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Evaluation of the Ameliorative Impact of ß, Epsilon-Carotene-3, 3'-diol on the Hepatic System after Ethanol-influenced Liver Toxicity in Adult Male Wistar Rats: Morphological and Biochemical Analyses

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ABSTRACT

The frequency of liver injury is rising universally due to the leisure and societal acceptance of ethanol (EtOH) consumption. Enzymes that break down ethanol are primarily found in the liver, as such, the effects of long-time ethanol drinking cause a more serious effect on the liver as opposed to other body parts like the heart, brain, and gastrointestinal organs. Ethanol-induced hepatic injury occurs through oxidative stress, lipid accumulation, and inflammation. This experiment was carried out to investigate the impacts of ß, Ɛ-Carotene-3,3'-diol on the hepatotoxicity induced by EtOH in Wistar rats. A total of 48 Wistar rats were purchased and randomly split into six distinct groups (n=8): Oral administration was carried out using oral cannula. The control group was Group A, Group B = EtOH-only, and Group C = EtOH, + ß, Epsilon-Carotene-3,3'-diol at 10 mg/kg, group D = EtOH, + ß, Epsilon-Carotene-3,3'-diol at 20 mg/kg, group E = EtO.H, + ß, Epsilon-Carot.ene-3,3'-diol at 40 mg/kg, and group F = EtOH + Silymarin 200 mg/kg. At sacrifice, blood samples were collected to determine antioxidant activity by assaying for superoxide dismutase (SOD), and catalase. The total protein level, malondialdehyde and the concentration of tumor necrosis factor-alpha (TNF-α) was evaluated for the effects of EtOH on inflammation and lipid peroxidation. The results showed ethanol caused macrovesicular and microvesicular steatosis. EtOH caused an increase in MDA and TNF-α concentration, and a reduction in catalase, SOD, and TP level. Remedy with ß, Epsilon-Carot.ene-3,3'-diol after ethanol-influenced hepatotoxicity highlights its ameliorative ability in a dose-reliance pattern.

Keywords: alcoholic liver disease, ethanol, hepatotoxicity, ß, Ɛ-Carotene-3,3'-diol, hepatocellular ballooning

INTRODUCTION

Data from the World Health Organization estimated that 400 million people globally live with alcohol use disorder [1]. Given the significant risks and global prevalence of alcohol use disorder (AUD), it accounts for 20% of all disability-adjusted life years (DALYs) attributed to suicide [2]. Despite the awareness of the liver damage caused by alcohol, its consumption is steadily increasing [3-4]. Some of the predictors of excessive ethanol use includes age at first drink, pleasure expectancies, family history of ethanol abuse, environmental factors. Ethanol is a major risk factor for several diseases, causing toxicity to vital organs like the liver, heart, brain, and gastrointestinal (GIT) organs. Its long-term and high consumption leads to liver damage, primarily because it serves as the principal site for ethanol-metabolizing proteins that catalyze specific biochemical reactions. It has also been posited by several authors that when excessive drinking happens over time, it causes alcoholic liver disease, which can be associated with conditions like steatosis or fatty liver caused by alcohol, steatohepatitis, cirrhosis, and liver cancer [3, 5-7]. Ethanol-induced liver injury occurs through destruction of hepatocytes via oxidative stress, lipid accumulation and inflammation [8]. For instance, it was reported that rats exposed to ethanol had hepatic injury due to necrosis, and presence of lipid droplets in the cytoplasm of the hepatocytes [9].

Alcoholic fatty hepatic disease is often observed in about 90% heavy drinkers [6, 10, 11], marked by the storage of lipid in the liver cells due to over-supply of lipid [12] that were not cleared. Fat clearance in the liver is controlled by mitochondrial β-oxidation and expression of excessive stored triglycerides that are packed into very-low density lipoprotein (VLDL) [13]. Continued alcohol consumption speeds up the

progression of alcoholic fatty liver to hepatitis. This is depicted by steatosis, hepatocytes ballooning, and penetration of neutrophils with or without fibrosis [14, 15]. Excess alcohol consumption promotes acetaldehyde increase, which in turn activates the action of reactive oxygen species (ROS) [16, 17]. The production of ROS rapidly accelerates iron overload in the cytoplasm [18] and promotes peroxidation of lipid, hence, hepatocyte death. Acetaldehyde is then transformed to acetate by aldehyde dehydrogenase enzyme (ALDH), which is converted into carbon dioxide (CO_2), fatty acids (FAs), and water (H_2O) [3, 19]. Ethanol causes limits enzymes such as glutathione peroxidase, super.o.xi.de dismutase, and catalase [20], thereby weakening the liver's antioxidant system.

The Nuclear factor kappa B (NF-κB) cells signaling pathway becomes activated when exposed to ethanol-derived metabolites and TNF-α concentration rises in macrophages [21]. Pharmacological therapeutics used for treating alcoholic liver disease (ALD) causes some side effects like Jaundice, neuropsychological activities, vomiting, stooling, dermal reactions and GIT problems. As a result, toxic effects of ethanol, the concern for alcoholism is still of global concern to social and health workersAs a result of the toxic effects of ethanol, alcoholism remains a global concern for social and health workers [22].

Over the years, plant extracts have been used worldwide to treat health complexities due to their countless advantages, accessibility [23] and phytochemical contents [24] such as phenol, flavonoids carotenoid, and vitamins [25, 26]. As a result, the consumption of plants rich in phytochemical constituents has been advocated by several authors [24, 27, 28]. One of such is ß, Epsilon-Carotene-3,3'-diol, the non-provitamin carotenoid type which is available in vegetables like spinach, broccoli, etc. Previous studies [29, 30] have reported its anticancer, counter-hypertensive and counter-diabetic effects. Its reparative effects as an anti-inflammatory and antioxidant agent on indomethacin-induced hepatotoxicity and indomethacin- induced gastric ulcer have also been documented [31]. Therefore, this research explored the effects of ß, Epsilon-Carot.ene-3, 3' diol on ethanol-induced hepatotoxicity in adult male Wistar rats.

MATERIALS AND METHODS

Experimental reagents

ß, Epsilon-Carotene-3,3'-diol was acquired from Ambeed Inc, United States of America, Ethanol was procured from SIGMA Aldrich, USA, Silymarin tablet was acquired at the medication station of the Obafemi Awolowo University Teaching Hospital Complex, (OAUTHC), Ile-Ife Osun State, Nigeria.

Ethical statement

This experimentation was achieved after getting consent from the Health Research Ethics Committee (HREC) of the University. The HREC identity of IPH/OAU/2655 was obtained.

Animal use

A total of forty-eight Wistar rats were obtained, and housed at the research building of the Department of Anatomy and Cell Biology, OAU, Ile-Ife, Nigeria under room temperature, and lighting. They were granted access to rat feed, distilled water *ad libitum* and were acclimatized within two weeks prior to the beginning of the experiment.

Study pattern

The rats were grouped into six of eight rats each (n=8). Using an oral cannula, the animals in group A received distilled water, while groups B-F rats received a once daily oral administration of 40% ethanol for 21 days. They were fasted for twenty-hours after the last ethanol administration for gastric emptying, and put through oral treatment with ß, Epsilon-Carotene-3,3'diol, 12 hourly for 21 days.

Animal sacrifice

Using ketamine as anesthetic agent, sacrifice was done 24 hours upon completion of the final treatment regimen. A thoracoabdominal incision was made, hematology samples were obtained with a hypodermic needle through cardiac puncture. Rat livers were expunged, swilled in normal saline, and preserved in 10% formol saline. Biochemical studies were carried out on the serum and liver tissue.

Determination of liver weight

The relative liver weight of the animal was measured thus:

$$\text{Relative liver weight} = \frac{liv.er\ we.ight}{fina.l\ body\ w.eight} x100$$

Histological analysis

Fixed tissues were processed and stained for histological demonstration using Oil Red O stain.

Statistical analysis

Figures were demonstrated as Mean ± SEM. Significant variability amo.ng means of the groups was determined employing a one-way analysis of variance (ANOVA). Values of result was regarded to be statistically substantial at $P < 0.05$.

Photomicrography

Liver sections we.re observed using a LEICA research microscope (DM750) fastened to a computerized camera (LEICA ICC50), and microphotographs with merged scale bars were captured.

RESULTS

Mac.ros.co.pic influence of ß, epsilon-caro.ten.e-3,3'-diol and silymar.in on the liver after ethanol – influenced liver toxicity

The macroscopic representation of the liver across the experimental groups is presented in figure 1. The image shows features of numerous fatty droplets and hepatocellular ballooning in the ethanol-treated group in contrast to the regular group. The B and C category demonstrated a mild reduction in fatty droplets, but the anti-steatotic effects of ß, Epsilon-Carotene-3,3'-diol was demonstrated via a high dosage. Silymarin also demonstrated a reduction in presence of fatty droplets, with features similar to control.

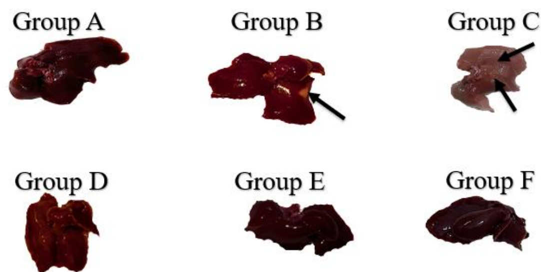

Figure 1: Macroscopic images showing liver managed with ß, Ɛ-Carotene- 3, 3'-diol and Silymarin following ethanol-induced hepatotoxicity. Black arrow shows ballooning of the liver and fatty droplets. A = Normal control; B = Ethanol only group; C = treated with 10 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol; D = treated with 20 mg/kg b.w of ß, Ɛ-Carote.ne- 3, 3'-diol; E = treated with 40 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, F = treated with 200 mg/kg Silymarin.

Histopathology for Oil Red O staining

The results in group A showed normal cytoarchitectural appearance alongside prominent central vein, linearly arranged hepatocytes with apparent nuclei. Group B was characterized by microvesicular and macrovesicular steatosis displayed within the green circle as shown in figure 2. The histoarchitectural observations in the ß, Epsilon-Caroten.e-3,3'-diol managed categories showed a dose-dependent ameliorative effects, with an highest efficacy observed in group E, a restoration that is comparable with normal control. The silymarin group exhibited little indication pertaining to steatotic accumulation.

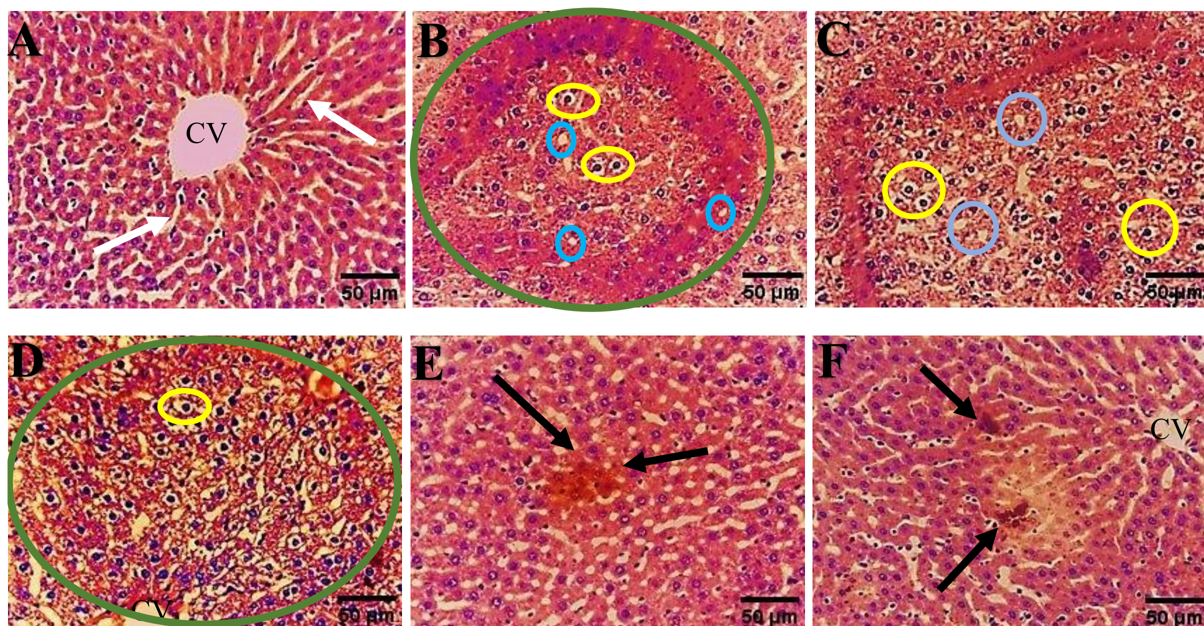

Figure 2: Representative microphotographs of the liver subjected to ethanol-induced hepatotoxicity then treated with ß, Ɛ-Carotene-3,3'diol and silymarin. Oil Red O Stain x 400 magnification. The reddish-orange coloration within the "green" circle indicates the area percolated by fatty droplets, showing features of microvesicular and macrovesicular steatosis (Blue and yellow circle). A = Normal control; B = Ethanol-treated group; C = treated with 10 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol; D = treated with 20 mg/kg b.w of ß, Ɛ-Carotene- 3, 3'-diol; E = treated with 40 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, F = treated with 200 mg/kg Silymarin.

Biochemical observation

Influence of ß, epsilon-carotene-3,3'-diol and silymarin on ethanol – influenced liver toxicity on serum antioxidants (catalase and superoxide dismutase) activities

The results revealed significant reduction ($p<0.05$) in catalase action in the negative control, in contrast to the typical control (Fig 3). Remediation with ß, Epsilon-Carotene-3,3'-diol promted significant dose-dependent increasement ($p<0.05$) across enzyme activity in groups C and D in contrast to group A. However, group E exhibited a non-significant difference ($p=0.1079$) in contrast to group A, group F (silymarin-treated) also showed a significant disparity ($p<0.05$) in contrast to group A. Group E (highest ß, Ɛ-Carotene-3,3'-diol group) exhibited a significant difference in catalase activity in contrast to group F. Ethanol significantly reduced SOD activity in group B in contrast to group A as shown in figure 3b. However, remediation with ß, Epsilon-Carotene-3,3'-diol effected a significant dose-dependent increase ($p<0.05$) in groups C, and D, in contrast to group A. However, groups E and F exhibited a non-significant difference ($p>0.05$) in contrast to group A. There was a non-significant difference ($p=0.7841$) in contrasting group E to group F.

Effects of ß, epsilon-carotene-3,3'-diol and silymarin on ethanol –influenced liver toxicity on malondialdehyde (MDA) concentration and total protein level

Fig 4a illustrates the influence of ß, Epsilon-Carotene-3,3'-diol regarding serum MDA concentration. Ethanol significantly increased ($p < 0.0001$) MDA activities in group B in c to group A. However, ß, Epsilon-Carotene-3,3'-diol and Silymarin caused a dose-dependent significant difference ($p < 0.0001$) in groups C, D, and F in contrast to group A, but a non-significant decrease was observed in contrasting category E with category A.
The Total Protein level of rats is shown in figure 4b. Ethanol significantly decreased ($p < 0.0001$) TP level in group B in contrast to group A. Remediation with ß, Epsilon-Carotene-3,3'-diol and silymarin influenced a dose-reliant significant increase ($p < 0.0001$) in groups D, E, and F when compared to group B, conversely, a non-significant difference was observed in group C ($p=0.1059$) in contrast to group B. A significant difference was displayed ($p < 0.0001$) in category E in contrast to F.

Effects of ß, epsilon-carotene-3,3'-diol and silymarin on ethanol–influenced liver toxicity on tissue tumor necrosis factor-alpha (TNF-α)

Ethanol significantly increased ($p < 0.0001$) TNF-α concentration in group B in contrast to group A as shown in figure 5. Remediation with ß, Epsilon-Carotene-3,3'-diol and silymarin influenced a dose-dependent significant increase ($p < 0.0001$) in groups D, E, and F in contrast to group B, conversely, a non-significant difference was observed in group C ($p=0.1059$) when compared to group B. It was also discovered that a significant difference ($p < 0.0001$) was in group E when compared with group F.

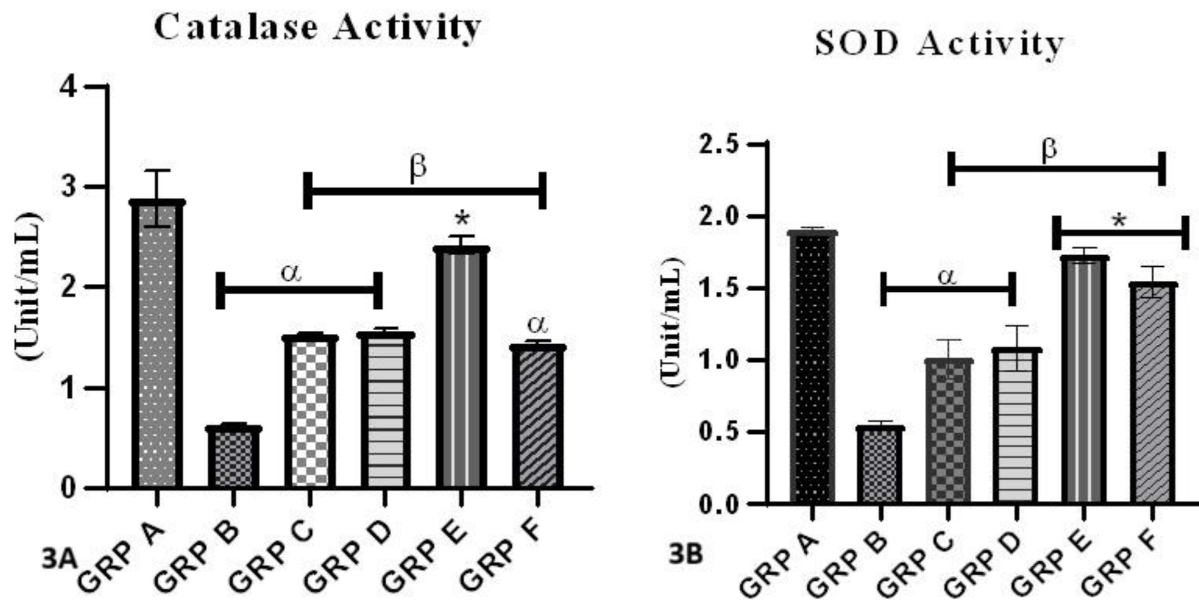

Figure 3a and 3b: Shows the activity of Catalase and Superoxide dismutase following remediation with ß, Epsilon-Carotene- 3, 3'-diol and silymarin after ethanol-influenced liver toxicity. n = 8, mean ± SEM, ANOVA, $p < 0.05$. A = Normal category; B = Ethanol-influenced category; C = 10 mg/kg b.w ß, Epsilon-Carotene- 3, 3'-diol; D = 20 mg/kg b.w of ß, Epsilon-Carotene- 3, 3'-diol; E = 40 mg/kg b.w ß, Epsilon-Carotene- 3, 3'-diol, F = 200 mg/kg Silymarin.

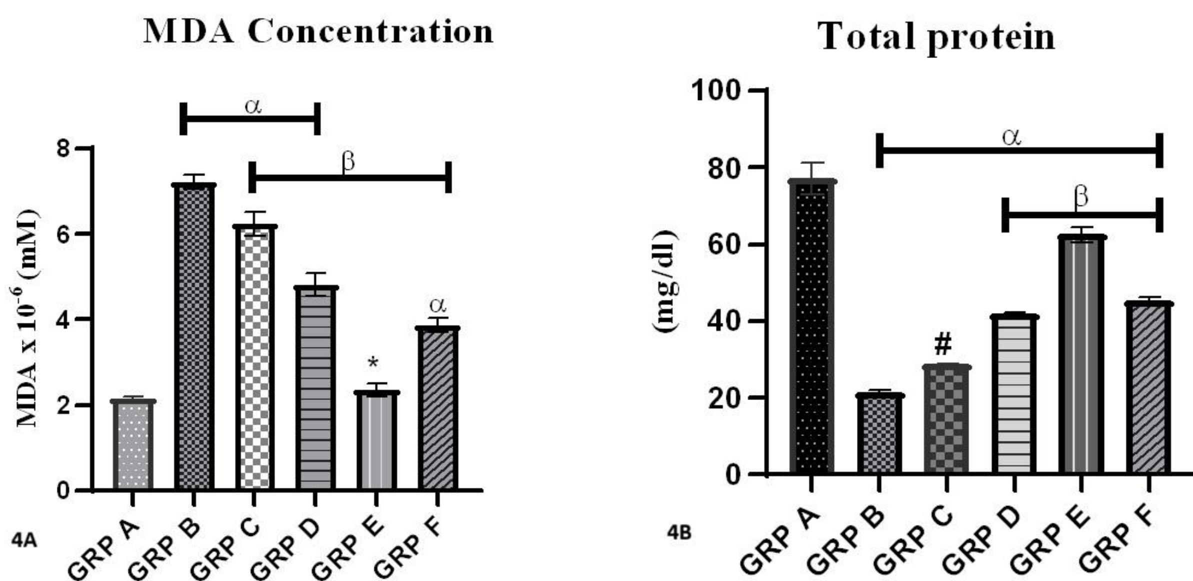

Figure 4a and 4b: Shows Malondialdehyde concentration and the total protein level following remediation with ß, Epsilon-Carotene- 3, 3'-diol and silymarin after ethanol-influenced liver toxicity. n = 8, mean ± SEM, ANOVA, $p < 0.05$. A = Normal category; B = Ethanol-treated category; C = 10 mg/kg b.w ß, Epsilon-Carotene- 3, 3'-diol; D = 20 mg/kg b.w of ß, Epsilon-Carotene- 3, 3'-diol; E = 40 mg/kg b.w ß, Epsilon-Carotene- 3, 3'-diol, F = 200 mg/kg Silymarin.

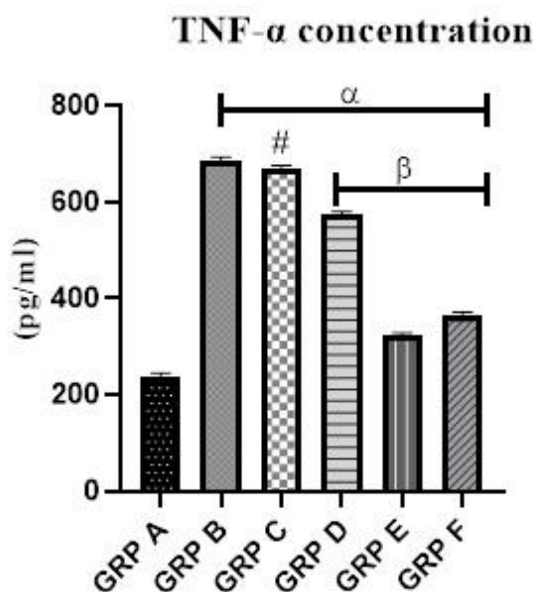

Figure 5: Shows Tumor Necrosis Factor - Alpha concentration following remediation with ß, Ɛ-Carotene- 3, 3'-diol and silymarin after ethanol-induced hepatotoxicity. n = 8, mean ± SEM, ANOVA, $p < 0.05$. A = Normal control; B = Ethanol-treated group; C = treated with 10 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol; D = treated with 20 mg/kg b.w of ß, Ɛ-Carotene- 3, 3'-diol; E = treated with 40 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, F = treated with 200 mg/kg Silymarin.

DISCUSSION

The liver is usually more damaged seeing that ethanol is metabolized there [19]. The mechanism of ethanol-induced hepatotoxicity occurs through systemic destruction to liver cells by way of redox imbalance, accumulation of lipid, and inflammation [8]. The features of hepatocyte ballooning and fatty droplets in the liver agrees with previous studies that observed that the administration of ethanol causes ballooning of the liver and presence of large and small lipid droplets [32-34]. However, remediation with ß, Epsilon-Carotene-3,3'-diol in the lesser, middle, higher dose, and the silymarin-treated group revealed a dose-dependent decrease in the lipid droplets and fatty infiltration. This is similar to the work of Ofusori [35], who posited that the observable features of fatty infiltration in the liver were abated in rats treated with ß, Epsilon-Carotene-3,3'-diol. This observation may be as a result of the antisteatotic ability of ß, Epsilon-Carotene-3,3'- diol.

Administration of ethanol initiates an inflammatory pathway, leading to an increase in tumor necrosis factor (TNF-α) concentration across the group. The activation of inflammatory pathway, underscores previous reports [36-37] which posited that ethanol and its metabolites overwhelm the liver thereby activating the IL-6 and TNF-α mediated pathways. This is because prolonged ethanol consumption activates Kupffer cells in the liver, making them responsive and sensitive to subsequent inflammatory stimuli. This increases their capacity for TNF-α production and release across liver tissues. ß, Epsilon-Carotene-3,3'-diol caused a significant depletion in TNF-α concentration in the remediated groups as opposed to the ethanol-only group. This observation highlights its ability in inhibiting inflammatory pathways, primarily by suppressing NF-kB pathway that regulates the production of inflammatory interleukins. The dose-dependent ameliorative effect indicates that higher dose provided greater protection, as similarly reported by Adekunle and Ofusori [31].

Ethanol overwhelms the body's antioxidant protection set-up by amplifying reactive oxygen species (ROS), alongside reducing essential antioxidants like catalase, superoxide dismutase and glutathione. This result corroborates the report of Moraes [38]. However, treatment with graded doses of ß, Epsilon-Carotene, 3, 3'diol, influenced an increment in Catalase and Superoxide dismutase activity in the remediated categories. This effect might be due to the capacity of ß, Epsilon-Carotene, 3, 3'diol in boosting endogenous anti-oxidant level, underscoring earlier findings [27, 39, 40]. These authors reported that the administration of exogenous antioxidants can enhance a balance reactive oxygen species when the capacity of endogenous antioxidants is limited. Therefore, the reparative effects of ß, Epsilon-Carotene, 3, 3'diol might be due to its ability in reducing ROS production and the imbalanced endogenous antioxidants.

Malondialdehyde is an ultimate derivative of the degeneration of polyunsaturated fatty acids [39]. This research showed that ethanol administration increased MDA concentration. This increased concentration might be due to the excessive production of free radicals alongside manifestation of facilitated lipid peroxidation. This underscores previous reports [42, 43] which

posited that increased lipid peroxidation leads to higher levels of MDA. The dose-dependent decrease in MDA concentrations in the ß, Epsilon-Carotene, 3, 3' diol-treated groups might be connected to its potency in neutralizing lipid peroxyl radicals, thereby interrupting the chain reaction of lipid oxidation caused by ethanol.

Hepatocytes are responsible for protein synthesis, the reduction in total protein may be partly due to their destruction following ethanol administration. Ethanol causes an increase in poly-ubiquitinated proteins by impairing the ubiquitin-proteasome pathway (UPP). The impairment of this system can result from the adduct formation between acetaldehyde and proteasome subunits, thereby leading to protein degradation. Similarly, the reduction in protein level may also be due to the inhibiting effect of acetaldehyde on hormones like IGF-1, insulin, and GH that stimulates and regulates protein synthesis and metabolism. This corroborates the earlier findings which reported that ethanol intake decreases demonstration of hepatocyte growth factor (HGF), growth hormone (GH), Insulin-like growth factor-1 (IGF-1) [44-45], which modulates synthesis of enzymes and transport of metabolites which influences protein synthesis. Similarly, according to McDonough [46], an alteration in the antioxidant defense system causes hydroxyl radical (OH), superoxide radical (O_{2s-}) and hydrogen peroxide (H_2O_2) to react with cellular proteins, lipids and DNA. However, treatment with varying doses of ß, EpsilonƐ-Carotene, 3, 3' diol in this study promoted an increase in protein level. This may be due to its ability to decrease ubiquitin conjugates by suppressing the expression of ubiquitin ligases like Atrogin-1 and MuRF1 which are involved in the UPP.

By removing these enzymes, ß, Epsilon-Carotene, 3, 3' diol decreased the number of ubiquitin conjugates, and protein breakdown was reduced. ß, Epsilon-Carotene, 3, 3' diol may have also promoted protein synthesis by stimulating growth-related factors like GH, HGF, leading to increased protein synthesis as seen in the low, mid, and high dose groups respectively. Some bioactive compounds, such as flavonoid, polyphenols, and saponins [47-50] are contained in ß, Ɛ-Carotene, 3, 3' diol, these compounds contribute to phenomena and overall properties that help in mitigating the overall ethanol damage to the liver.

CONCLUSION

In conclusion, this study has provided information that treatment with ß, Epsilon-Carotene-3,3'-Diol displayed ameliorative effects, demonstrating a stronger dose–reliant reduction in liver inflammation, improving antioxidant enzyme activity, and promoting reduction of fatty droplets. This result was particularly evident at a high dosage of 40 mg/kg body weight, highlighting it as a potential anti-steatotic, antioxidant and anti-inflammatory agent.

Declarations

This research obtained no specific grant from any sponsor agency

Conflict of interests

The authors have no conflicting interests to declare.

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