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ETHANOL AND BENZENE INDUCED TOXICITY IN WISTAR RATS: AMELIORATIVE EFFECTS OF EXTRA-VIRGIN OLIVE OIL ON HAEMATOLOGICAL INDICES AND SPLEEN DAMAGE

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ABSTRACT

Background: The metabolic intermediates of benzene and ethanol are relatively poisonous to humans. Extra virgin olive oil (EVOO) is considered a natural source of bioactive compounds with beneficial effects on multiple organs. There is a dearth of empirical information on its protective roles in relieving symptoms associated with ethanol and/or benzene intoxication. **Objective:** This study was conducted to investigate the effects of extra virgin olive oil benzene and/or ethanol-induced haematological and splenic tissue aberrations in rats. **Methodology:** Forty-eight female Wistar rats (110kg-170kg) were randomly divided into 8 groups (n=6); Negative Control, EVOO= Extra Virgin Olive Oil, 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. Animals were sacrificed and blood samples were collected for food blood count (FBC) analysis. Data were analyzed with a graph pad prism (version 5.03). $p<0.5$ was considered significant. The spleen was excised and processed for the histopathological assessment using Haematoxylin & Eosin (H&E) and Periodic Acid Schiff (PAS) staining techniques. **Results:** The FBC analysis showed alteration in macrophages, neutrophils and monocytes following treatment with ethanol and/or benzene, while also showing marked distortion of the histoarchitectural integrity of the splenic tissues. However, treatment with EVOO significantly ($p<0.05$) normalized haematological indices while also ameliorating splenic tissue distortions. **Conclusion:** Data obtained from this study revealed that extra virgin olive oil has anti-inflammatory potential on the Spleen.

KEYWORDS: Extra Virgin Olive Oil, Benzene, Ethanol, Spleen, hematology.

INTRODUCTION

Drug use incorporates the non-medical exercise of psychoactive substances, including legal drugs such as alcohol (ethanol).[1] Ethanol and benzene are heavy chemicals which are used commonly for various industrial processes.[2] Ethanol is a clear, colourless liquid which is a major component of alcoholic beverages.[3] Benzene is a highly volatile industrial chemical which is commonly present in petroleum products and combustion effluents.[4] Active and passive exposure to tobacco smoke may also serve as a very common source of benzene contamination[5].

Chronic human exposure to either ethanol or benzene has been implicated in a host of human pathological conditions in multiple organs.[6],[7],[8] Alcohol intoxication can potentially cause a range of diseases within the gastrointestinal tract (GIT),[9] central nervous system,[10] gonads,[11] circulatory system,[12] cardiovascular system.[13],[14],[15]

Epidemiological evidence has documented the roles of alcohol in a host of malignant diseases.[16],[17] Whereas prolonged benzene exposure has been associated with acute myeloid and acute non-lymphocytic

leukaemia,[18],[19],[20] aplastic anaemia, necrosis, headache, dizziness, drowsiness, confusion, tremors, and loss of consciousness.[21],[22],[23] The toxic dynamics of both benzene and ethanol have been reported to elicit deleterious genotoxicity.[24] A higher risk of genetic mutation has been reported among individuals who abuse both cigarettes and alcohol than those who consume either of them.[25] Of course, the toxicity of benzene can be potentiated by ethanol.[26]

Extra virgin olive oil is an essential oil that is commonly extracted from the seed of the olive tree plant.[27] Its prolonged incorporation as an essential component of Mediterranean cuisines has been associated with an increased life span.[28],[29]

Notably, the pharmacological activities of extra virgin olive oil have been scientifically proven to relieve pathological symptoms in multiple organs.[30],[31], [32] As a result, it has been demonstrated to possess antioxidative,[33],[34] analgesic, anti-inflammatory,[35], [36] antimicrobial,[37], [38] antiviral,[39] anti-atherogenic,[40] and anti-mutagenic,[41] properties. In our previous study, we demonstrated its curative effect on experimental nephrotoxicity.[42] Importantly, its classic pharmacological properties are usually attributed to its constituent bioactive components.[43] However, there is a dearth of empirical information concerning the protective effects of extra virgin olive oil on ethanol and benzene induced hematotoxicity and splenic tissue injury. The present, therefore, study is aimed at elucidating its ameliorative properties on ethanol and benzene induced toxicity using a biochemical and histomorphological approach.

MATERIAL AND METHODS

Chemicals and Reagents

Analytical grades of benzene, ethanol (75%) and extra virgin olive oil were procured from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were obtained from either the British Drug House (Poole, England) or Randox laboratory (Aldren, USA). Benzene, ethanol and extra virgin olive oil were kept in pigmented bottles and kept cool at room temperature.

Animal Procurement, use and care.

Female Wistar rats (110kg-170kg) were inbred at the animal house facility at the Department of Anatomy, Babcock University, Ilisan Remo, Ogun State Nigeria. Rats were kept in plastic rat cages (Mediwise animal cage, 430 × 270 × 15 mm) under standard atmospheric conditions. The animals were given unrestricted access to water and rat chow (grower's mash). Ethical approval for the study was obtained from Babcock University Health Research Ethics Committee (BUHREC 751/19), Ilishan Remo, Ogun State, Nigeria. Ethanol, Benzene, and Extra Virgin Olive Oil were administered orally using gastric gavage. Rats were treated humanely in compliance with the guidelines of the Institutional Animal Care and Use Committee (IACUC).

Animal grouping and treatment protocol

Before the commencement of the study, rats were kept for at least two weeks to allow for acclimatization. Rats were randomized and grouped into 8 (n=6); group 1 was designated to be the control (CTR) group and was administered distilled water (2 mL/kg), and group 2 received extra virgin olive oil {EVOO} (2 mL/kg), group 3 received 25% (2 mL/kg) Ethanol {ETH}, group 4 received benzene {BEN} (200 mg/kg), group 5 received ETH and BEN, group 6 received ETH and EVOO, group 7 received BEN and EVOO while group 8 received ETH, BEN and EVOO. Rats in all groups were treated twice a week for two weeks (14 days). The rat grouping, treatment protocol and dosage regimen are shown in table 1.

Table 1: Rat grouping, dosage and administration Schedule.

Group	Administration	Dose regimen	Duration
A	Distilled Water	2 mL/kg	2 weeks
B	EVOO	2 mL/kg	2 weeks
C	ETH	2 mL/kg of 25%	2 weeks
D	BEN	200mg/kg	2 weeks
E	ETH + BEN	2 mL/kg of 25% and 200mg/kg respectively	2 weeks each
F	ETH + EVOO	2 mL/kg of 25% and 2 mL/kg respectively	2 weeks each
G	BEN + EVOO	200mg/kg and 2 mL/kg respectively	2 weeks each
H	ETH + BEN + EVOO	2 mL/kg of 25%, 200mg/kg and 2 mL/kg respectively	2 weeks each

Animal Sacrifice

After the last day of administration, the experimental animals were euthanized by cervical dislocation. Blood was collected through the ocular puncture into EDTA tubes for haematological assay. The Spleen was carefully excised and fixed in 10% formol-saline for routine histological processing.

Haematological assays

Haematological analysis of the blood samples was performed using an automated haematology analyzer (2800 Hematology AutoAnalyzer). Full blood count (FBC) analysis was carried out including packed cell volume (PCV), haemoglobin (HB), white blood cells, neutrophils, lymphocytes (L) and monocytes (M).

Histopathology Examination

The spleen was dehydrated with increasing concentrations of isopropyl alcohol (50%, 70%, 90%, and 100%), cleared in xylene, and then impregnated in paraffin wax of melting point between 55^0C–56^0C for infiltration. Paraffin sections at a thickness of 5 µm were then mounted on glass slides. Haematoxylin & Eosin (H&E) stain was used to highlight the general microstructure of the spleen while Periodic Acid Schiff (PAS) stain was used to highlight collagenous connective tissue fibres. The pancreas was also stained with Masson Trichrome to visualize collagen fibres. The stained tissue sections were then observed under the microscope (Leica, DM 750) interfaced with a camera (Leica, ICC 50), and photomicrographs taken at x40 objective lens were archived.

Data Presentation and Statistical Analysis

Significant differences in data were determined by one-way analysis of variance (ANOVA). This was followed by Tukey's *post hoc* test on Graph Pad Prism version 5.03 (GraphPad Software, Inc. CA, 92037 USA). Data were expressed as mean ± standard error of the mean (SEM). $p < 0.05$ was considered significant.

RESULTS

Effects of Extra virgin olive oil, Ethanol and Benzene on Organ Weight and Relative Organ Weight

Comparison of data showed no significant difference ($p > 0.05$) when all groups were compared.

Following the administration of ethanol and benzene, and treatment with extra virgin olive oil, there was no significant difference in the organ weight (figure 1). Also, the relative organ weight (figure 2) showed no significant difference across all treatment groups in comparison with the control group.

Effects of Extra virgin olive oil, Ethanol and Benzene on Hematological Parameters

Administration of ethanol and benzene alone or when combined resulted in significant alteration of the haematological parameters (Table 2). Interestingly, treatment with extra virgin olive oil significantly ($p<0.05$) reversed the hematotoxic effects of ethanol, benzene and ethanol combined with benzene.

Histopathological Assessment

Section showed that ethanol and benzene resulted in the remarkable distortion of the splenic tissue compartment using H and (Plate 1) as well as periodic acid Schiff staining techniques (Plate 2) However, improved splenic tissue histoarchitecture was observed among the group of animals treated with extra virgin olive oil.

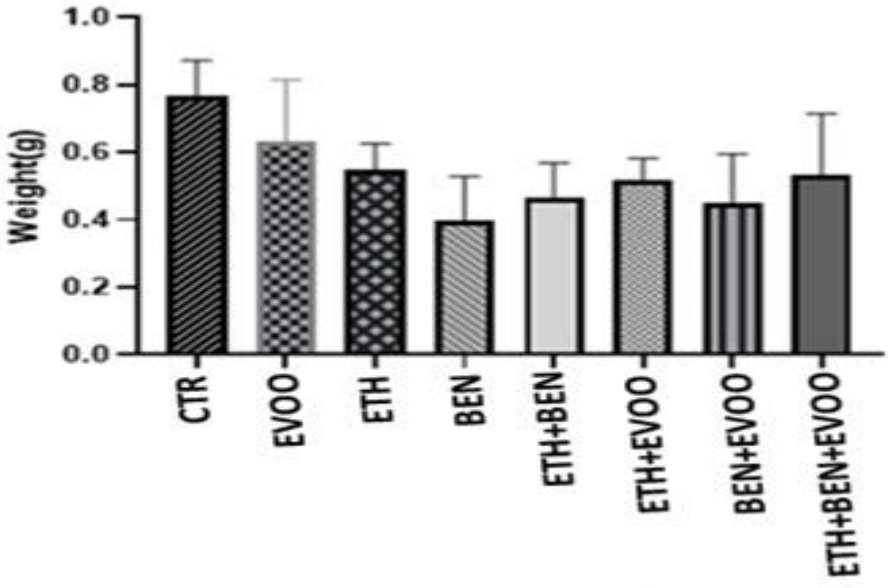

Figure 1: Bar chart represents the weight of Spleen following the effect of extra virgin olive oil, ethanol and benzene. Data are expressed as mean ± SEM. Values are not statistically significant across the groups at (p>0.05).

Abbreviations: Control (CTR), Extra Virgin Olive Oil (EVOO), Ethanol (ETH), Benzene (BEN), Ethanol + Benzene (ETH + BEN), Ethanol+ Extra Virgin Olive Oil (ETH+EVOO), Benzene+ Extra Virgin Olive Oil (BEN+EVOO), Ethanol+Benzene+Extra Virgin Olive Oil (ETH+BEN+EVOO).

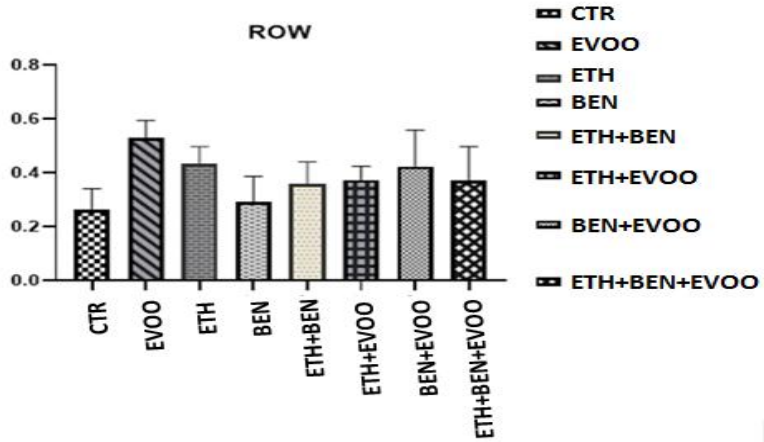

Figure 2: Bar chart of Relative Organ Weight (ROW).

Abbreviations: Control (CTR), Extra Virgin Olive Oil (EVOO), Ethanol (ETH), Benzene (BEN), Ethanol + Benzene (ETH + BEN), Ethanol+ Extra Virgin Olive Oil (ETH+EVOO), Benzene+ Extra Virgin Olive Oil (BEN+EVOO), Ethanol+Benzene+Extra Virgin Olive Oil (ETH+BEN+EVOO).

Table 2: Effect of Ethanol, Benzene and Extra virgin olive oil on haematological parameters.

GROUPS	PCV (%)	Hb (gm/dl)	WBCs (mm^3)	N (%)	L (%)	M (%)
CTR	34.50±6.922	11.90±0.6061	5.550±0.2306	25.00±2.646	78.33±3.694	1.333±0.6670
EVOO	35.83±1.815	11.47±2.3020	6.050±1.3320	28.67±2.171	75.67±2.716	1.000±0.4472
ETH	41.67±0.843*	13.82±0.2600	5.783±1.1330	33.00±1.308*	68.33±2.98*	3.833±0.9098*
BEN	32.33±6.525	12.90±0.3564	3.700±0.4219*	36.00±2.828*	66.33±3.84*	3.667±0.7601*
ETH+BEN	35.50±7.210	14.20±0.5079*	5.200±0.7956	34.00±1.414*	66.17±3.08*	4.667±0.8028*
ETH+EVOO	38.17±1.376**	12.68±0.4438	8.783±1.3840	33.00±1.838	64.00±1.713	1.000±0.4472
BEN+EVOO	34.83±1.167	11.74±0.4556	6.140±0.8931**	33.60±1.470	67.33±3.676	2.333±0.6164**
ETH+BEN+EVOO	23.67±7.513	7.883±2.5030	4.483±1.9570	32.00±1.826	70.83±3.167	2.667±0.6667**

Table 1: Showing Full Blood Count Analysis.
Data are significant (*p<0.5) in comparison to CTR and EVOO groups while data are significant (**p<0.5) with ETH, BEN and ETH + BEN groups.

Abbreviations: Control (CTR), Extra Virgin Olive Oil (EVOO), Ethanol (ETH), Benzene (BEN), Ethanol + Benzene (ETH + BEN), Ethanol+ Extra Virgin Olive Oil (ETH+EVOO), Benzene+ Extra Virgin Olive Oil (BEN+EVOO), Ethanol+Benzene+Extra Virgin Olive Oil (ETH+BEN+EVOO), Packed Cell Volume (PVC), Hemoglobin (HB), White Blood Cells (WBCs), Neutrophil (N), Lymphocyte (L), Monocyte (M), Percentage (%), grams per deciliter (g/dL).

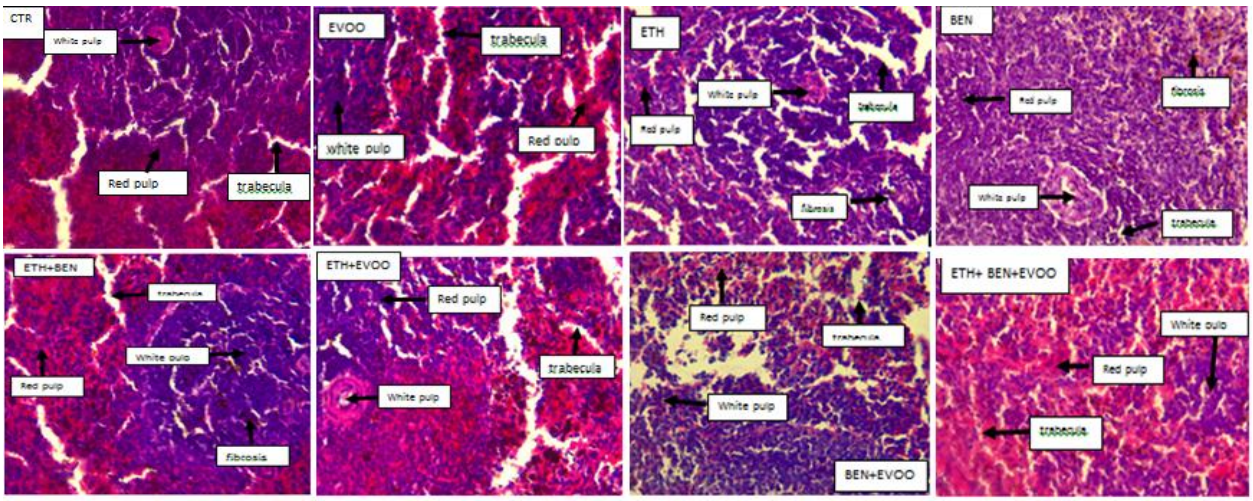

Plate 1: Representative light photomicrograph of the Splenic tissue subjected to H and E stain Mag. (× 400). Unlike the CTR and EVOO groups, the ETH, BEN and ETH+BEN groups presented with splenic tissue distortions characterized

by remarkable fibrosis. There was no evidence of fibrosis in the ETH+EVOO, BEN+EVOO, and ETH+BEN+EVOO groups.

Abbreviation: Control (CTR), Extra Virgin Olive Oil (EVOO), Ethanol (ETH), Benzene (BEN), Ethanol + Benzene (ETH + BEN), Ethanol+ Extra Virgin Olive Oil (ETH+EVOO), Benzene+ Extra Virgin Olive Oil (BEN+EVOO), Ethanol+Benzene+Extra Virgin Olive Oil (ETH+BEN+EVOO).

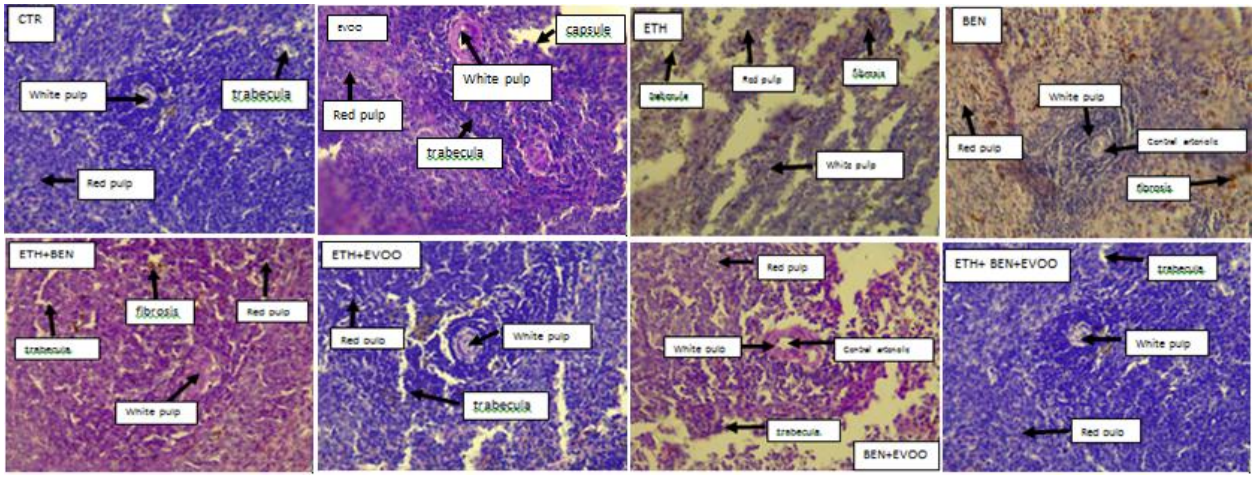

Plate 2: Representative light photomicrograph of the Splenic tissue subjected to Periodic Acid Schiff (PAS) stain (× 400). Sections revealed normal splenic capsule, white pulp, red pulp, and trabecular with no evidence of fibrosis in the CTR and EVOO groups. Whereas, the spleen of the ETH, BEN, and ETH + BEN groups had remarkable fibrosis. There were no traces of fibrosis in the splenic tissue of the ETH+EVOO, BEN+EVOO, and ETH+BEN+EVOO groups. **Abbreviation**: Control (CTR), Extra Virgin Olive Oil (EVOO), Ethanol (ETH), Benzene (BEN), Ethanol + Benzene (ETH + BEN), Ethanol+ Extra Virgin Olive Oil (ETH+EVOO), Benzene+ Extra Virgin Olive Oil (BEN+EVOO), Ethanol+Benzene+Extra Virgin Olive Oil (ETH+BEN+EVOO).

DISCUSSION

The present study investigated the ameliorative potentials of extra virgin olive oil on the hematotoxic and splenotoxic effects of ethanol and benzene either when administered alone or when coadministered.

When all the groups were compared, there was no significant alteration in the weight of the spleen following the administration of ethanol, benzene and extra virgin olive oil (EVOO). This may likely be due to the relatively short period of the administration. However, chronic ethanol or benzene exposure with EVOO for 28 days significantly reduced the weight of the spleen in Wistar rats.[44],[45],[46]

Ethanol[47], [48] and benzene[49], [50] intoxication has been implicated in certain disease conditions which are associated with pathologically relevant levels of haematological indices. In the present study, the administration of ethanol or benzene alone or when coadministered resulted in significant alteration of haematological parameters. In comparison with the rats in the control group, a significantly low level of PCV was obtained from the ethanol-treated group but not from the benzene treated group. A reduced PCV level is usually indicative of anaemia. The mechanism of ethanol-induced anaemia is a function of several metabolic aberrations. These include upregulation of inflammation signalling, compromised hepatocellular integrity, defective erythropoiesis or even malnutrition.[51],[52] Interestingly, supplementation with extra virgin olive oil significantly curtailed the debilitating effects of ethanol on the PCV level. This indicated that extra virgin olive oil may likely contain bioactive mechanisms which may potentially modulate downstream biochemical pathways of ethanol-induced anaemia.

Consequently, benzene administration significantly ($p<0.5$) reduced the white blood cell (WBCs) level. A condition of reduced WBCs is known as leukopenia. The debilitating effect of benzene on WBCs has been implicated in certain biochemical pathways associated with the initiation and progression of leukaemia.[53] However, treatment with extra virgin olive oil ameliorated the hematotoxic effect of benzene by significantly ($p<0.5$) increasing the WBC level. This indicated that certain bioactive compounds in EVOO may likely curtail the carcinogenic effect of benzene by inhibiting the reduction of white blood cells. Whereas, a previous study has documented that supplementation with EVOO significantly reduced the blood levels of haemoglobin and leucocytes in pregnant Sprague Dawley rats,[54] the present study couldn't establish a similar effect of EVOO on ethanol and/or benzene hematotoxicity.

Moreover, a previous study has shown that olive oil can potentially curtail elevated blood levels of neutrophils and monocytes.[55] Notwithstanding, our findings in this

study revealed that EVOO supplementation significantly ($p<0.5$) lowered the blood level of monocytes alone, but not neutrophils. Although monocytes are essential components of the immune response, their activation has been linked with other haematological conditions such as anaemia and leukopenia.[56] Elevated monocytes can also trigger a chronic inflammatory response which has been associated with cardiomyopathy,[57], [58] autoimmune diseases and certain malignancies.[59],[60]

The histopathological assessment showed remarkable fibrosis in the splenic tissue compartments of the groups administered with only ethanol and benzene as well as the coadministration group, relative to the control group. The debilitating effects of ethanol and benzene on the histoarchitectural integrity of the spleen may likely explain the reason for the elevated haemoglobin levels in the blood. This is particularly possible because extramedullary hematopoiesis occurring during postnatal life in the spleen has been implicated as a pathological symptom.[61] It is noteworthy that supplementation with extra virgin olive oil preserved the splenic tissue while equally ameliorating the toxic effects of ethanol and benzene. This indicated that extra virgin olive oil may therefore possess an essential bioactive mechanism whose pharmacological properties are relevant in improving haematological indices and protecting vital organs.

CONCLUSION

This study demonstrated the toxicity effects of ethanol and benzene on blood cells as well as the spleen. However, supplementation with extra virgin olive oil elicited outstanding pharmacological potential in ameliorating the toxicity attributable to blood and the spleen. Nevertheless, our future studies will focus on identifying the specific natural compounds which are responsible for the pharmacological potentials of EVOO in relieving pathological symptoms associated with ethanol and benzene induced toxicities.

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Nil

CONFLICT OF INTERESTS

Nil.

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