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STUDY OF PHYTOEXTRACT FOR ANTIDIABETIC AND HYPOLIPIDEMIC EFFECT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

Allium humile and Origanum vulgare is traditionally used for treating nocturnal emissions, abdominal pain, diarrhea, sexual dysfunction and asthma. This study aimed at investigating the antidiabetic and hepatoprotective activities of the ethanolic fraction extract of Allium humile and Origanum vulgare. For the antidiabetic activity, rats were induced with diabetes by intraperitoneal injection of STZ and NA injections. Animals were sacrificed after the study and the fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), HDL, malondialdehyde (MDA) catalase, superoxide dismutase and glutathione levels were determined. The hepatoprotective assay, mice were pretreated for seven days with EEAH and EEOV (200 mg/kg and 400 mg/kg) and Glibenclamide. Blood samples were obtained and serum enzymes ALT, AST, ALP, SOD, GSH, CAT, MDA were assessed. Allium humile and Origanum vulgare significantly decrease FBG, serum TG, TC, MDA levels and significant increased HDL, SOD, GSH and CAT activities in the diabetic rats. In conclusion, the antidiabetic and hepatoprotective effect of Allium humile and Origanum vulgare may be associated with its antioxidant and its ability to inhibit the pro-inflammatory mediators.

Keywords: Allium humile and Origanum vulgare; Antidiabetic, Hepatoprotective.

1. INTRODUCTION

Diabetes mellitus (DM) is considered as the most prevalent and most common chronic disorder. It is characterized by abnormal rise in blood glucose levels as well as alterations in protein and lipids metabolism due to the inability of the pancreas to produce sufficient insulin and/or as a result of the body's inability to effectively utilize the insulin it produces.1,2 The increasing prevalence of DM is alarming, based on recent studies approximately 420 million people are suffering from DM globally in 2014 and it is envisaged that this figure will rise to over 650 million by the year 2040.3,4 DM is associated with several complications that accounts for most of the fatalities reported in diabetic patients. Notable among these complications includes diabetic angiopathy, neuropathy, retinopathy, cardiovascular diseases, kidney failure and leg amputation. Currently, the therapeutic approach for managing DM is focused on controlling the blood glucose levels through a combination of diet and the use of insulin or oral hypoglycemic drugs.5,6 However, most of these drugs have unpleasant side effects and the accessibility of the drugs are limited. Due to these

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 3054-3064

short comings, alternative therapies which are readily available and with little or no side effects are becoming the primary focus for the treatment and management of DM.7,8 Natural products, especially medicinal plants have been considered as potential sources of safe and effective antidiabetic agents due to the presence of secondary metabolites such as polyphenols and flavonoids which exert antioxidant properties and hypoglycemic effects.9,10

Alpine Onion Allium humile, belongs to family Alliaceae, is a species of onion found in the Himalayas, at altitudes of 3,000– 4,000 m. Leaves and inflorescences are commonly used as seasoning agents.11

Origanum vulgare, also called origanum or wild marjoram, aromatic perennial herb of the mint family (Lamiaceae) known for its flavorful dried leaves and flowering tops.12

2. MATERIAL AND METHODS

2.1 Collection of plant

Allium humile (AH) and Origanum vulgare whole plants were collected from various areas.

2.2 Preparation of Extracts

The plants of A. humile and Origanum vulgare were dried in shade and room temperature for 2 days followed by drying [40– 50°C] for 3–4 h and powdered to obtained coarse powder. 980 gm of powder of A. humile and Origanum vulgare were extracted with ethanol and Petroleum Ether using Soxhlet extraction technique to get extracts.

2.3 Animals

A total of 42 male Wistar rats procured from NIN, Hyderabad were used in the present study. Rats were kept in clean polypropylene cages; temperature was maintained at 24-26oC. Three rats were housed per each cage. Standard care was provided to the animals as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. All animals were allowed to acclimatize for one week before the experiment. The study was initiated only after the written approval from the Institutional Animal Ethics Committee.

2.4 Diabetes induction procedure

Streptozotocin and nicotinamide (STZ-NA) model was used in the study. DM was induced by giving intraperitoneal STZ and NA injections.

A single dose of NA 110 mg/kg, dissolved in freshly prepared 0.9% normal saline, in a volume of 4.8 mL/kg body weight, was given intraperitoneally. Later, after 15 min STZ, 50 mg/kg, dissolved in freshly prepared 0.1 Citrate buffer with a pH 4.5, in a volume of 4.8 mL/kg body weight, was administered intraperitoneally. Citrate buffer (0.1 M), which has been used as solvent for mixing STZ, was made by dissolving solution of trisodium citrate (294.10 mg in 100 mL of distilled water) with citric acid solution (210.10 mg in 100 mL of distilled water) to attain pH 4.5. Ice was kept around the glass beaker containing Citrate buffer to maintain citrate buffer at lower temperatures. Normal control rats were administered with Citrate buffer alone intraperitoneally.

On the 4th day, after the diabetes induction procedure rats with fasting blood glucose value (FBG) > 126 mg/dL were included in the study.

2.5 Experimental design

Group I	Control animal model
Group II	Streptozotocin and nicotinamide induced diabetic animal model (DC)
Group III	Diabetic animal model treated with the extract (200 mg/kg and 400 mg/kg) for both the extract
Group IV	Diabetic rats treated with standard drug (Glibenclamide)

2.6 Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed on overnight fasted rats by pricking the tip of the tail. Initially fasting blood glucose levels were measured, and then 2g/kg body weight glucose was administered orally. There after blood glucose levels were measured at 30, 60 and 120 mins.

2.6.1 Estimation of serum triglycerides

0.01mL of sample was mixed with 1.0 mL of working reagent and incubated at 350C for 5 min. measured the absorbance of the standard and test sample at 505 nm at a light path of 1cm against the blank, within 60 min.

2.6.2 Estimation of Serum total cholesterol

0.01ml sample was mixed with 1.0 mL of cholesterol reagent and incubated at 350C 5min. measured the absorbance of the standard and test at 505 nm at a light path of 1cm against the blank, within 60 min.

2.6.3 Estimation of serum High-Density Lipoprotein Cholesterol

0.05 mL of sample was mixed with 1.0 mL of working reagent and incubate at 350C for 5 min. measure the absorbance of the Standard and test sample at 505 nm at a light path of 1cm against the blank, within 60 min.

2.6.4 Estimation of Serum Glutamate Oxaloacetate Transaminase- SGOT

1.0ml of working reagent is incubated at 350 C for 1 min and added 0.1 ml of starter reagent. Mixed well and read the initial absorbance A0 and repeat the absorbance reading after every 1,2 and 3 min. calculate the mean absorbance changer per minute (ΔA/min).

2.6.5 Estimation of serum SGPT

1.0ml of working reagent is incubated at 350 C for 1 min and adds 0.1 ml of starter reagent. Mix well and read the initial absorbance A0 and repeat the absorbance reading after every 1, 2, and 3 min. calculate the mean absorbance change per minute(ΔA/min).

2.6.6 LDL

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 3054-3064

0.020 ml sample was mixed with 1.0 ml of 0.800 ml of reagent 1 and incubated at 350C 5min, followed by 0.200 ml of reagent 2, incubate 1 min. at 37°C and then measure the initial absorbance of calibrator and sample against reagent blank. Measure the absorbance change exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

2.7 ESTIMATION OF BILIRUBIN

Pipette into test tubes marked	Blank	Standard	Test
Working reagent	500 µl	500 µl	500 µl
Distilled water	25 µl	-	-
Standard	-	25 µl	-
Test	-	-	25 µl

2.7.1 Gamma glutamyl transferase

1 ml of reagent blank is mixed with0.1 ml of sample, mixed and after 1 min. incubation (at 37oC), 0.250 ml of reagent 2 was added, mixed and incubated at 37oC and then initial absorbance of calibrator and sample was measured against blank. Change in absorbance was measured exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

2.7.2. Estimation of MDA

One ml of patient or control serum was combined with 4ml of Trichloroacetic acid (TCA)-Thiobarbituric acid (TBA)- Hydrochloric acid (HCl) solution and mixied thoroughly, when heated for 15 mins in boiling water bath. After cooling, the precipitate was removed by centrifugation at 3000 rpm for 10 mins. The absorbancy was determined at 535nm against reagent blank, which was containing all the reagents minus the serum.

3. RESULTS

3.1 Effect of EEAH and EEOV on oral glucose tolerance test

The mean OGTT levels of normal control group was 232 ± 2.6 mg-h/dL, which was significantly increased to 587 ± 2.6 mg-h/dL in diabetic control group. These increased levels were significantly decreased by the treatment with EEAH, EEOV, and glibenclamide. Glucose levels at various time intervals were reported.

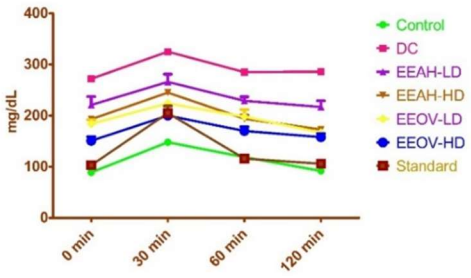


Figure1. Effect of EEAH and EEOV on OGTT

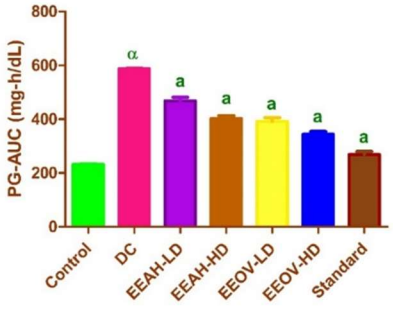


Figure 2. Effect of EEAH and EEOV on AUC

3.2 Effect of EEAH and EEOV on serum glucose levels

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 3054-3064

The diabetic control group has shown significant increase in serum glucose levels during the experimental period. At the end of the study, serum glucose levels of the diabetic control group were significantly increased to 296 ± 9.8 mg/dL as compared to normal control group. The increased serum glucose level was significantly decreased with treatment with EEAH, EEOV, and glibenclamide.

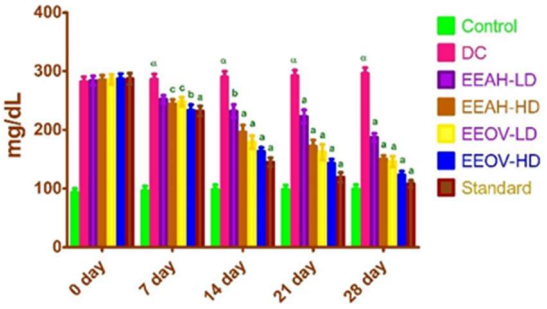


Figure 3: Effect of EEAH and EEOV on serum glucose levels

3.3 Effect of EEAH and EEOV on lipid parameters

3.3.1 Triglyceride levels

The mean serum triglyceride level of normal control group was 72 ± 3.0 mg/dL, which significantly increased to 121 ± 3.5 mg/dL in the diabetic control group. The increased serum triglyceride level was decreased by treatment with EEAH-LD, EEAH-HD, EEOV-LD, EEOV-HD, and glibenclamide.

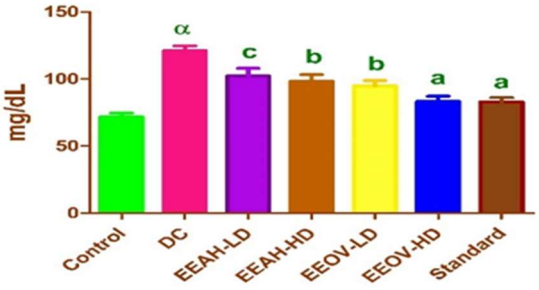


Figure 4: Effect of EEAH and EEOV on TG levels

3.3.2 Cholesterol levels

The mean serum total cholesterol level of the normal control group was 77 ± 4.0 mg/dL, which was significantly increased to 156 ± 5.6 mg/dL in the diabetic control group. These increased levels were decreased by the treatment with EEAH-HD, EEOV-HD, and glibenclamide, whereas treatment with EEAH-LD and EEOV-LD, showed no significant decrease in total cholesterol levels when compared to diabetic control group.

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 3054-3064

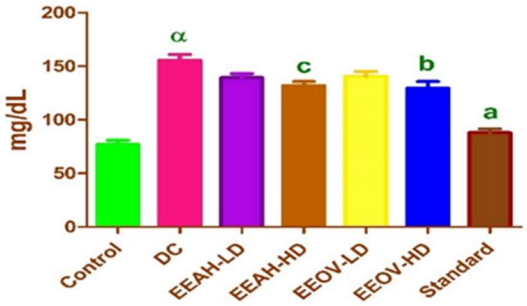


Figure.5: Effect of EEAH and EEOV on TC levels

3.3.3 HDL-c levels

Diabetes induction caused significant decrease in serum HDL-cholesterol levels of 28 ± 1.1 to 12 ± 1.1 mg/dL when compared to normal control group. Treatment with EEAH-LD, EEAH-HD, EEOV-LD, EEOV-HD, and glibenclamide, caused significant increase in serum HDL-c levels, when compared to diabetic control group.

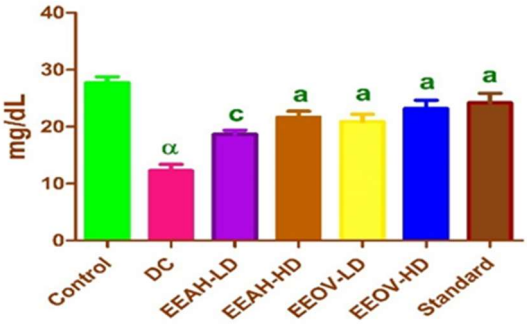


Figure.6: Effect of EEAH and EEOV on HDL levels

3.4 Effect of EEAH and EEOV on liver parameters

3.4.1 LDH levels

Diabetes induction caused significant increase in liver LDH levels from 215 ± 6.4 U/mg protein to 558 ± 9.7 U/mg when compared to normal control group. The increased LDH levels were decreased by treatment with EEAH-LD, EEAH-HD, EEOV-LD, EEOV-HD, and glibenclamide.

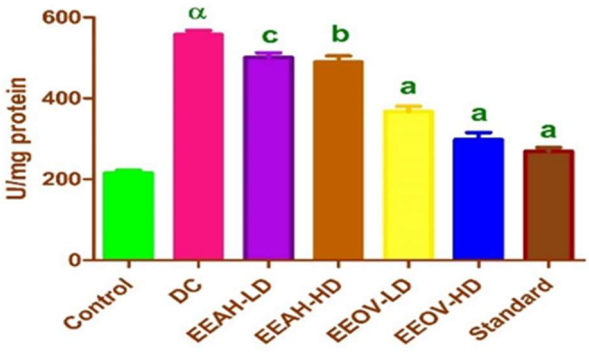


Figure 7: Effect of EEAH and EEOV on LDH levels

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 3054-3064

3.4.2 SGOT levels

Induction of diabetes caused significant increase in liver SGOT levels from 43 ± 3.5 U/L to 167 ± 6.9 U/L when compared to the normal control group. The increased SGOT levels were decreased by treatment with EEAH-LD, EEAH-HD, EEOV-LD, EEOV-HD, and glibenclamide.

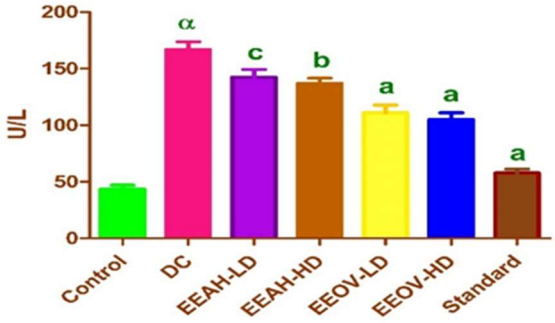


Figure 8: Effect of EEAH and EEOV on SGOT levels

3.4.3 SGPT levels

Induction of diabetes caused a significant increase in liver SGPT levels from 21 ± 2.9 U/L to 166 ± 5.0 U/L when compared to the normal control group. The increased SGPT levels were decreased by treatment with EEAH-HD, EEOV-LD, EEOV-HD, and glibenclamide, whereas treatment with EEAH-LD did not show any significant increase in SGPT levels when compared to diabetic control group.

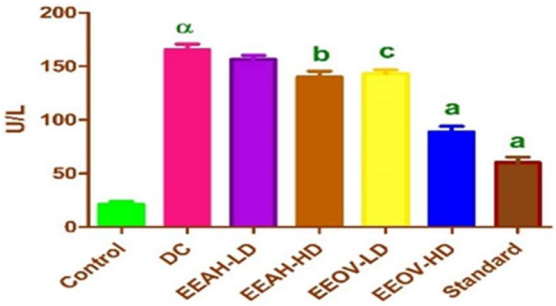


Figure 9: Effect of EEAH and EEOV on SGPT levels

3.4.4 ALP Levels

Diabetes induction caused a significant increase in liver ALP levels from 88 ± 7.3 U/L to 135 ± 3.4 U/L when compared to the normal control group (Table 4). The increased ALP levels were decreased by treatment with EEAH-HD, EEOV-HD, and standard, whereas EEAH-LD and EEOV-LD failed to show any significant increase in ALP levels compared to diabetic control group.

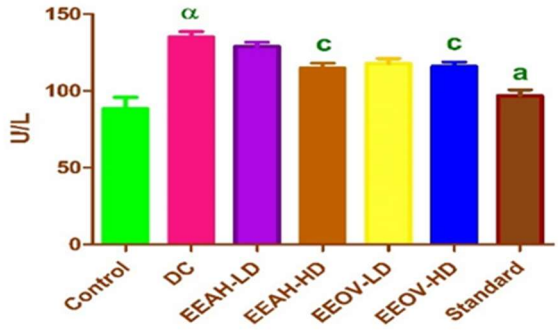


Figure10: Effect of EEAH and EEOV on ALP levels

3.5 Total bilirubin levels

Induction of diabetes caused a significant increase in liver total bilirubim levels from 0.53 ± 0.067 µmol/L to 1.1 ± 0.065 µmol/L when compared to the normal control group. The increased total bilirubin levels were decreased by treatment with EEAH-HD, EEOV-LD, EEOV-HD, and glibenclamide, whereas treatment with EEAH-LD, failed to show any significant decrease in total bilirubin levels when compared to diabetic control group.

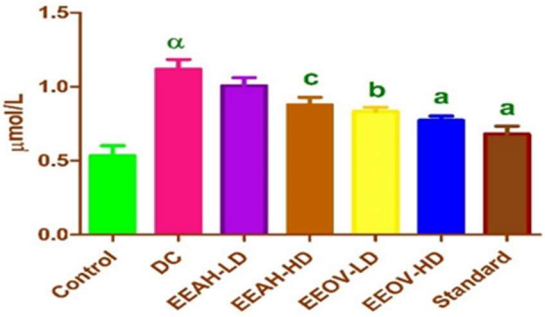
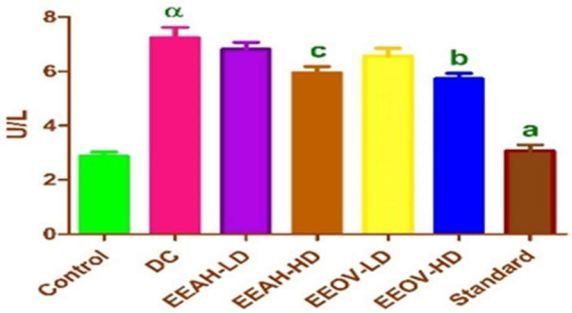


Figure11: Effect of EEAH and EEOV on Total bilirubin levels

3.6 γ glutamyl transferase levels

Diabetes induction caused a significant increase in liver γ glutamyl transferase levels from 2.9 ± 0.16 U/L to 7.2 ± 0.38 U/L when compared to the normal control group. The increased γ glutamyl transferase levels were decreased by treatment with EEAH-HD, EEOV-HD, and glibenclamide, and treatment with EEAH-LD and EEOV-LD did not show any significant decrease in γ glutamyl transferase levels when compared to diabetic control group.



Catalyst Research Volume 23, Issue 2, October 2023 Pp. 3054-3064

Figure12: Effect of EEAH and EEOV on γ glutamyl transferase

3.7 Effect of EEAH and EEOV on anti-oxidant levels

3.7.1 Effect of EEAH and EEOV on catalase level

The mean serum catalase level of normal control group was 8.8 ± 0.41 nmol/min/mL, which was significantly decreased in diabetic control group to 3.4 ± 0.33 kU. The decreased catalase levels were increased in EEAH-HD, EEOV-LD, EEOV-HD, and standard group, whereas EEAH-LD did not show any significant increase in catalase levels when compared to diabetic control group.

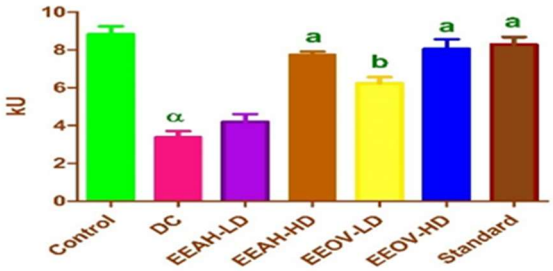


Figure 13: Effect of compounds EEAH and EEOV on serum catalase level

3.7.2 Effect on GSH level

The mean serum GSH level of normal control group was 38 ± 1.7 mg/dL which was significantly decreased in diabetic control group to 19 ± 2.6 mg/dL. The decreased level of GSH were significantly increased in EEAH-HD, EEOV-HD, and the standard group when compared to diabetic control group. However, no significant changes were observed in EEAH-LD and EEOV-LD groups.

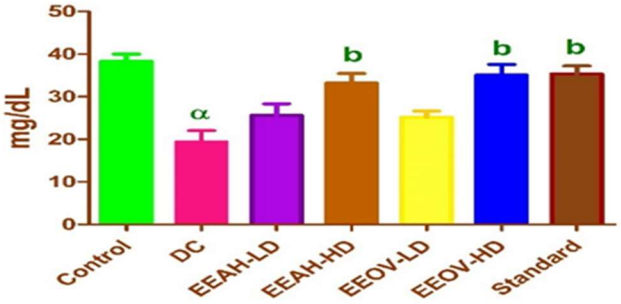


Figure14: Effect of compounds EEAH and EEOV on serum GSH level

3.8 HISTOPATHOLOGY STUDY

Control

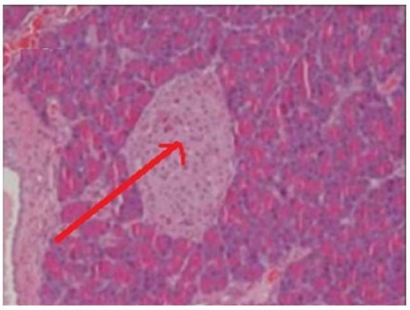


Figure 15: Islets of pancreas with mild hemorrhages

DC

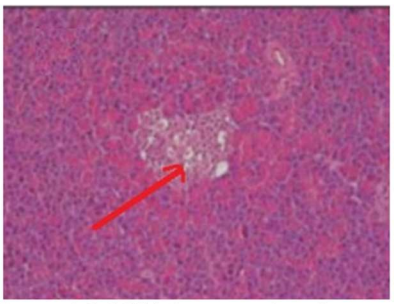


Figure 16: Mild degenerative changes in acinar cells

EEAH-LD

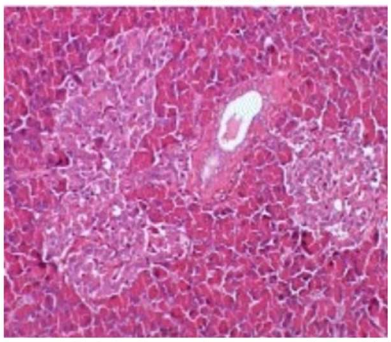


Figure 17. Moderate hypertrophy of islets of pancreas

EEAH-HD

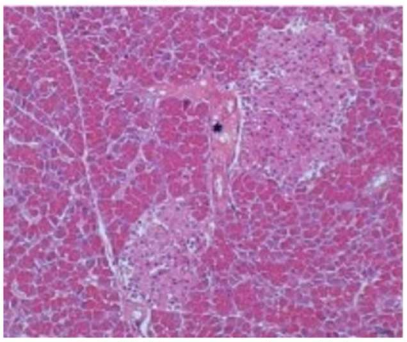


Figure 18. Mild hypertrophy of islets of pancreas

EEOV-LD

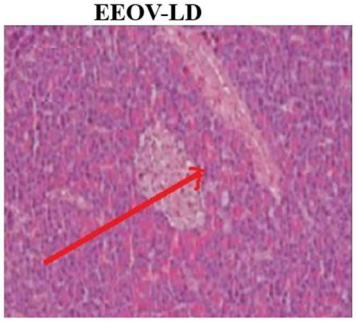


Figure 19. Mild hypertrophy of islets of pancreas

EEOV-HD

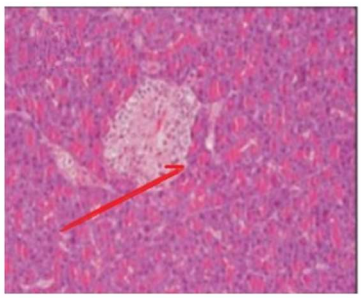


Figure 20. islets of pancreas and degenerative changes in beta cells

Standard

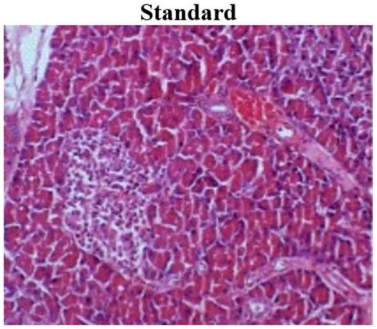


Figure 21. Mild hypertrophy islets of pancreas

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 3054-3064

4. CONCLUSION

Ethanolic extracts of both Allium humile and Origanum vulgare have shown protective effect against glucose intolerance shown in STZ-Nicotinamide induced diabetic model also it has reduced the triglyceride, cholesterol, and HDL levels. Both the extracts have shown both hepatoprotective and antioxidant. The protective effect of these extracts may be because of stimulating the beta cells of the pancreas, which results in insulin secretion and regulation of glucose levels, which further results in a positive effect on lipid and liver parameters.

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