin, sectioned at 5um, stained with hematoxylin-eosin(H.E.) stain for histopathological examination. using Light microscope with a digital camera.

Statistical analysis:

Data were presented as mean±SD. Statistical significance was determined by unpaired sample T test for differences between two groups. The correlations between parameters were analyzed using Pearson's correlation. $p<0.05$ was considered statistically significant. Statistical methods used in this study for analysis of data were according to SPSS 20 for Windows(SPSS inc. chicago,IL,USA) were used.

RESULTS

It was found that chronic soft drink consumption caused significant increase in level of serum A.S.T($p<0.001$), A.L.T.($p<0.001$), serum glucose($p<0.001$), insulin($p<0.001$) and HOMA-IR($p<0.001$) in cola group when compared to control group. However, there was insignificant change in total protein, albumin, globulin($p>0.05$) between both groups(table 1).

Also, it was found that chronic soft drink consumption caused fatty changes with marked cloudy swelling, hydropic degeneration, congested central vein with haemorrhage in liver histopathological study(figure 1).

Regarding kidney function, this study showed significant increase in serum urea($p<0.001$) level in cola group when compared to control group. However, there was insignificant change in creatinine level($p>0.05$) between both groups(table 2).

Regarding histopathological changes, renal tissue sections showed mild to marked tubular necrosis, hemorrhage, cyst formation, fibrosis, inflammatory cells infiltration(figure 2b,c).

Our results revealed a significant decrease in serum Vit.D($p<0.001$), calcium($p<0.001$), accompanied by significant increase in serum phosphorus level($p<0.001$) in group II when compared to group I(table 2).

Moreover, the results of the present study showed a significant negative correlation between Vit.D, A.S.T($p<0.001$), A.L.T.($p<0.001$) in cola group. However, there was insignificant correlation between these

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parameters, Vit.D in control group(p>0.05). Moreover, there was insignificant correlation between Vit.D, total protein, albumin, globulin(p>0.05) in both groups(table4).

Regarding the association between serum Vit.D, renal function tests, there was insignificant correlation between Vit.D, either urea or creatinine in both groups(p>0.05)(table4).

Additionally, in this study, there was significant increase in M.D.A. level(p<0.001), significant decrease in T.A.C.(p<0.001), G.S.H.(p<0.001), S.O.D.(p<0.001) levels in group II when compared to group I(table 3). Moreover, there was significant negative correlation between Vit.D, M.D.A. in both groups(p<0.001). However, there was significant positive correlation between Vit.D, T.A.C.(p<0.001) in cola group. In addition, there was significant positive correlation between Vit.D, G.S.H.(p<0.001, p<0.01;respectively), S.O.D.(p<0.001,p<0.01; respectively). in both Groups. While, insignificant correlation was found between Vit.D, T.A.C. in control group(p>0.05)(table 4).

Table 1: liver function tests and metabolic parameters in all studied groups

	Group I	Group II
Serum ALT (U/L)	56.08 ±2.03	77.24 ±7.36***
Serum AST (U/L)	132.66 ± 7.29	160.41 ± 4.62***
Total protein (g/dl)	6.54 ±0.42	6.65± 0.35
albumin (g/dl)	3.57±0.10	3.55±0.09
globulin (g/dl)	3.17± 0.19	3.14 ± 0.20
Glucose(mg/dl)	85.19± 9.42	148.00±25.06***
Insulin(µIU/ml)	19.66±2.29	32.27±6.15***
HOMA-IR	4.06±0.70	12.11±3.91***

Data presented as mean±SD, ***: p<0.001

Table 2: Renal function tests and serum vitamin D in all studied groups

	Group I	Group II
Serum Urea (mg/dl)	32.17 ±0.89	36.11 ±2.05***
Serum creatinine (mg/dl)	0.40± 0.01	0.40± 0.01
Serum Calcium (mg/dl)	11.66 ±0.31	9.32± 0.20***
Serum phosphorus (mg/dl)	5.27 ± 0.17	6.67 ± 0.40***
Serum vitamin D (ng/ml)	26.99 ± 2.47	13.76 ± 1.68***

Data presented as mean±SD, ***: p<0.001

Table 3: Oxidative stress biomarkers in all studied groups

	Group I	Group II
Serum MDA (nmol/ml)	11.33 ±2.58	21.71 ±2.72 ***
Serum TAC (ng/ml)	0.29 ± 0.016	0.09± 0.02 ***
Serum GSH (mg/dl)	4.57±0.88	2.28±0.43 ***
Serum SOD(U/ml)	12.26±1.27	7.03±0.65 ***

Data presented as mean±SD, ***: p<0.001

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Table 4: correlation coefficient (r) between serum vitamin D and (liver, kidney function and oxidative stress parameters) in all groups

	Group I	Group II
AST	r= -0.480	r= -0.927***
ALT	r= 0.183	r= -0.870***
Total protein	r= 0.615	r= 0.095
Albumin	r= -0.064	r= -0.230
Globulin	r= 0.323	r= 0.473
Urea	r= -0.055	r= 0.097
Creatinine	r= -0.181	r= -0.166
Calcium	r= 0.281	r= 0.911***
Phosphorus	r= -0.742	r= -0.818***
MDA	r= -0.818***	r= -0.784***
TAC	r= 0.326	r= 0.735***
SOD	r= 0.843***	r= 0.672**
GSH	r= 0.846 ***	r= 0.691**

**: p< 0.01, ***: p<0.001

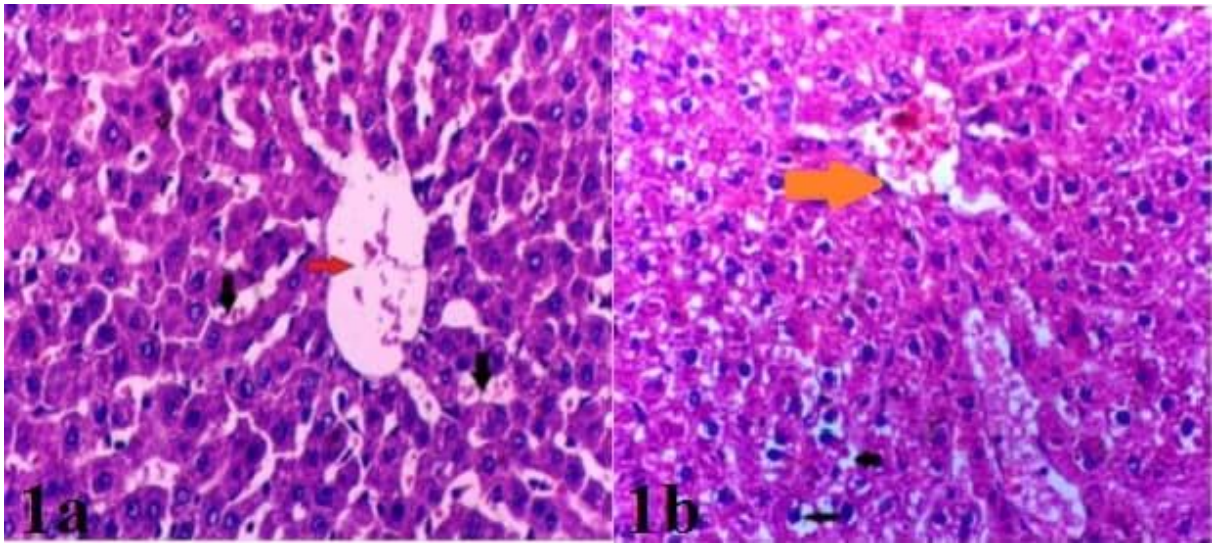

Fig 1: liver histopathological analysis of all studied groups

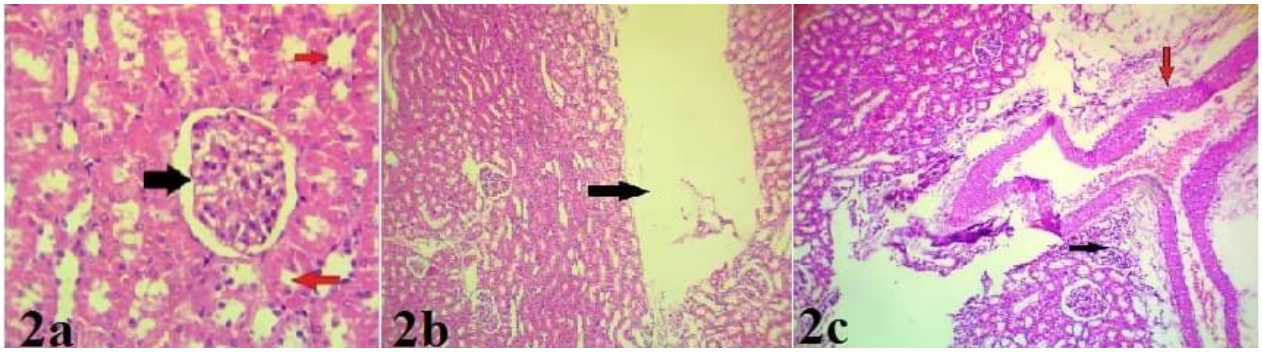

Fig 2: kidney histopathological analysis of all studied groups

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Fig 1: (a): Liver tissue section from the control group showing a normal morphology with normal central vein (red arrow), sinusoids and liver lobules (black arrow) (H&E 400). (b) Liver section from cola consumption group showing marked cloudy swelling and hydropic degeneration (black arrow), with congested central vein (red arrow) (H&E 400).
Fig 2: (a) Renal tissue section from control group with a normal morphology, normal glomeruli (black arrow) and normal tubule (red arrow) (H&E 400). (b): Renal tissue section from cola consumption group with marked tubular necrosis and cyst formation (black arrow) (H&E 400). (c): Renal tissue section from cola consumption group with fibrosis (red arrow) and inflammation (black arrow) (H&E 400).

DISCUSSION

Regarding liver function, chronic soft drink consumption in the present work for 3 months was associated with significant increase in level of serum A.S.T, A.L.T. in cola group when compared to control group, which was accompanied by fatty changes with marked cloudy swelling, hydropic degeneration of hepatic cells, additionally, liver sections showed congested central vein with haemorrhage in the histo-pathological analysis of the same group. However, there was insignificant change in total protein, albumin, globulin in both groups. These observations were consistent with the results of **Ma et al.[11]** who reported that soft drinks consumption was positively associated with increased A.L.T. levels with no change in plasma proteins**.**

The increased liver enzymes could be explained by the presence of hepatic inflammation, that could be attributed to the intake of a large amount of sugar which could induce a high glycemic load, high C-reactive protein level[**12]**. As when hepatocytes get damaged, liver-specific macrophages(Kupffer Cells) get activated, secrete tumor necrosis factor-alpha(T.N.F.-α), interleukin-6 into the blood, that could induce hepatic production of C-reactive protein level[**13].**

Interestingly, hepatic inflammation decreases insulin sensitivity, resulting in hepatic insulin resistance, which is aggravated by oxidative stress, aberrant inflammatory signals. Insulin resistance has become one of the main factors contributing to liver damage. Insulin resistance subsequently leads to more chronic, potentially fatal conditions such as cirrhosis, end-stage liver failure[**14].**These observation is in accordance with our results that showed significant increase in serum glucose ,insulin and HOMA-IR.

The development of steatosis has been explained by the accumulation of fat in hepatocytes because of excessive triglycerides due to an imbalance in the lipid metabolism as a consequence of insulin resistance[**15].**

These results are in agreement with other studies that found that cases consuming soft drinks suffered from non-alcoholic fatty liver disease(N.A.F.L.D.) with or without metabolic syndrome when compared to healthy controls. Moreover, soft drink consumption is a strong predictor of fatty liver independent of metabolic syndrome, C-reactive protein level. However, the underlying mechanism remains unknown[**12].**

Accumulated evidence shows that insulin resistance, dyslipidemia, inflammation all play an important role in the development of N.A.F.L.D.. Therefore, soft drinks consumption may be an important predictive factor or therapeutic target for the development, progression of N.A.F.L.D.[**12].**

The mechanisms by which soft drinks could promote N.A.F.L.D. development are unclear, but accumulated evidence has shown that fructose might play an important role[**12].**

Sugary beverages contain high fructose corn syrup, which can increase the synthesis, deposition of liver triglyceride, reduce its clearance rate. A long-term high-fructose diet might lead to N.A.F.L.D.**[16].**

In addition, **Meng et al.[12]** reported that over-loaded fructose increases hepatic metabolic burden, stimulating the overproduction of acetyl-CoA in mitochondria, which enters the cytoplasm to be used for fatty acid, cholesterol synthesis.

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Meanwhile, the intake of fructose can significantly increase the expression of fructose kinase, fatty acid synthase in the livers of N.A.F.L.D. cases, which accelerates fructose metabolism, fatty acid synthesis, promotes lipid deposition in the liver, thus forming a vicious circle**[**[16]**.**

Interestingly, artificial sweeteners also are widely used in beverage products. Individuals who consume artificially sweetened beverages are at greater risk for further weight gain, increased abdominal fat deposition. Artificial sweeteners may promote food intake, induce metabolic changes. These effects on metabolism may be derived from stimulation of adipogenesis, suppression of lipolysis by artificial sweeteners[[17]**.**

Furthermore, the variable progression of N.A.F.L.D. has led to the description of a 'multiple-hit' hypothesis suggesting that multiple events act in parallel including lipotoxicity, proinflammatory cytokines, increased oxidative stress, mitochondrial dysfunction, genetic or environmental susceptibilities[[18]**.**

Previous studies reported that oxidative stress has been associated with the etiology, pathogenesis of various chronic diseases, plays a vital role in the aging process[[19]**.**

High levels of free radicals or reactive oxygen species(R.O.S.) can cause direct damage to lipids inside cells, induce peroxidation. The mitochondria, plasma membrane, endoplasmic reticulum, peroxisomes are the primary sources of endogenous R.O.S. production through a variety of mechanisms, including enzymatic reactions and/or auto-oxidation of several compounds, including catecholamines, hydroquinone[[20]**.**

Interestingly, the results of the present work showed significant increase in serum M.D.A. level, significant decrease in serum T.A.C., G.S.H., S.O.D. activities in chronic cola consumption group when compared to control group, which indicated a state of oxidative stress in this group adding another explanatory factor for the development of N.A.F.L.D. in cola group.

The liver is among the primary organs susceptible to the effects of hyperglycaemia-induced oxidative stress, which may lead to liver tissue injury. This is followed by derangement of protein, carbohydrate, lipid metabolism, thereby leading to increased oxidative stress, further triggering the inflammatory cascade[[14]**.**

Excessive R.O.S. production results in several deleterious events, including an irreversible oxidative modification of lipids, proteins, carbohydrates. In addition, it induces hepatocytes apoptosis, the release of inflammatory cytokines, thereby increasing the expression of adhesion molecules, the infiltration of leukocytes. A combination of all of these processes causes massive hepatic tissue destruction[[21]**.**

However, the liver is equipped with potent antioxidants such as superoxide dismutase(S.O.D.), catalase(C.A.T.), the glutathione(G.S.H.) enzyme family, including glutathione-S-transferases(G.S.Ts.), glutathione peroxidases(G.P.Xs.) so as not only to neutralize free radicals but also to protect the liver cells from oxidative damage. Previous research has proven that a decrease in S.O.D., C.A.T. activities within a hyperglycemic state leads to an increase in ROS, which eventually contributes to oxidation-induced liver damage**[**[14]**.**

The present results are supported by the findings of **Friedman et al.[**[22]** who showed that all of the metabolic Alterations in case of steatosis could increase the pro-inflammatory cytokine activity leading to oxidative stress-mediated lipotoxicity, impaired hepatocyte apoptosis, inflammasome activation, mitochondrial dysfunction, contributing to fatty acid accumulation, hepatocellular injury, inflammation, progressive accumulation of excess extracellular matrix.

oxidative stress could be triggered by pro-inflammatory cytokines(such as T.N.F.-α, interleukin-6, interleukin-8) or the reduction of

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anti-inflammatory cytokines such as adiponectin, may further exacerbate insulin resistance, hepatocyte injury induced by genetic or environmental susceptibilities[23].

The present results are consistent with the findings of **El Terras et al.**[24] who showed that chronic consumption of carbonated soft drinks induced oxidative stress, Alterations in antioxidants, the expression levels of certain genes associated with brain function of Wistar rats.

In contrast to our results, **Celec,Behuliak.**[6] reported that there were no differences in plasma malondialdehyde, fructosamine, T.A.C. in cola-consuming group when compared to control. This disagreement could be attributed to genetic, species, environmental differences, /or duration of SD administration.

Regarding kidney function, this study showed significant increase in serum urea level in cola group when compared to control group. However, there was insignificant change in creatinine level in both groups. These findings were consistent with that of **Akande, Banjoko.**[25] who reported increasing of urea concentration in rats treated with energy beverage without change in creatinine

Regarding histopathological analysis of renal tissue, it showed mild to marked tubular necrosis, with hemorrhage, cyst formation, together with fibrosis, inflammatory cells infiltration. These findings agreed with the results of **Otero-Losada et al.**[26] who revealed some congestion, diffuse glomerulonephritis, tubular necrosis, distortion, disruption of cytoarchitecture of renal cortex in kidney of rats by chronic consumption of Soda pop drinks.

The pathological changes in renal tissue, function could be explained by the effect of cola drinking to predispose mild renal insufficiency, which is associated with inflammation, insulin resistance[27].

Necrosis that observed in renal sections may be due to high consumption of cola beverages which leads to change in A.T.P. production that reduced amount of blood arrived to cells(hypoxia) consequently causing cells necrotic[28].

Moreover, consumption of sugar-sweetened soft drinks is also associated with many diseases including hypertension which causes expansion of blood vessels, help aggregation of blood cells that lead to congestion which could aggravate cellular hypoxia[29].

In addition, these renal injurious effects could be caused by the effect of free radicals that generated from long -term carbonated beverages, damaging membranes of cells**,** causing the disturbance in renal tissue structure after cola consumption, which could explain the change in renal function, the accumulation of nitrogenous wastes as urea[30] .

Furthermore, the effects of cola intake on glomerular structure might be secondary to metabolic syndrome and/or they might be related to other factors such as increased fluid overload, intravascular expansion[31].

Interestingly, the results of the present work showed significant increase in serum phosphorus, accompanied by significant decrease in serum calcium, Vit.D in cola group in comparison to control group.

These observations provide another, evidence supporting the plausibility of a link between cola beverages, kidney disease, as cola beverages are generally acidified using phosphoric acid. Phosphorus may have an effect on the risk of kidney disease[32].

It was reported that heavy cola consumption, are characterized by hypocalcemia, secondary hyperparathyroidism, reduced 1α,25-dihydroxyvitamin-D level**.** That is explained by the inhibition of renal 1α hydroxylase secondary to hyperphosphatemia, which suggested that exogenous phosphate loads interfere with earlier metabolic steps in Vit.D synthesis. Additionally, the developed hyperparathyroidism is not enough to prevent sustained hypocalcemia, but further studies are required to test this hypothesis[33].

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Moreover, the results of the present study showed significant correlation between Vit.D, liver, kidney function. It showed significant negative correlation between Vit.D, serum A.S.T, A.L.T. in cola group. However, there was insignificant correlation between these parameters in control group. Moreover, there was insignificant correlation between Vit.D, total plasma protein, albumin, globulin in both groups.

In line with the findings of the present study, **Barchetta et al.[34]** reported the wide presence of Vit.D receptors in the liver, its inverse correlation with the severity of inflammation, as it acts as an immune-modulator suppressing fibroblast proliferation, collagen production in cases with viral or metabolic hepatitis, thereby suggesting that Vit.D would influence the progression of liver diseases.

Furthermore, **Chung et al.[35]** demonstrated that Vit.D insufficiency was associated with disease progression and/or poor outcomes in cases with chronic liver disease such as N.A.F.L.D. which was independent from metabolic syndrome, diabetes, insulin-resistance profile. Additionally, it was shown that stellate cell activation was inhibited by Vit.D receptor ligands.

However, **Ha et al.[36]** reported quite contradictory results, as they argued that Vit.D insufficiency alleviated accumulation of liver fat. In addition, a study by **Patel et al.**[37] also showed no difference or correlation between Vit.D status, the presence or severity of N.A.F.L.D..

These contradictory findings could be related to species, genetic and/ or environmental factors differences. Additionally, neither of these studies was on chronic cola beverage consuming-rats.

It worth noticing that, there was significant positive correlation between Vit.D, calcium in cola group. however, there was significant negative correlation between Vit.D, phosphorus in both groups. While, there was insignificant correlation between Vit.D, either serum urea or creatinine in both groups.

Interestingly, Vit.D deficiency is common in cases with chronic kidney disease(C.K.D.), serum levels of Vit.D appear to have an inverse correlation with kidney function. Growing evidence has indicated that Vit.D deficiency may contribute to deteriorating renal function, impaired glomerular filtration rate as well as increased morbidity, mortality in cases with C.K.D.[38].

However, the insignificant correlation between serum Vit.D, either serum urea or creatinine in the present work could be attributed to the differences in the species, duration of the study, or the sample size.

It worth noticing that, our results showed significant negative correlation between Vit.D levels, serum M.D.A., accompanied by significant positive correlation between its levels, serum S.O.D., G.S.H., T.A.C. in cola consumption group.

These findings are in line with the results of **Franca Gois et al.[4]** who reported that Low levels of 25(OH.)-Vit.D have been associated with increased markers of oxidative stress in kidney disease. Vit.D deficient animals showed increased thiobarbituric acid reactive substances, decreased glutathione levels, a biomarker of oxidative stress, a major endogenous antioxidant, respectively**.**

Moreover, **Wang et al.[39]** reported that Vit.D deficiency was associated with increased oxidative stress in the elderly, the obese, in those suffering from diabetes, severe Asthma. Additionally, they did not reveal a clear link between Vit.D status, oxidative stress biomarkers in the absence of any of previously mentioned conditions.

It was found that Vit.D reduced T.N.F.-α, oxidative stress markers in the adipose tissue of rat model of high fat diet induced N.A.F.L.D.**.** Additionally**,** 25-(OH.) Vit.D repletion reduced oxidative stress, inflammation through increasing nuclear factor-erythroid-2-related factor2(Nrf2), activating the

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antioxidant response element in case with C.K.D.[40].

CONCLUSION

Soft drink consumption plays a vital role in the development of N.A.F.L.D., associated with deterioration of kidney function, structure. Additionally, high intake of cola had lowered the circulating 25-hydroxyVit.D with a hazardous impact on serum calcium, phosphorus. These effects could be attributed to the development state of an oxidative stress which could link Vit.D deficiency to the deterioration of liver, kidney functions.

RECOMMENDATION

Further studies are needed to evaluate the cellular effects, pathways of the injurious impact of soft drink on liver, kidney functions. Moreover, additional studies are required to ascertain the minimal amounts, minimal time of exposure of soft-drink needed to produce deleterious effects such as those described in the present study. In addition, other studies with long follow up are also needed to evaluate long term effects of cola on body systems.

Further clinical studies need to be undertaken to verify whether there is a beneficial effect of V.D. supplementation on redox balance in subjects with low 25(OH.)-V.D. levels

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