INNOVARE JOURNAL OF MEDICAL SCIENCES

Vol 10, Issue 2, 2022

ISSN - 2321-4406

Research Article

TREATMENT OF EXTRA-VIRGIN OLIVE OIL ON ETHANOL- AND BENZENE-INDUCED ACINAR CELLS DAMAGE IN ADULT WISTAR RATS

ADETUNJI OPEYEMI ADEBOLA[1]*, ADERUPOKO IDAYAT TOMILOLA[1], ADETUNJI OLUWASEYI ADEGOKE[2], OGUNBIYI BABAFEMI TOSIN[2], ADETUNJI IYABODE TOYIN[3], NWOBI NNENNA LINDA[4], OGUNBIYI OLUBUNMI ESTHER[1], NWOBI JOSEPH CHIGBOGU[2], BEJIDE AYODELE ROLAND[1]

[1]Department of Anatomy, School of Basic Medical Sciences, Babcock University, Ogun State, Nigeria. [2]Department of Biochemistry, School of Basic Medical Sciences, Babcock University, Ogun State, Nigeria. [3]Department of Pure and Applied Biology, Ladoke Akintola University, Ogbomoso, Oyo State, Nigeria. [4]Department of Chemical Pathology, School of Basic Clinical Sciences, Babcock University, Ogun State, Nigeria. Email: addturng1809@gmail.com

Received: 10 January 2022, Revised and Accepted: 28 February 2022

ABSTRACT

Objective: The study is focused on the effect ethanol and benzene on the acinar cells and extra- virgin olive oil (EVOO) as an ameliorative agent.

Methods: Forty-eight Wistar rats were divided into eight groups of six animals per group: (1) Control group (a placebo of water), (2) EVOO, (3) 25% ethanol, (4) 200 mg/kg benzene, (5) 25% ethanol+200 mg/kg benzene (EB), (6) 25% ethanol+EVOO (EO), (7) 200 mg/kg benzene+EVOO (BO), and (8) 25% ethanol+200 mg/kg benzene+EVOO (EBO). The ethanol, benzene, and EVOO were administered orally.

Results: All the treatment groups lost body weight except Groups 1 and 2. The histology of the pancreas showed significant ($p \leq 0.05$) damage to the acinar cells of groups exposed to 25% ethanol, 200 mg/kg benzene, and EB when compared to the control, EVOO, EO, BO, and EBO. The enzymatic activities of amylase and lipase evaluated showed significant ($p \leq 0.05$) decrease in EO and BO as compared to 25% ethanol, 200 mg/kg benzene, and EB-treated groups.

Conclusion: There was an elevated glucose concentration in 25% ethanol and EBO as compared to control and EVOO has anti-inflammatory potential across the ameliorated groups.

Keywords: Ethanol, Benzene, Extra-virgin olive oil, Acinar cells.

 DOI: http://dx.doi.org/10.22159/ijms.2022v10i2.44088. Journal homepage: https://innovareacademics.in/journals/index.php/ijms

INTRODUCTION

Chronic alcohol consumption has been considered to be one of the major causes of pancreatic damage for a long time. Ethanol abuse has been repeatedly identified as the most common cause of pancreatic damage [1,2] because the negative evidence on pancreatic damage induced by prolonged ethanol consumption is insufficient and the putative mechanism underlying it is still unknown. Oxidative impairment may lead to pancreatic cell dysfunction and disruption in glucose homeostasis [3]. Benzene is an organic compound, used as an industrial solvent and a component of petrochemicals. 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 [4]. Pancreatic cells are easily prone to oxidative stress caused by increased levels of reactive oxygen species due to decreased levels of antioxidant enzymes [5]. Moreover, the resulting oxidative stress may result in pancreatic cell dysfunction and a disruption in glucose homeostasis [3].

The limited information regarding the early pathophysiologic steps involved in pathogenesis of alcoholic pancreatic injury may have been attributable to the unavailability of an easily reproducible animal model of this disease [6]. Repeated attacks of pancreatic inflammation with loss of pancreatic parenchyma and replacement with fibrosis, variable pain, and symptoms of pancreatic insufficiency (malabsorption and diabetes) may stimulate or coexist with pancreatic carcinoma [7]. Histologic changes from the normal pancreatic architecture include irregular fibrosis, acinar cell loss, islet cell loss, and inflammatory cell infiltrates several studies [8,9].

There have been techniques for pancreatic damage with ethanol and benzene separately but not a combined effect. The improved sensitivity of diagnostic tests leads to be believed that there are more patients with pancreatic damage than initially suspected. Ethanol and benzene have been observed to have inflammatory effect on the acinar cells of the pancreas. This study is aimed at using extra-virgin olive oil (EVOO) to check its ameliorative effect. The pancreas plays an important role of producing pancreatic hormone. Studies have shown that EVOO has anti-inflammatory properties and awareness on this is very low [10].

METHODS

Procurement and preparation of reagent

Ethanol (75%) was gotten from Babcock University Anatomy Department, Ilishan-Remo, Ogun state. EVOO was purchased from Jumia online store, Lagos, Nigeria. Benzene was gotten from the Department of Anatomy, University of Ibadan, Ibadan, Oyo state. Moreover, the appropriate quantity was prepared for the experiment (Table 1). Ethanol (25%) was obtained by measuring 10 ml of the 75% ethanol with an addition of 20 ml of distilled water measured using a measuring cylinder, this was done consecutively when 25% of ethanol was required during administration.

Animal housing, treatment, and grouping

All rules and regulations guiding animal research and teaching were strictly followed in accordance with the Babcock University Health Research Ethics Committee (BUHREC: 746/19). Forty-eight adult Wistar rats (120 g) were gotten and housed in Babcock University animal housing facility Ilishan-Remo, Ogun state. They were placed in plastic cages with net covers for ventilation. Distilled water and pelletized food

were given to the Wistar rats daily. Wood shavings were used as beddings for the Wistar rats. The beddings were changed every 3 days to avoid build-up of toxic ammonia levels. The 48 Wistar rats were acclimatized for 7 days and were divided into eight groups of six animals per group. The period of experimentation lasted for 14 days for Groups 1, 2, 3, 4, and 5, while the period of experimentation lasted for 28 days for Groups 6, 7, and 8 dues to amelioration for 14 days after induction. Furthermore, a number of deaths were recorded during the course of the experiment.

Measurement of body weight and organ weight

The body weight of the rats was measured twice a week throughout the duration of the study with the use of a measuring balance, this was done to check the weight gain or loss in various animals of each group.

Animal sacrifice

Following the 4 weeks added experiment, the rats were fasted overnight and euthanized under ketamine anesthesia (100 mg/kg). The pancreas was carefully excised through lower abdominal incision using scalpel and forceps. Blood was collected from the left ventricle with 2 ml syringe and then stored in a sample bottle so as to carry out pancreatic functional tests. The excised pancreas was kept in a sample bottle filled with 10% formal saline solution for histological analysis.

Histological techniques

The pancreas was excised and was fixed in 10% formal saline and was processed using the routine tissue processing protocols, and the tissue slides were prepared using hematoxylin and eosin (H and E) for general histological appearance and Masson trichrome for visualizing connective tissue, especially collagen in tissue sections.

Biochemical tests

The animals were sacrificed by cervical dislocation. Blood samples were collected through cardiac puncture into plain bottles under aseptic conditions. The blood samples were centrifuged at 4000 revolutions/min for 15 min using Gulfex Medical and Scientific Centrifuge, England. The serum was separated. Randox kits were used to measure the amylase and lipase activity in the blood [11].

Glucose tests

Blood samples were obtained from the rats through tail puncture after massage with warm towel to enable blood flow. The blood sample was placed on the glucose strip which was already inserted into the glucometer. The glucose levels were obtained from each animal [11].

Statistical analysis

All the results were represented as a grouped data and analyzed using the GraphPad Prism 8.0 software using one-way analysis of variance. The results were expressed as Mean±SEM. Newman–Keuls *post hoc* test was used to compare the means.

RESULTS

The difference in body weight for the final day of the experiment and beginning of the experiment across the groups shows that the body weight difference of ethanol (5.25±0.854), benzene (−0.75±4.029), E+B (−10.75±4.479), E+O (5.00±1.683), B+O (−4.00±3.367), and E+B+O (−2.750±0.750) was significant ($p<0.05$) when compare to the control group (21.25±8.320) and olive oil (30.00±4.848), as shown in Fig. 1. The relative organ weight across the experimental group shows that groups, benzene (0.20±0.0365), E+B (0.18±0.0365), and E+O (0.20±0.0365), were significant ($p<0.05$) when compare to the control group (0.40±0.0816), as shown in Fig. 2.

Serum amylase activity

The serum amylase activity of EO (50.45±6.252) and BO (46.7±7.935) is significant ($p<0.05$) when compare to EO (85.78±3.342).

Serum lipase activity

The serum lipase levels of benzene (87.88±5.131) and EB (88.75±5.692) are significant ($p<0.05$) when compare to EO (58.48±5.712).

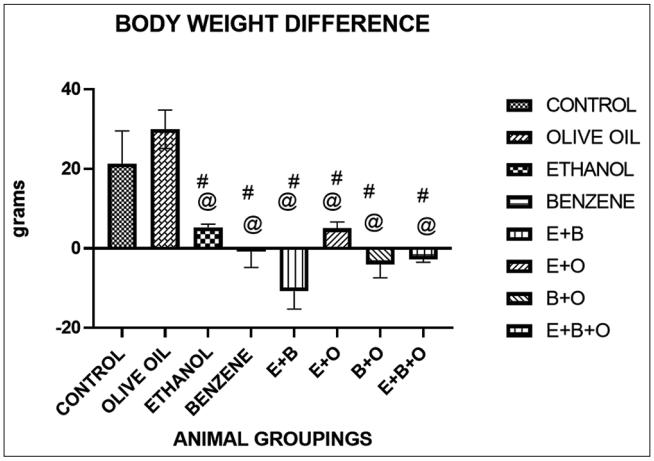

Fig. 1: The difference in body weight between the final day of the experiment and beginning of the experiment across the groups, values were expressed as Mean±SEM of data obtained, where @=Significantly different ($p<0.05$) from control and #=Significantly different ($p<0.05$) from olive oil. SEM: Standard error of the mean, E+B: Benzene+ethanol, E+O: Ethanol+olive oil, B+O: Benzene+olive oil, and E+B+O: Ethanol+benzene+olive oil

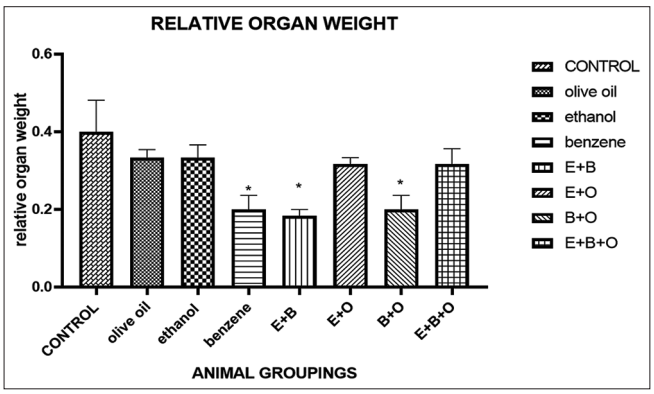

Fig. 2: The relative weight of the pancreas in each experimental group, values were expressed as Mean±SEM of data obtained, where *=Significantly different ($p<0.05$) from control. SEM: Standard error of the mean, E+B: Benzene+ethanol, E+O: Ethanol+olive oil, B+O: Benzene+olive oil, and E+B+O: Ethanol+benzene+olive oil

Initial blood glucose levels

The initial blood glucose levels of ethanol (108.5±2.179), E+O (88.0±6.416), BO (111.3±2.780), and olive oil (103.0±1.871) were significant ($p<0.05$) when compared to EBO (138±1.780). Furthermore, ethanol (125.3±3.119) and EB (113±4.270) are significantly ($p<0.05$) different when compare to E+O (88.0±6.416). The initial blood glucose levels of E+B+O (138±1.780) are significant ($p<0.05$) when compare to control (103±11.77).

Final blood glucose levels

The final blood glucose levels of E+O (73.0±1.789) are significant ($p<0.05$) when compare to B+O (93.20±2.478).

Histological studies

H and E

Heamatoxylin and eosin [12] demonstrated (Fig. 3)morphology.

Masson trichrome

Masson Trichrome [13] demonstrated (Fig. 4) connective tissues, particularly collagen; it also demonstrated cytological and differential stain.

DISCUSSION

The body weight difference of the rats showed significant difference between the treatment groups (ethanol, benzene, EB, EO, BO, and EBO) and control. Furthermore, there was a significant difference body weight between the treatment groups and EVOO [10]. The relative organ weight shows the ratio of the pancreas weight of the animals to the body weight. It shows the proportionality in growth of the pancreas with that of the body weight of the animal. The relative organ weight of the rats was significantly different ($p<0.05$) in the treatment groups (EB and B+O) as compared to control. It was discovered that the groups treated with benzene only have a relative decrease in the weight of the organ (pancreas).

Two histological methods were studied to describe possible morphological changes in the pancreatic tissue in response to various treatments given across the animal groupings. (Fig. 3) Heamatoxylin and eosin [12] demonstrated morphology while masson Trichrome [13] demonstrated (Fig. 4) connective tissues, particularly collagen; it also demonstrated cytological and differential stain. These methods revealed that the control, EVOO, EO, BO, and EBO groups had blood vessels, islet of Langerhans, normal pancreatic ducts, and acini cells with no fibrosis, whereas the ethanol, benzene, and EB groups had blood vessels, islet of Langerhans, normal pancreatic ducts, and acini cells with fibrosis; while ethanol, benzene, and EB groups showed blood vessels, islet of Langerhans, normal pancreatic ducts, and acini cells with a remarkable fibrosis. These changes may be attributed to the destruction of the acinar cells [14]. Trypsin is an enzyme stored in its inactive form, trypsinogen, in the zymogen granules. Inflammation may occur as a result of trypsin being activated by other activators, such as lysosomal protease cathepsin, in the pancreas rather than the duodenum. Due to the accumulation of protein (trypsin) in the pancreas, there is the formation of protein plugs [15,16].

Amylase and lipase are enzymes produced normally by the pancreas. Serum levels of amylase and lipase are commonly used as reliable indicators of the pancreatic functions in human and experimental animals [17]. In this study, the function of pancreatic cells was evaluated by measuring the levels of serum pancreatic enzymes such as amylase and lipase. There was an elevated concentration of serum amylase levels (Fig. 5) in EB group, but relatively low EO

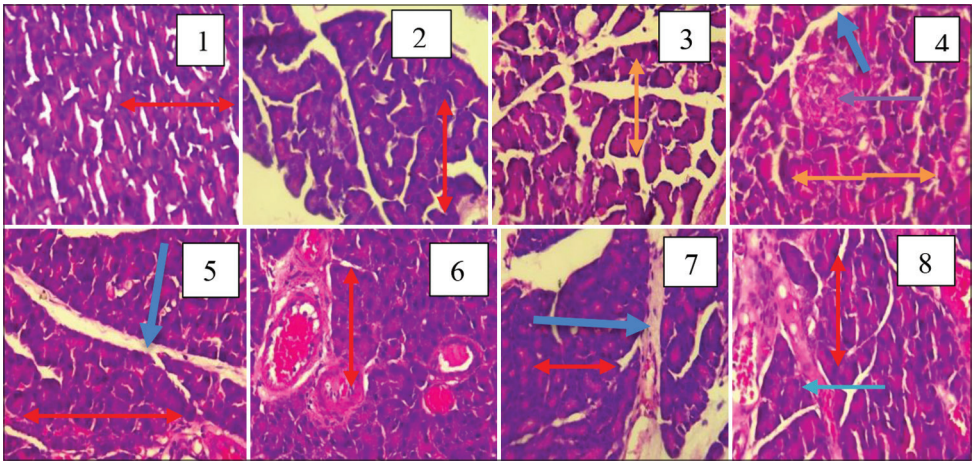

Fig. 3: Hematoxylin and eosin photomicrographs showing pancreatic tissue consisting of blood vessel, islet of Langerhans, and acini for the various animal groups (H and E ×400). 1=Control, 2=Extra-virgin olive oil (EVOO), 3=Ethanol, 4=Benzene, 5=Ethanol+benzene (EB), 6=Ethanol+EVOO (EO), 7=Benzene+EVOO (BO), and 8=Ethanol+benzene+EVOO (EBO); where, blue arrow represents normal pancreatic duct, yellow arrow represents islets of Langerhans, black arrow represents blood vessels, and red arrow represents the acini cells without fibrosis

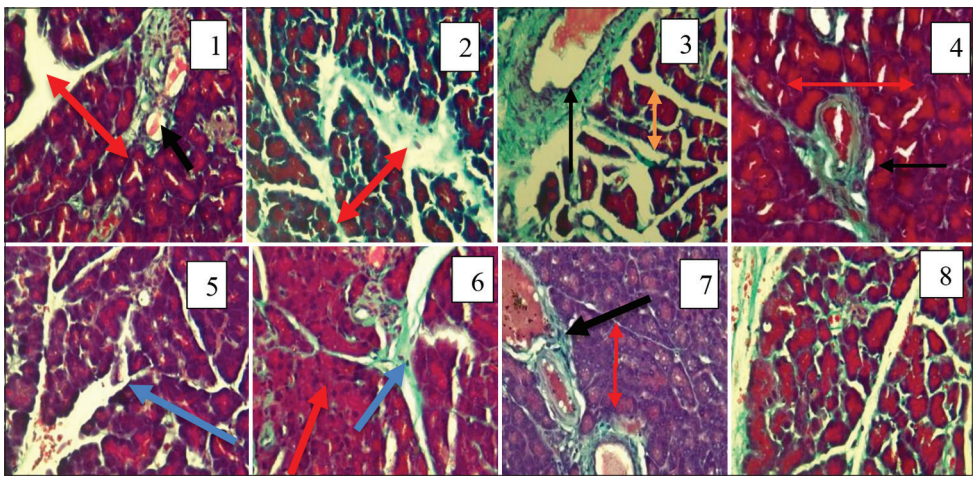

Fig. 4: Masson trichrome photomicrographs showing pancreatic tissue consisting of blood vessel, islet of Langerhans, and acini for the various animal groups (mt ×400). 1=Control, 2= Extra-virgin olive oil (EVOO), 3=Ethanol, 4=Benzene, 5=Ethanol+benzene (EB), 6=Ethanol+EVOO (EO), 7=Benzene+EVOO (BO), and 8=Ethanol+benzene+EVOO (EBO); where, blue arrow represents normal pancreatic duct, yellow arrow represents islets of Langerhans, black arrow represents blood vessels, and red arrow represents the acini cells without fibrosis

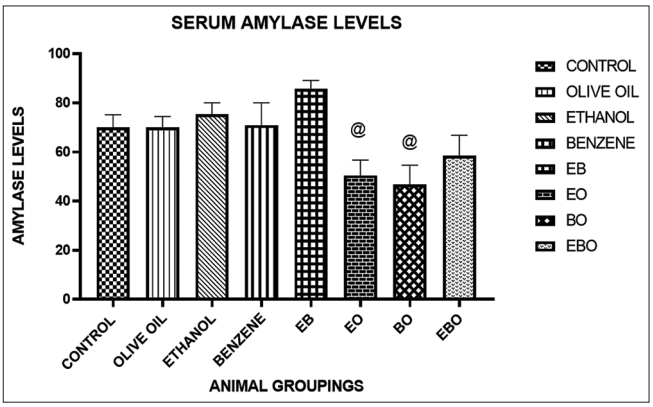

Fig. 5: The serum amylase activity across the groups, values were expressed as Mean±SEM of data obtained, where @=Significant difference (p<0.05) from EB. SEM: Standard error of the mean, EB: Benzene+ethanol, EO: Ethanol+olive oil, BO: Benzene+olive oil, and EBO: Ethanol+benzene+olive oil

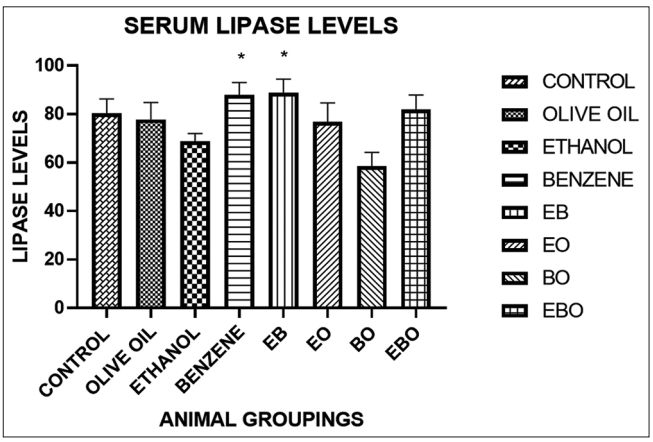

Fig. 6: The serum lipase levels across the groups. Values were expressed as Mean±SEM of data obtained, where *=Significant difference (p<0.05) from BO. SEM: Standard error of the mean, EB: Benzene+ethanol, EO: Ethanol+olive oil, B+O: Benzene+olive oil, and EBO: Ethanol+benzene+olive oil

Table 1: Grouping of experimental rats

Group	Number of animals	Treatment schedule
1	6	A placebo of water
2	6	Extra-virgin olive oil (EVOO) at 2 ml/kg.bw per day
3	6	25% ethanol 2 ml/kg.bw twice a week
4	6	Benzene at 200 mg/kg.bw, twice a week
5	6	25% ethanol+benzene twice in a week
6	6	25% ethanol+extra-virgin olive oil (EO) twice a week
7	6	Benzene+extra-virgin olive oil (BO) twice a week
8	6	25% ethanol+benzene+extra-virgin olive oil (EBO) twice in a week

and BO treated group. This result possibly describes pancreatitis for EB, EO, and BO groups. There was an elevated concentration of serum lipase levels (Fig. 6) in Benzene, EB, and EBO groups and relatively low in ethanol- and BO-treated groups. This result possibly

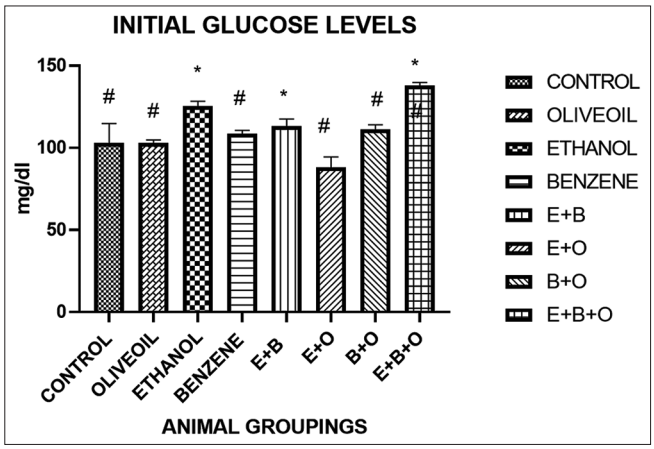

Fig. 7: The serum lipase levels across the groups. Values were expressed as Mean±SEM of data obtained, where *=Significant difference (p<0.05) from EO and #=Significantly different (p<0.05) from E+B+O. SEM: Standard error of the mean, E+B: Benzene+ethanol, E+O: Ethanol+olive oil, B+O: Benzene+olive oil, and E+B+O: Ethanol+benzene+olive oil

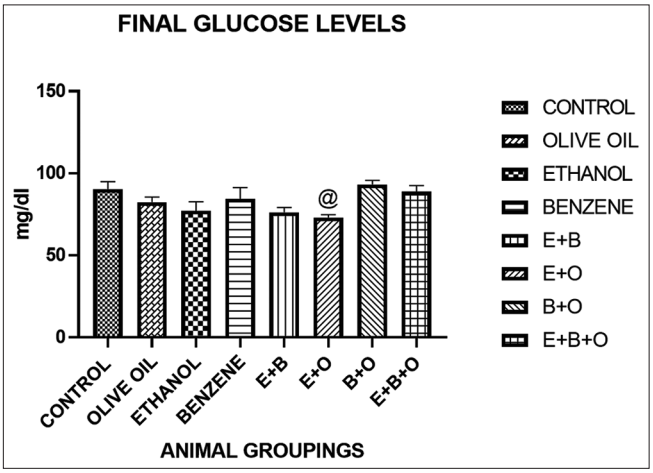

Fig. 8: The final glucose levels across the groups. Values were expressed as Mean±SEM of data obtained, where @=Significantly different (p<0.05) for 200 mg/kg BW benzene+2 ml olive oil. SEM: Standard error of the mean, E+B: Benzene+ethanol, E+O: Ethanol+olive oil, B+O: Benzene+olive oil, and E+B+O: Ethanol+benzene+olive oil

describes pancreatitis for the ethanol-, BE-, BO-, and EBO-treated groups. Pancreatitis is caused by the abnormal activation of digestive enzymes within the pancreas. The reason for variations in individual test results is suggestive of varying tolerance ability of the rats since the production of amylase and lipase is under the control of the pancreas. Glucose is one of a group of carbohydrates known as simple sugar, glucose levels are associated with insulin production. The production of insulin is under the control of the pancreas. There was an elevated glucose concentration in the ethanol and EBO groups for the initial glucose test (Fig. 7) although it was within the normal glucose range of 140 mg/dL, while in the final glucose test (Fig. 8) there was no elevated glucose level. However, results obtained from the microanatomical structures show significant damage to the acinar cells of the ethanol, benzene, and EB groups as compared to the control, ethanol, EO, BO, and EBO groups.

CONCLUSION

The study conducted to carry out the effect of EVOO on ethanol- and benzene-induced pancreatic damage in adult Wistar rats showed that EVOO has anti-inflammatory potential from the histological analysis.

There are also variations in the enzymes levels which suggest possible anti-inflammatory effect.

ACKNOWLEDGMENT

Nil.

REFERENCES

1. Deng X, Wang L, Elm MS. Chronic alcohol consumption accelerates fibrosis in response to induced pancreatitis in rats. Am J Pathol 2005;166:93-106.
2. Saluja AK, Bhagat L. Pathophysiology of alcohol-induced pancreatic injury. Pancreas 2003;27:327-31.
3. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Is oxidative stress activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes 2003;52:1-8.
4. Uzma N, Kumar BS, Hazari MA. Exposure to benzene induces oxidative stress, alters the immune response and expression of p53 in gasoline filling workers. Am J Ind Med 2010;53:1264-70.
5. Modak MA, Parab PB, Ghaskadbi SS. Pancreatic islets are very poor in rectifying oxidative DNA damage. Pancreas 2009;38:23-9.
6. Tsukamoto H, French SW, Benson N, Delgado G, Rao GA, Larkin EC, *et al*. Severe and progressive steatosis and focal necrosis in rat liver induced by continuous intragastric infusion of ethanol and low-fat diet. Hepatology 1985;5:224-32.
7. Jain MD. Chronic Pancreas. Available from: http://www.pathologyoutlines.com/topic/pancreaschronic.html [Last accessed on 2021 Apr 24].
8. Kloppel G, Maillet B. A morphological analysis of 57 resection specimens and 9 autopsy pancreata. Pancreas 1991;6:266-74.
9. Kloppel G, Maillet B. Pathology of acute and pancreatic damage. Pancreas 1993;8:659-70.
10. Adebola AO, Adegoke AO, Toyin AI, Linda NN. Role of extra virgin olive oil on the liver of alcohol and benzene-treated Wistar rats. World J Med Sci 2021;18:51-9.
11. Adebola AO, Toyin AI, Afolayan S, Busayo AO. Role of progesterone treatment on the microanatomy of the prefrontal cortex of streptozotocin induced diabetic Wistar rats. Glob J Pharmacol 2020;14:8-16.
12. Shoobridge MP. A new principle in polychrome staining: A system of automated staining, complementary to hematoxylin and eosin, and usable as a research tool. Stain Technol 1983;58:245-58.
13. Suvik A, Effendy AW. The use of modified masson's trichrome staining in collagen evaluation in wound healing study. Mal J Vet Res 2012;3:39-47.
14. Sato S, Stark HA, Martinez J. Receptor occupation, calcium mobilization, and amylase release in pancreatic acini: Effect of CCK-JMV-180. Am J Physiol 1989;257:G202-9.
15. Aghdassi AA. Pathogenesis of Chronic Pancreatitis; 2016. Available from: https://www.pancreapedia.org/reviews/pathogenesis-of-chronic-pancreatitis
16. Crampton L. Pancreas Functions, Pancreatitis, and Diabetes; 2019.
17. Watt T. Amylase Blood Tests. Grahams Rogers; 2017. https://www.healthline.com/health/amylase-blood [Last accessed on 2021 Apr 12].