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Role of Extra Virgin Olive Oil on the Liver of Alcohol and Benzene-Treated Wistar Rats

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Abstract: This study aimed at demonstrating the activities of extra virgin olive oil (EVOO) against the effect of alcohol and benzene on the liver of wistar rats. Forty-eight (48) female Wistar rats were used for this study. They were divided into eight (1-8) groups (n = 6). Group 1-control, Group 2-25% Ethanol, Group 3-200 mg/kg/b.w Benzene, Group 4-25% Ethanol + 200mg/kg/b.w Benzene, Group 5-25% Ethanol + 2ml EVOO, Group 6-200mg/kg/b.w Benzene + 2ml EVOO, Group 7-25% Ethanol + 200mg/kg/b.w Benzene + 2ml EVOO, Group 8-2ml EVOO. Animals were euthanized through cervical dislocation after the last day of administration and the liver were excised and part was fixed in formalin solution of 10 % for histological processing and the other part were homogenized for biochemical assay in phosphate buffer before centrifugation. Histological evidence led us to understand the cellular defect (hepatic degeneration) in which benzene and ethanol manifests likewise the mitigated ability of extra virgin olive oil. The relative organ weight provides information on the extent of the hepatic degeneration. Liver function test level is significantly expressed. *Conclusion*: Ethanol and benzene in combination cause several damage; also, they separately induce hepatic dysfunction. Extra virgin olive oil was shown to mitigate these hepatic damages.

Key words: Ethanol · Benzene · Extra Virgin Olive Oil · Hepatic

INTRODUCTION

The uncontrolled exposure to substances such as benzene and ethanol has been labeled as a global burden by the World Health Organization (WHO), apparent role in liver damage leading to various disease conditions such as cirrhosis and eventually hepatocellular carcinoma was estimated to cause over one million deaths in 2010, which is approximately 2% of all deaths [1]. Benzene and alcohol have been highly rated differently by causing hepatotoxic and hypertrophy and likewise elevating biochemical parameters of the liver [2-4]. Both have been a major toxicant substance within the society that causes injury to the liver, kidney and pancreas such as inflammation. Inflammation is a biological response of the immune system that can be triggered by a variety of factors, including pathogens, damaged cells and toxic compounds leading to a process by which the immune system recognizes and removes harmful stimuli and begins the healing process [5]. These factors may induce acute and/or chronic inflammatory responses in the heart, pancreas, liver, kidney, lung, brain, intestinal tract and reproductive system, potentially leading to tissue damage or disease [6].

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Benzene occurs naturally as a product of pyrolysis, mostly through anthropogenic sources [7] and its major source of exposure are tobacco smoke, automobile service stations, exhaust from motor vehicles and industrial emissions; about 50% of the entire nationwide exposure to benzene results from smoking tobacco, smokes, vapors from products that contain benzene such as glues, paints, furniture wax and detergents can also be sources of exposure [8]. Waterborne and food-borne benzene contributes only a small percentage of the total daily intake in non-smoking adults; benzene was detected in approximately 40% of surface water samples with levels ranging from non-detectable to 100μg/L [9]. Benzene after being metabolized in the liver and bone marrow produces toxic metabolites and free radicals, which are known to be responsible for oxidative stress [10]. The properties of membrane phospholipids were changed, some structural and functional changes occur under benzene treatment in rat microsomes; it has also been shown that benzene induces oxidative stress, benzene toxicity is related to the ability of its reactive intermediates to bind to DNA and proteins, cell cycle alterations and programmed cell death in cultured cells [11]. The increase in the enzymes activities in serum of benzene-treated rats is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream; in other words enzymes activities increase in serum when cellular degeneration or destruction occurs in liver; the major metabolites found in the liver are muconic acid and hydroquinone [12].

Alcohol consumption is a major risk factor for chronic diseases, based on 58 studies from 17 Global Burden of Diseases (GBD) regions, alcohol use disorder accounted for 9.6% (7.7-11.18%)of age-standardized disability-adjusted life years (DALYs) worldwide in 2010 [13]. Alcohol induced liver cirrhosis which was responsible for 0.9% of all global deaths and 47.9% of all liver cirrhosis deaths in 2010 [14]. Ethanol induces fatty liver by increasing the ratio of reduced form of nicotinamide adenine dinucleotide to oxidized form of nicotinamide adenine dinucleotide in hepatocytes; increasing hepatic sterol regulatory element-binding protein (SREBP)-1, plasminogen activator inhibitor (PAL)-1 and early growth response-1 activity and decreasing hepatic peroxisome proliferator-activated receptor-α activity; it activates the innate immune system and induces an imbalance of the immune response which is followed by activated Kupffer cell-derived tumor necrosis factor (TNF)-α overproduction, which is in turn responsible for the damages in the hepatic SREBP-1 and PAL-1 activity [15].

WHO expert Group defined traditional medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing [16]. Essential oils extracted from aromatic plants are natural products and represent an important part of traditional pharmacopeia; they have been reported to inhibit several phytopathogens, human pathogens and insects as well as their effective uses in food and pharmaceutical industries [17].

The chemical composition of olive oil varies depending on the extraction technology that is applied in order to obtain oil from the fruits; the process of extraction of olive oil depends on crushing olives and then separating the oil from the fruit pulp under elevated pressure [18]. Oil obtained by chemical extraction can be consumed after refining; the process of refining is to purify the extracted oil from any residual solvent and other impurities, however refined olive oil is devoid of vitamins, polyphenols, phytosterols and other low molecular natural ingredients [19]. Olive oil mostly consists of triacylglycerols (98-99%) which are a diverse group of glycerol esters with different fatty acids, the fatty acid majorly present in olive oil tricylglycerols is mono saturated oleic acid (up to 83%) [20]. Extra virgin olive oil by its low yield is more expensive than other types of olive oil, but it contains the highest level of polyphenols; it also has a delicate flavor, aroma and light color due to the removal of free fatty acids [21]. 1-2% of the weight of Extra Virgin Olive Oil (EVOO) is represented by other minor constituents such as: (i) An apolar fraction, represented by squalene, triterpenes, sterols, tocopherols and pigments, which can be extracted with solvents and (ii) A polar fraction, where phenolic compounds are prominent and to which many beneficial effects on human health have been attributed [22]. These phenolic compounds exert potent anti-inflammatory actions [23]. Oleocanthal (OC), a naturally occurring phenolic secoiridoid exclusively found in extra-virgin olive oil (EVOO), is a potential nutraceutical therapeutic for inflammation, neurodegenerative diseases and many malignancies, especially breast cancer [24]. These protective effects manifest through the modulation of various enzymatic pathways that reduce inflammation and oxidative stress, prevent hepatic tissue damage and promote Fatty acid oxidation [25]. This study was based on the effect of these conjoint environmental toxic substances on live and effectiveness of the EVOO.

MATERIALS AND METHODS

Ethical Approval: This experiment was carried out in conformity with the rules and guidelines of the Animal Ethics Committee of the Babcock University Ilishan, Ogun State. All rules and regulations in the guide, care and compliance with the Institutional Animal Care and Use committee (IACUC) and teaching are abide and approved by Babcock University Health Research Ethics Committee (BUHREC 796/19), Ilishan Remo, Ogun State, Nigeria.

Preparation and Procurement: Benzene was purchased from University of Ibadan. An appropriate quantity was measured and kept in an amber bottle, Ethanol (75%) was gotten from Sigma-Aldrich, 10mls of the ethanol was mixed with 20mls of distilled water to give 30mls of 25% ethanol, while EVOO was also purchased from Sigma-Aldrich and kept in a cool dry cabinet when not in use.

Study Design: Forty-eight (48) female Wistar rats were purchased from the animal house facility of Babcock University, for the experiment. They were placed in plastic cages with net covers for ventilation. The rats were bred at the Department of anatomy animal house, Babcock University. After two weeks' acclimatization period, the animals were divided into eight (1-8) groups (n = 6).

Group 1 - (CTR) Control group
Group 2 - 25% (2ml) Ethanol (ETH) twice a week for 2 weeks.
Group 3 - 200mg/kg/b.w Benzene (BEN) twice a week for 2 weeks.
Group 4 - 25% (2ml) Ethanol and 200mg/kg/b.w Benzene (ETH + BEN) simultaneously twice a week for 2 weeks.
Group 5 - 25% (2ml) Ethanol twice a week for 2 weeks after which EVOO (2ml) treatment started twice a week for the next two weeks (ETH + EVOO)
Group 6 - 200mg/kg/b.w Benzene twice a week for 2 weeks after which EVOO (2ml) treatment started twice a week for the next two weeks (BEN + EVOO)
Group 7 - 25% (2ml) Ethanol and 200mg/kg/b.w Benzene simultaneously twice a week for 2 weeks after which EVOO (2ml) treatment started twice a week for the next two weeks. (ETH + BEN + EVOO)
Group 8 - EVOO (2ml) twice a week for 2 weeks

Excision of Target Tissue: After the last day of administration, the experimental animals were euthanized using cervical dislocation (Liver were harvested and fixed in 10% formol-saline for routine histological processing using Haematoxylin & Eosin (H&E) stain to highlight the general microstructure of Liver, while Masson's Trichrome Stain to highlight collagenous connective tissue fibers. The animal blood was collected through Ocular puncture after which it was subjected to centrifugation at a resolution of 3000 rpm for 30 minutes. Cleared supernatants were aspirated into plain bottles and refrigerated at 4°C before the liver function test.

RESULTS

Table 1: Showing RBW= Rats Body Weight, M ± SD= Mean ± Standard Deviation, M ± SEM= Mean ± Standard Error of Mean, *= Significant at $p < 0.05$ (2 tail), CTR= Control, ETH= Ethanol, BEN= Benzene, ETH+BEN= Ehanol + Benzene, ETH+EVOO= Ethanol+Extra Virgin Olive Oil, BEN+EVOO= Benzene+Extra Virgin Olive Oil, ETH+BEN+EVOO= Ethanol+Benzene+Extra Virgin Olive Oil, EVOO= Extra Virgin Olive Oil

	RBW (g)			
Groups	M ± SD	M ± SEM	*T*	*p*
CTR	187.4 ± 12.76	187.4 ± 4.51	2.723	0.034?
ETH	121.9 ± 9.98	121.9 ± 3.53	0.718	0.500
BEN	139.9 ± 11.01	139.9 ± 3.90	0.089	0.932
ETH+BEN	140.6 ± 11.49	140.6 ± 4.06	1.415	0.207
ETH+EVOO	138.0 ± 14.26	138.0 ± 5.04	0.467	0.657
BEN+EVOO	107.1 ± 12.62	107.1 ± 4.46	0.287	0.784
ETH+BEN+EVOO	127.9 ± 11.37	127.9 ± 4.02	0.319	0.760
EVOO	157.0 ± 20.38	157.0 ± 7.21	3.122	<0.021?

Results are presented as M ± SD= Mean ± Standard Deviation, M ± SEM= Mean ± Standard Error of Mean, *= Significant at $p < 0.05$.

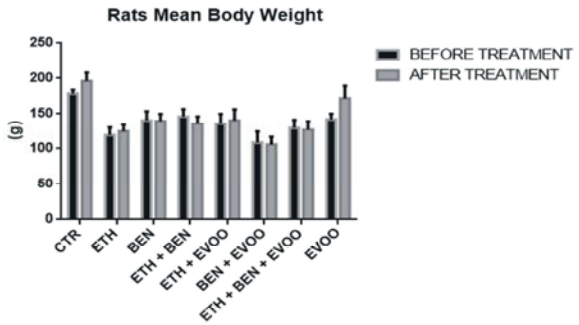


Fig. 1: Bar chat of Mean body weight showing CTR= Control, ETH= Ethanol, BEN= Benzene, ETH+BEN= Ehanol + Benzene, ETH+EVOO= Ethanol+Extra Virgin Olive Oil, BEN+EVOO= Benzene+Extra Virgin Olive Oil, ETH+BEN+EVOO=Ethanol+Benzene+ExtraVirgin Olive Oil, EVOO= Extra Virgin Olive Oil.

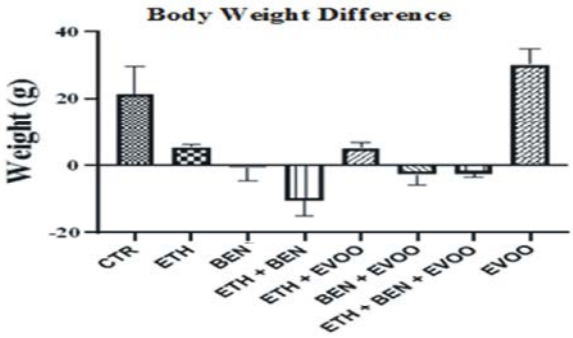
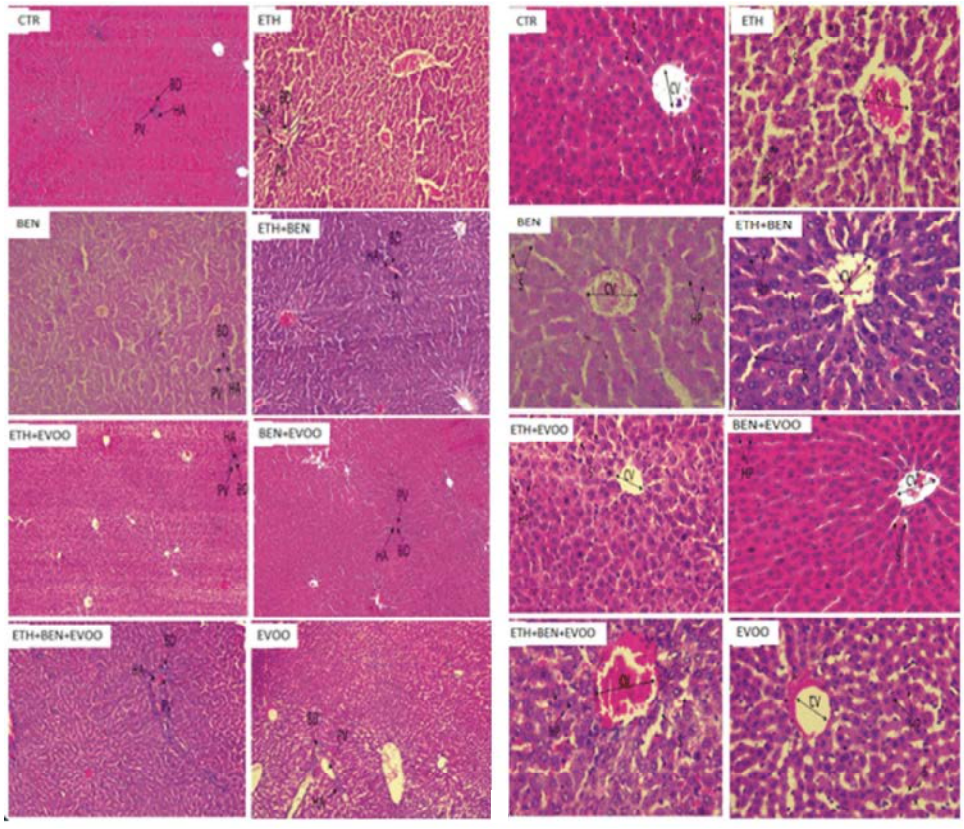


Fig. 2: Bar graph showing the Body Weight Difference in the CTR= Control, ETH= Ethanol, BEN= Benzene, ETH+BEN= Ethanol + Benzene, ETH+EVOO= Ethanol +Extra Virgin Olive Oil, BEN+EVOO= Benzene +Extra Virgin Olive Oil, ETH+BEN+EVOO= Ethanol + Benzene +Extra Virgin Olive Oil, EVOO= Extra Virgin Olive Oil



A B

Fig. 3: Hepatocytes of Wistar Rats Exposed to CTR= Control, ETH= Ethanol, BEN= Benzene, ETH+BEN= Ethanol + Benzene, ETH+EVOO= Ethanol +Extra Virgin Olive Oil, BEN+EVOO= Benzene +Extra Virgin Olive Oil, ETH+BEN+EVOO= Ethanol + Benzene + Extra Virgin Olive Oil, EVOO= Extra Virgin Olive Oil. Fig. 4A; Mag. x100 and Fig. 4B; Mag. × 400 Haematoxylin and Eosin Stains.
LEGEND: BD: Bile Duct, HA: Hepatic Artery, PV: Portal Vein, CV: Central Vein, HP: Hepatocytes, S: Sinusoids

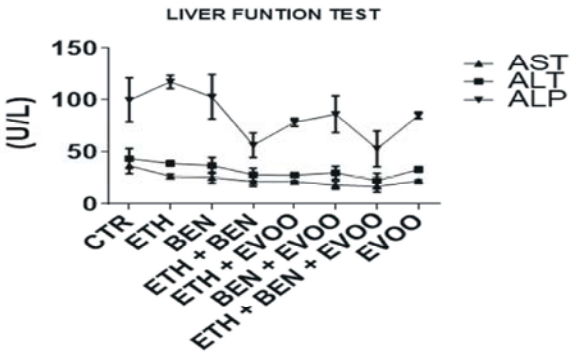
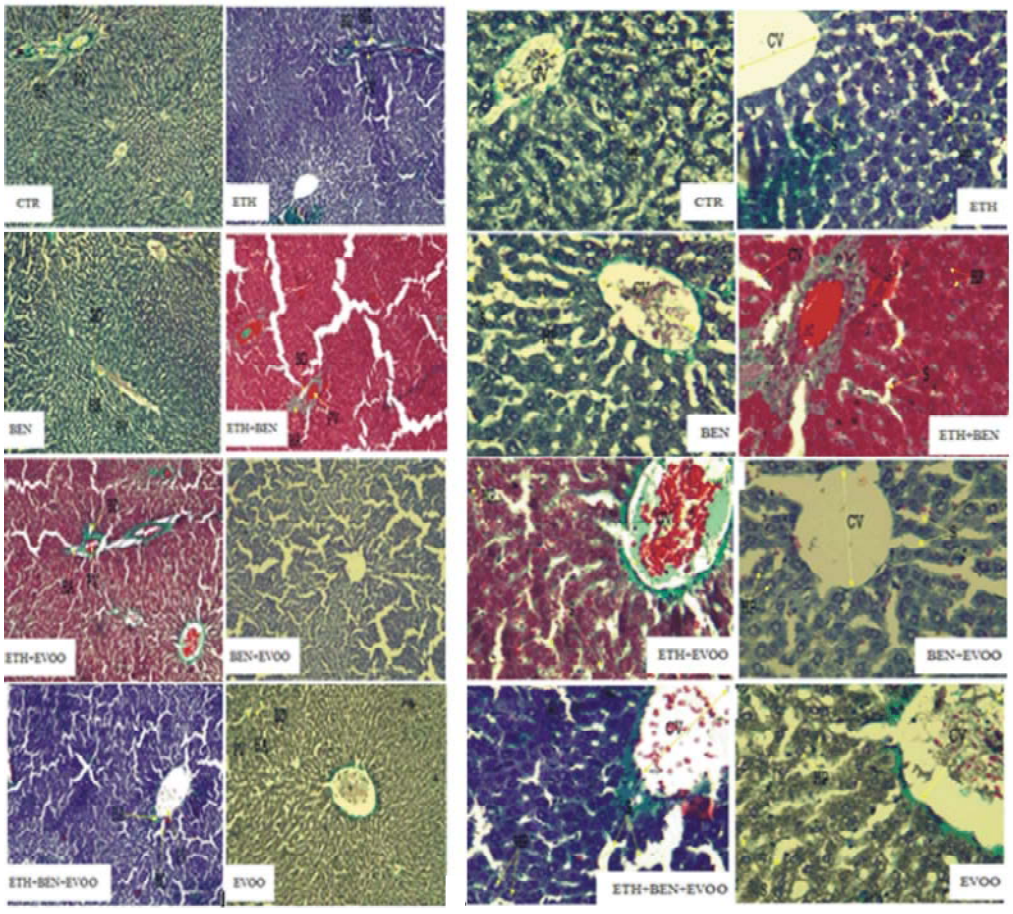


Fig. 4: Graph line showing the AST= Aspartate Aminotransferase, ALT= Alanine Aminotransferase, ALP= Alkaline Phosphatase, CTR= Control, ETH= Ethanol, BEN= Benzene, ETH+BEN= Ethanol + Benzene, ETH+EVOO= Ethanol +Extra Virgin Olive Oil, BEN+EVOO= Benzene +Extra Virgin Olive Oil, ETH+BEN+EVOO= Ethanol + Benzene + Extra Virgin Olive Oil, EVOO= Extra Virgin Olive Oil.



A B

Fig. 5: Hepatocytes of Wistar Rats Exposed to CTR= Control, ETH= Ethanol, BEN= Benzene, ETH+BEN= Ethanol + Benzene, ETH+EVOO= Ethanol +Extra Virgin Olive Oil, BEN+EVOO= Benzene +Extra Virgin Olive Oil, ETH+BEN+EVOO= Ethanol + Benzene + Extra Virgin Olive Oil, EVOO= Extra Virgin Olive Oil. Fig. 6A: Mag. × 100 and Fig. 6B; x400 Masson Trichrome Stains.
LEGEND: BD: Bile Duct, HA: Hepatic Artery, PV: Portal Vein, CV: Central Vein, HP: Hepatocytes, S: Sinusoids

DISCCUSION

Ethanol and benzene are agents used in inducing oxidative stress. Although less common, the exposure of these agents to the Nigerian population causes this study to deal with understanding the effects of ethanol and benzene on liver damage and the effects of EVOO in animal models over a period of 6 weeks. Induced oxidative stress has been reported to affect the hepatocytes which causes fibrosis and over time develops into cirrhosis eventually affecting liver function. The liver loses its function in these models and there is observable damage as seen in biochemical "liver function tests" results in which the levels of aspartate transaminase (AST), Alanine Transaminase (ALT) and Alkaline phosphatase (ALP) gives the impression that the amount in the blood is directly associated with the amount of tissue damage, as the experiment shows a significant decrease in the treated group compared to untreated group and control, in AST and ALP via the statistical analysis indicates that alkaline phosphate levels, aspartate aminotransferase levels and aspartate aminotransferase levels of the ETH, BEN and BEN+ETH groups were significantly higher when compared to their counterpart EVOO treated groups, which infers that ethanol and benzene in isolation or combination possess significant harm to the liver which could be greatly reduced with the aid of extra virgin olive oil [26-30].

Influence (Alcohol, Benzene and Extra Virgin Olive Oil) on body weights shows that the statistical analysis of relative body weight is significant, at a P value of <0.05 (95% confidence interval) although values are closely related; it indicates that EVOO group had the highest relative organ weight, while ETH+EVOO had the lowest along with the mean organ weight, suggesting that they suffered body weight increase relative to their organ weight loss. The mean relative organ weight of rats in EVOO was highest due to treatment with extra virgin olive oil, the rats suffered body weight increase relative to the increase organ weight. According to the results obtained from the body weight our experimental animals were subjected to two inflammatory agent benzene and alcohol that impaired the hepatocyte greatly, in which P value of <0.05 (95% confidence interval), showed that both CTR and EVOO recorded the highest mean weight gains across the grouping, EVOO being the highest weight gaining group explained the existence of growth supplements in extra virgin olive oil [22].

The organ weight of the CTR was recorded to be the highest and ETH which was the least, although rats in CTR group had the highest organ weight, it was only slightly higher than the EVOO group; this could be as a result of extra virgin olive oil improving hepatocyte generation in the liver. Therefore, an increase in the liver would lead to a relative increase in total body weight as it is the largest organ. This inference could mean that extra virgin olive oil has a positive effect on the liver weight. The ETH, BEN and BEN+ETH groups all had significantly lower means when compared to CTR, indicating significant negative effect. ETH+EVOO, BEN+EVOO and BEN+ETH+EVOO groups showed no significant difference when compared to the corresponding groups not treated with extra virgin olive oil indicating no ameliorating effect of extra virgin olive oil which could be as a result of short term treatment.

In the body weight difference ETH had insignificant weight gain when compared to CTR and EVOO, but had a higher weight gain when compared to BEN and BEN+ETH which recorded weight losses and not gains. In the experiment, only ETH+EVOO showed a weight gain significantly slightly related to ETH alone, while BEN+EVOO and BEN+ETH+EVOO showed a weight loss significantly when compared to that of BEN and BEN+ETH. The extra virgin olive oil (EVOO) explained the increasing effect on body weight and ameliorative effects with the increase weight in ETH+EVOO, BEN+EVOO and BEN+ETH+EVOO [23], Likewise benzene could have a more significant negative effect on animal weight compared to ethanol [7].

Histological results of the Masson Trichrome Stain show that the CTR and EVOO groups were seen to have well-arranged and integrated hepatocyte with eosinophilic cytoplasm, rounded vascular nuclei: No prominent nucleoli with intact endothelium of blood and lymph vessels and bile ducts. The ETH and BEN groups was seen to have a slightly altered arrangement compared to the CTR and EVOO groups with abnormal distribution of collagen and many enlarged vacuolated degenerating cells having indistinct boundaries and pale cytoplasm with fibrosis, steatosis and collections of eosinophilic alcoholic hyaline Mallory bodies. Apoptotic cell number was increased showing shrinkage, hypereosinophillic cytoplasm and fragmented nuclei, portal tracts were infiltrated with many inflammatory cells. While that of the BEN+ETH group was highly altered with hepatic pathologies; The BEN+EVOO and ETH+EVOO groups

showed less altered arrangement compared to the ETH and BEN groups with almost normal distribution of collagen without remarkable fibrosis and almost no inflammatory cells infiltration. Hepatocytes appeared with distinct boundaries, moderate amount of vacuoles, mild cytomegaly and almost normal cytoplasmic acidophilia. Apoptotic cells were minimally increased while alcoholic Mallory bodies were almost absent among minimal degenerating hepatocytes. And the BEN+ETH+EVOO group showed no significant reduction in abnormal collagen distribution, fibrosis and inflammatory cells infiltration with many enlarged vacuolated degenerating cells having indistinct boundaries and pale cytoplasm with fibrosis and steatosis. The Hematoxylin and Eosin shows blood sinusoids not apparent between the vacuolated hepatocyte, slight distortion in the architecture of the hepatic plates and boundaries with fibrosis, steatosis and collections of eosinophilic alcoholic hyaline Mallory bodies in the cytoplasmic of the ETH, BEN and ETH+BEN groups; the ETH+EVOO and BEN+EVOO groups are less affected; the BEN+ETH+EVOO group showed no significant reduction in fibrosis and inflammatory cells and the EVOO group showed similar hepatic standard to that of the CTR group with adially arranged cords of hepatocytes with eosinophilic cytoplasm, rounded vascular nuclei and prominent nucleoli while the sinusoids, intact endothelium, the hepatic stroma and parenchyma within normal range proving that extra virgin olive oil has no toxicity in the liver, which is in agreement with many similar studies which have reported that extra virgin olive oil does not affect the liver tissue and can therefore be accepted as a safe natural product with no health hazards or significant side effects [31, 32].

CONCLUSIONS

Evidence has shown the dangers of ethanol and benzene that is implicated in the hepatic degeneration associated with liver damage as seen in alcoholic liver disease as well as cirrhosis. The relative organ weight provides information on the extent to which the liver is defected to reduce in size due to hepatic degeneration. Histological experiments have also led us to understand the cellular effects in which benzene and ethanol manifests as well as the effect of extra virgin olive oil. Finally, having supported the presence of the induced liver damage with serum Alkaline phosphate (ALP), Alanine transaminase (ALT) and Aspartate transaminase (AST) levels, the study suggested that extra virgin olive oil could be a potential treatment for the induced liver damage.

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