The Pharmaceutical and Chemical Journal, 2024, 11(2):96-109

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Evaluation of Anti-diabetic Activity of *Eugenia jambolana* in dexamethasone induced diabetes in experimental animal

Rahul Saktawat[1*], Sachin K Jain[2], Sudha Vengurlekar[2]

[1]Research Scholar, Oriental University, Near Aurobindo Hospital Sanwer Road Indore MP India 453555
[2]Faculty of Pharmacy, Oriental University, Near Aurobindo Hospital Sanwer Road Indore MP India 453555

Abstract This study has assessed the phytochemical and pharmacological screening of the antidiabetic activities of *Eugenia jambolana*. There is evidence to suggest that *Eugenia jambolana* contains potentially beneficial chemicals. The various extracts were produced and assessed for their potential to prevent diabetes in vivo using the Dexamethasone-induced diabetes paradigm. Following therapy with *Eugenia jambolana*, there was a reduction in the weight of the liver, kidney, and pancreas. This might be attributed to the avoidance of inflammation, necrosis, and MNC infiltration as a result of the oxidative stress caused by dexamethasone. The reduction in inflammation, necrosis, and MNC infiltration in pancreatic tissue, as well as the antioxidant capability of EEEJ, are indicative of the protection against histopathological harm to the pancreas. Moreover, it has been claimed that lowered levels of bilirubin, SGOT, and SGPT successfully protected against liver impairment caused by oxidative stress caused by dexamethasone.

Keywords *Eugenia jambolana,* antidiabetic, Phytochemical, Dexamethasone

Introduction

Herbal medications can be defined as the whole extracts or fractions of whole extracts that can be made from the entire plant, a particular part of the plant, or a combination of different parts of the plant, which are then used to treat and prevent diseases or to advance health and wellbeing [1]. Herbal medicine plants have a long history in many indigenous tribes and are constantly being used to treat a wide range of disorders [2]. The primary source of the pharmacological or therapeutic strategy is, nonetheless, the many plants used in traditional remedies. In recent years, individuals have started using herbal items either alone or in conjunction with other substances to renew their health or as a therapeutic ingredient. According to the World Health Organization, many communities used herbs and herbal products for their fundamental medical requirements. [3, 4]. The usage of herbal products has greatly revived in recent years due to the limited efficacy of modern medicines in chronic illnesses. Approximately 75% of the plant-based medicinal medicines were derived from traditional folk drugs that were used all over the world. Approximately 70% of contemporary medications in India are derived from natural sources, and a number of synthetic analogs are made from isolated chemicals of prototype plants [5]. Previous research has shown that around 60% of anticancer medications on the market are of natural origin. As of right now, over 80% of drugs used for cardiovascular, immunomodulatory, anti-infective, and anti-cancer purposes have a botanical origin. Out of the 177 authorized anti-cancer drugs, about 70% have a natural origin. Approximately 25% of prescription medications available worldwide have their origins in botanical sources. Thirteen natural product medications were licensed in

the United States between 2005 and 2007, while more than 100 medicines based on natural products are the subject of ongoing clinical investigations. Furthermore, it has been estimated that 11% of the 252 total medications listed in the WHO Essential Drugs list have a natural origin [6]. In traditional Indian medicine, a variety of botanicals are employed. Ayurvedic medicine uses between 1200 and 1800 plants, Siddha medicine uses between 500 and 900 plants, the Unani system uses between 400 and 700 plants, and Indian traditional healers employ over 7,500 therapeutic herbs for a variety of diseases [7, 8].

The Current State of Herbal Medicine

In the past decade and a half, there has been an increase in awareness of herbal remedies and medicinal plants in India. This has led to a surge in financing for research; several departments, including the department of herbal science, as well as several herbal institutions, have been founded or are emerging. Regretfully, there is a lack of plant-based phytomedicines or traditional chemical intelligence medications to meet the need. The overall global market for the application of phytomedicine is 60–100 billion US dollars, but it is just 0.1 billion Indian dollars. A number of well-known pharmaceutical businesses have recently begun to produce an increasing number of herbal remedies. The need for raw materials of superior quality has grown significantly. The industry centered around medicinal plants is growing at a pace of 7–15 % each year. Take note of any records pertaining to herbal medication that aren't readily available. As to the research, in 1991, the market share of herbal pharmaceuticals in Germany, France, and Italy was 3.0, 1.6, and 0.6 billion, respectively. In certain countries, such as Germany and France, herbal extracts are offered in addition to prescribed medications.

Material and methods

Collection and Authentication of Plant Material

Aerial pieces of *Eugenia jambolana* were gathered from the Malwa area. Botanist Dr. S.N. Dwivedi discovered, verified, and certified the plant. In January 2020, leaves were taken from the surrounding area of Kota, Rajasthan's local market.

Phytochemical Screening

Preliminary Phytoprofile

The powder of the air dried leaves of *Eugenia jambolana* extracted in soxhlet apparatus with solvents of increasing polarity. Every time, the material was dried before being extracted with the subsequent solvent. Each extract was concentrated by removing the spent solvent using distillation. The concentrated extracts were then dried and weighed using a rotary evaporator. Plant material that had been air dried was used to determine the color and percentages of the extracts.

Qualitative Phytochemical Analysis

Various extracts of leaves were subjected to preliminary phytochemical analysis

Acute toxicity study

In the present investigation, the doses of the *Eugenia jambolana* extracts were selected on the basis of literature reports.

Pharmacological studies

***In-Vivo* Studies**

In vivo antidiabetic activity was performed by dexamethasone induced model.

Experimental animals

For the study, Wistar rats of both sexes weighing between 230 and 250 g were used. The animals were maintained in colony cages with unrestricted access to food and water, and were housed in typical laboratory settings with a temperature of 25 ± 2°C, a 12-hour light-dark cycle, and 50 ± 5 % relative humidity. Prior to the test, the animals were adjusted to the laboratory environment. There were six (n = 6) animals in each group. All of the experiments

were conducted between the hours of 8:00 and 16:00. The Committee for the Purpose of the Committee for Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India, provided guidelines for conducting investigations, which were followed.

Experimental design-II (*Eugenia jambolana*)

Group 1: Vehicle treated (Distilled water, 5 ml/kg, and p.o.)
Group 2: Dexamethasone sodium phosphates, (10mg/kg/day, s.c.)
Group 3: HFD + Dexamethasone sodium phosphates,(10 mg/kg/day, s.c.)
Group 4: HFD + Dexamethasone sodium phosphates, (10mg/kg/day, s.c.)+ RHEJ (200 mg/kg p.o.)
Group 5: HFD + Dexamethasone sodium phosphates, (10mg/kg/day, s.c.) + RHEJ (400 mg/kg p.o.)
Group 6: HFD + Dexamethasone sodium phosphates,(10 mg/kg/day, s.c.) + Glibenclamide (500 mcg/kg/day, p.o.)
Group 7: HFD+ Dexamethasone sodium phosphates, (10 mg/kg/day, s.c.) + Atorvastatin 10 mg/kg

For the purpose of estimating biochemical parameters, blood samples from each animal in the corresponding treatment groups were taken by retro orbital on days 0, 10, and 20 of the therapy. On the twentieth day, which is the conclusion of the trial period Diethyl ether was used as an anesthesia, and blood samples were obtained using the retro-orbital method. Serum was separated for the purpose of estimating different biochemical parameters, and the animals were sacrificed by cervical dislocation. The viscera was exposed to remove various tissues for the purpose of estimating physical and biochemical parameters as well as Histopathological study.

Following parameters were evaluated in above paradigms:

Physical parameters:

- Change in body weight
- Weight of pancreas
- Weight of liver
- Weight of kidney
- Pancreas weight / Body weight ratio
- Liver weight / Body weight ratio
- Kidney weight / Body weight ratio

Antioxidant Parameters:

- Malondialdehyde (MDA) level in liver tissue
- Glutathione (GSH) level in liver tissue

Histopathology of pancreas

Serum biochemical parameters:

- Glucose level
- Total protein
- Total cholesterol
- Triglycerides level
- SGOT (serum-glutamate-oxalo acetate transferase)
- SGPT (serum glutamate pyruvate transferase)
- Total bilirubin
- Direct bilirubin

Estimation of various biochemical and tissue parameter:

Serum glucose level was estimated by GOD/ POD method as per the procedure describe in the estimation kit.

Principle:

Glucose oxidase (GOD) oxidizes glucose to gluconic acid and hydrogen peroxide. In the presence of enzyme peroxidase, released hydrogen peroxide is coupled with phenol and 4-amino antipyrine (4-AAP) to form colored quinoneimine dye. Absorbance of colored dye is measured at 500 nm and is directly proportional to glucose concentration in the sample.

$$\text{Glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AAP} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + H_2O$$

Procedure:

- Pipetted the samples in to a clean dry test tubes labeled as Blank (B), Standard (S) and test (T) as follows
- Mixed well and incubate at R. T. for 10 min.
- Measured absorbance of the Standard (Abs. S) and Test sample (Abs. T) against reagent blank (Abs. B)

Serum glutamate oxaloacetate transaminase (SGOT) / aspartase amino transaminase (AST) was estimated by liquid stable modified IFCC method as per the procedure describe in the estimation kit

Principle:

SGOT/ASAT catalyses the transfer of amino group between L-aspartase and α- ketoglutarate to form oxalo-acetate and glutamate, The oxalo-acetate formed reacts with NADH in the presence of MDH to form NAD. The rate of change of absorbance
(Decreasing) is directly propotional to the SGOT/ASAT activity in the serum.

L-Aspartate + α- Ketoglutarate $\xrightarrow{\text{SGOT}}$ Oxalo-acetate + L-glutamate

Oxalo-acetate + NADH + H^+ $\xrightarrow{\text{MDH}}$ Malate + NAD^+

Glutamate pyruvate transaminase (SGPT)/ alanine amino transaminase(ALT) was estimated by liquid stable modified IFCC method as per the procedure describe in the estimation kit.

Principle:

SGPT/ALAT catalyses the transfer of amino group between L-Alanine and α- Ketoglutarate to form pyruvate and glutamate, The pyruvate formed reacts with NADH in the presence of LDH to form NAD. The rate of change of absorbance (decreasing) is directly propotional to the SGPT/ALAT activity in the serum.

Procedure:

- Pipetted the samples in to a clean dry test tubes labeled as test (T).
- Mixed well and incubate at R. T. for 10 min.
- Measured absorbance at an interval of 60 sec. for 2 min. at 340 nm.

Cholesterol was estimated by roeschlau method as per the procedure describe in the estimation kit.

Principle:

In hot acidic medium, a cholesterol oxidases ferric ion to a brown coloured complex which absorbs at 530 nm and its intensity is directly proportional to cholesterol concentration**.**

Procedure:

- Pipetted the samples in to a clean dry test tubes labeled as Blank (B), Standard (S) and test (T).
- Mixed well and incubate at R. T. for 10 min.
- Measured absorbance of the Standard (Abs. S) and Test sample (Abs. T) against reagent blank (Abs. B).

Triglycerides was estimated by tinder method as per the procedure describe in the estimation kit.

Lipoprotein lipase hydrolyzes plasma triglycerides to produce glycerol and free fatty acids. Glycerol is transformed into glycerol-3-phosphate in the presence of ATP and glycerol kinase. Glycerol phosphate oxidase subsequently

oxidizes this compound to produce hydrogen peroxide. A rose-colored dye with a wavelength of 500 nm is produced by the oxidative condensation of DHBSA and Amino-4-antipyrine in the presence of peroxidase and hydrogen peroxide. The amount of triglycerides in the sample directly correlates with the color intensity that forms.

Procedure:

- Pipetted the samples in to a clean dry test tubes labeled as Blank (B), Standard (S) and test (T).
- Mixed well and incubate at R. T. for 05 min. or ten min. at 37^{o}C.
- Measured absorbance of the Standard (Abs. S) and Test sample (Abs. T) against
- Reagent blank (Abs. B).

Total protein was estimated by biuret method as per the procedure describe in the estimation kit

Principle:

In an alkaline solution, copper II ions and protein peptide bonds combine to generate a blue-violet complex known as the biuret reaction. Iodide is utilized to stop the alkaline copper complex from auto-reducing, and tartarate is added as a stabilizer to each copper ion complex containing five or six peptide links. The color that forms is measured at 546 nm (520-560 nm) and is proportionate to the protein content.

Reagent preparation: All reagents are ready to use.

Procedure:

- Pipetted the samples in to a clean dry test tubes labeled as Blank (B), Standard (S) and test (T).
- Mixed well and incubate at R. T. for 10 min.
- Measured absorbance of the Standard (Abs. S) and Test sample (Abs. T) against reagent blank (Abs. B), within 60 min.

Creatinine was estimated by alkaline picrate method as per the procedure describe in the estimation kit.

Principle:

Creatinine forms an orange coloured complex with picric acid in an alkaline medium. The intensity of the colour formed within fixed time directly propotional to the amount of creatinine present in the sample.

Procedure:

1. Pipetted the samples in to a clean and dry test tubes labeled as standard (S) and test (T).
2. Mixed and aspirated. Recorded the absorbance of standard (S) and test (T) at 30 seconds (S1, T1) and again at 90 seconds (S2, T2) at 505 nm, against distilled water

Direct bilirubin was estimated by mod. jendrassik and grof's method as per the procedure describe in the estimation kit.

Principle:

Bilirubin reacts with diazotized sulphanilic acid to form a coloured azobilirubin compound. The unconjugated bilirubin coulpes with the sulphanilic acid in the presence of a caffeine-benzoate accelerator, the intensity of the colour formed is directly propotional to the amount of bilirubin present in the sample.

Total bilirubin was estimated by mod. jendrassik and grof's method as per the procedure describe in the estimation kit

Principle:

Bilirubin reacts with diazotized sulphanilic acid to form a coloured azobilirubin compound. The unconjugated bilirubin coulpes with the sulphanilic acid in the presence of a caffeine-benzoate accelerator, the intensity of the colour formed is directly propotional to the amount of bilirubin present in the sample.

Bilirubin + Diazotized sulphanilic acid → Azobilirabin compound

Determination of reduced glutathione from liver tissue homogenate by Moron et al. (1979).

Preparation of solutions:

Trichloroacetic acid (50% w/v)

Fifty grams of trichloroacetic acid was dissolved in sufficient quantity of distilled water and the final volume was made up to 100 ml of distilled water.

DTNB reagent (0.01 M)

One gram of 5, 5'-dithiobis (2-nitrobenzoic acid) was dissolved in 50 ml of buffer and the final volume was adjusted to 100 ml with buffer.

Standard glutathione

10 mg of reduced glutathione was dissolved in 60 ml of distilled water and the final volume was made up to 100 ml with distilled water.

Principle:

Colored complex formed by sulphydryl group of GSH with DTNB, the absorbance of colour compound measured at 412 nm.

Procedure:

GSH was determined by spectrophotometric method, based on the use of Ellman's reagent. Tissue (liver) homogenate were mixed with 50 % trichloroacetic acid in distilled water in glass tube and centrifuged at 3000rpm for 15 min. The supernatants were mixed with 0.4 M Tris buffer, pH 8.9 and 0.01 M 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) was added. After shaking the reaction mixture, its absorbance was measured at 412 nm.

Determination of tissue (liver / kidney / pancreas) weight:

Procedure:

- Weighed the animal.
- Sacrificed the animal by spinal dislocation.
- Cut the abdomen, and removed tissue (liver / kidney / pancreas).
- Washed with cold saline and removed the blood and other tissues.
- Dried it by blotting on filter paper and weighed.

Determination of tissue (liver / kidney / pancreas) to body weight ratio.

Procedure:

- Weighed the animal.
- Sacrificed the animal by spinal dislocation.
- Cut the abdomen, and removed tissue (liver / kidney / pancreas).
- Washed with cold saline and removed the blood and other tissues.
- Dried it by blotting on filter paper and weighed.
- Calculated the ratio by using following formula.

Preparation of liver tissue homogenate

Procedure:

- After completed of the treatment, killed the animal by spinal dislocation.
- Perform midline abdomen incision and quickly removed liver tissue.
- Washed the tissue in ice cold solution, dried tissue on filter paper and weighed.
- Taken 1 gm of tissue and added buffer 0.05M phosphate buffer (pH 7.4) up to 10 ml. (10%).
- Prepared a homogenate by using tissue homogenizer at the speed 2000 rpm for 10 min speed.
- Taken this tissue homogenate for further investigation.

Statistical analysis:

The values were expressed as mean±SEM (n=6). The statistical significance was assessed using Student's t-test or one-way analysis of variance (ANNOVA) followed by Dunnett's test and $p<0.05$, $p<0.01$ and $p<0.001$ were considered as statistically significant.

Results and Discussion

Organoleptic features

Organoleptic and microscopic characteristics: The aerial portions of *Eugenia jambolana* are dark green in color with a leathery texture, and the abaxial surface is slightly lighter in green than the adaxial surface. The leaves are greenish-yellow in the early stages and becoming darker as they develop. The leaves had no smell, but they tasted somewhat bitter and astringent, which might have been caused by the presence of tannins.

Preliminary phytochemical screening

Several phytochemical analyses corroborated the extracts' contents, which are shown in Table, of tannins, diglycosides, carbohydrates, flavonoids, and phenolic compounds. Comparing the aqueous extract to the methanolic and ethyl acetate extract, it was discovered that the alkaloids were present in a more negative proportion.

Table 1: Results of phytochemical screening of different extracts of *Eugenia jambolana* aerial parts extracts

Constituent	Extracts		
	Aqueous extract	**Ethyl acetate extract**	**Ethanolic extract**
Alkaloids	-ve	+ve	+ve
Carbohydrates	+ve	+ve	+ve
Proteins & amino acids	-ve	-ve	-ve
Fixed oils & fats	-ve	-ve	-ve
Flavonoids	+ve	+ve	+ve
Phenolic compounds	+ve	+ve	+ve
Tannins	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve
Saponins	-ve	-ve	-ve

+ ve: Present; -Ve: Absent

Pharmacological activity

In-Vivo Antidiabetic Activity

Diabetes Induced by Dexamethasone

Table 2: Effect of EEEJ on Body weight in dexamethasone-induced diabetes mellitus

Group No.	Groups	Change in body weight (gm)		
		0 Day	**10 Days**	**20 Days**
I	Normal Control (Normal Saline)	3.166±0.945	7.500±1.945	3.333±3.062
II	Diabetic control	2.500±1.335#	19.010±1.788#	-6.833±2.441##
III	D-HFD + Dexamethasone 10 mg/kg	1.500±2.974	20.500±3.273	27.833±4.126*
IV	EEEJ 200 mg/kg	1.833±4.094	2.833±4.126	3.667±3.818**
V	EEEJ 400 mg/kg	2.833±1.249	11.833±2.700	3.666±2.951**
VI	Glibenclamide 0.5 mg/kg	1.666±5.194	13.010±5.773*	4.333±6.014**
VII	Atorvastatin 10 mg/kg	0.500±1.477	12.833±2.613*	3.500±3.253**

The data are presented as mean±standard error (n = 6). Compared it to the normal group (Students' test) with ##p<0.01, to the diabetic control group (One way ANOVA followed by Dunnett's test) with *p<0.05 and **p<0.01.

When compared to the normal group, the administration of dexamethasone (10 mg/kg, s.c.) demonstrated a substantial (P<0.01) reduction in body weight changes on days 10, 20, and of the observational period. As glibenclamide (500 mg/kg, p.o.) was administered, there was a substantial (P<0.01) increase in body weight change as compared to the diabetic control group. When compared to the diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) showed a significant increase in body weight change (p<0.05 and p<0.01), while the

administration of HFD + dexamethasone (10 mg/kg) showed a negligible effect in this regard. A substantial ($p<0.01$) increase in body weight change was seen when atorvastatin 10 mg/kg was compared to the diabetes control group.

Table 3: Effect of EEEJ on Serum Glucose level in dexamethasone induced diabetes mellitus

Group No.	Groups	Serum glucose level		
		0 Day	10 Days	20 Days
I	Normal Control (Normal Saline)	89.104±4.336	86.383±3.646	87.561±3.626
II	Diabetic control	90.2±21.44	171.61±28.9##	196.88±28.06##
III	HFD Dexamethasone 10 mg/kg	88.71±45.750	194.77±9.532	158.28±5.576*
IV	EEEJ 200 mg/kg	95.98±19.952	178.21±18.461	123.61±40**
V	EEEJ 400 mg/kg	98.28±6.686	164.16±4.844*	108.543±3.37**
VI	Glibenclamide 0.5 mg/kg	97.47±17.420	149.27±9.237*	97.817±4.104**
VII	Atorvastatini10 mg/kg	92.54±35.585	154.86±26.71*	102.029±5.58**

The data are presented as mean±standard error (n = 6). Compared it to the normal group (Students' test) with ##$p<0.01$, to the diabetic control group (One way ANOVA followed by Dunnett's test) with *$p<0.05$ and **$p<0.01$.

When compared to the normal group, the administration of dexamethasone (10 mg/kg, s.c.) demonstrated a substantial ($P<0.01$) rise in blood glucose levels on days 10, 20, and of the observational period. As glibenclamide (500 mg/kg, p.o.) was administered, there was a substantial ($P<0.01$) drop in blood glucose levels as compared to the diabetic control group. When compared to a diabetic control group, the administration of EEEJ (200 and 400, p.o.) resulted in a substantial ($p<0.05$ and $p<0.01$) drop in blood glucose levels. When compared to a diabetic control, the administration of 10 mg/kg of atorvastatin demonstrated a substantial ($p<0.01$) drop in blood glucose levels.

Table 4: Effect of EEEJ on Kidney weight, Liver weight, and Pancreas weight in Dexamethasone induced diabetes mellitus

Group No.	Groups	Liver weight (gm)	Kidney weight (gm)	Pancreas weight (gm)
I	Normal Control (Normal Saline)	7.004±0.304	0.729±0.022	0.776±0.051
II	Diabetic control	7.829±0.243#	0.852±0.027#	1.067±0.066 ##
III	HFD+ Dexamethasone 10img/kg	7.733±0.255	0.831±0.023	1.001±0.05
IV	EEEJ 200 mg/kg	7.577±0.281	0.808±0.021	0.929±0.032
V	EEEJ 400 mg/kg	7.299±0.304	0.766±0.027	0.809±0.05**
VI	Glibenclamide 0.5 mg/kg	7.213±0.307*	0.745±0.039*	0.798±0.052**
VII	Atorvastatin 10 mg/kg	7.004±0.253*	0.738±0.034*	0.701±0.024**

The data are presented as mean±standard error (n = 6). *$p<0.05$, **$p<0.01$, compared it to the diabetic control group (one-way ANOVA followed by Dunnett's test) #$p<0.05$, ##$p<0.01$ as compared to normal group (Student 't' test).

When compared to the normal group, the administration of dexamethasone (10 mg/kg, i.p.) resulted in a substantial ($P<0.05$) increase in median weight. When glibenclamide (10 mg/kg, p.o.) was administered, there was a substantial ($P<0.05$) reduction in insulin weight when compared to the diabetic control group. Conversely, as compared to diabetic control, EEEJ (200 and 400 mg/kg, p.o.) demonstrated no effects in this respect. When compared to the diabetic control, the administration of 10 mg/kg of atorvastatin demonstrated a substantial ($p<0.05$) reduction in liver weight.

As dexamethasone (10 mg/kg, i.p.) was administered, there was a statistically significant ($P<0.05$) increase in kidney weight as compared to the normal group. As glibenclamide (10 mg/kg, p.o.) was administered, there was a substantial ($P<0.05$) reduction in kidney weight as compared to the diabetic control group. When compared to

diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) revealed little effects in this respect. When compared to a diabetic control, the administration of 10 mg/kg of atorvastatin demonstrated a substantial ($p<0.05$) reduction in kidney weight.

When compared to the normal group, the administration of dexamethasone (10 mg/kg, i.p.) exhibited a substantial ($P<0.01$) increase in pancreatic weight. as glibenclamide (10 mg/kg, p.o.) was administered, there was a substantial ($P<0.01$) reduction in pancreatic weight as compared to the diabetes control group. When compared to diabetes control, the administration of EEEJ (400 mg/kg, p.o.) exhibited a substantial ($P<0.01$) reduction in pancreatic weight, while EEEJ (200 img/kg, p.o.) showed an insignificant impact. When compared to a diabetic control, the administration of 10 mg/kg of atorvastatin demonstrated a substantial ($p<0.01$) reduction in pancreatic weight.

Table 5: Effect of EEEJ on SGOT and SGPT in dexamethasone induced diabetes mellitus

Group No.	Groups	SGOT (U/L)	SGPT (U/L)
I	Normal Control (Normal Saline)	48.308±4.604	33.494±1.683
II	Diabetic control	92.635±7.374 ##	60.091±2.116 ##
III	HFD + Dexamethasone 10 mg/kg	84.662±7.695	56.378±2.453
IV	EEEJ 200 mg/kg	80.968±6.15	55.99±3.467
V	EEEJ 400 mg/kg	66.806±7.278*	43.208±3.238**
VI	Glibenclamide 0.5 mg/kg	52.156±3.38**	39.491±1.989**
VII	Atorvastatin	57.21±5.496**	39.471±2.538**

The data are presented as mean±standard error (n = 6). ##$p<0.01$, *$p<0.05$, **$p<0.01$, compared it to the diabetic control group (one-way ANOVA followed by Dunnett's test) and the normal group (Students t test).

When dexamethasone (10 mg/kg, s.c.) was administered, there was a substantial ($P<0.01$) rise in SGOT when compared to the normal group. When glibenclamide (500 u/kg, p.o.) was administered, there was a substantial ($P<0.01$) reduction in SGOT when compared to the diabetic control group. When compared to a diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) exhibited a substantial ($P<0.05$ and $P<0.01$) reduction in SGOT, although HFD + dexamethasone (10 mg/kg) showed an inconsequential impact. When compared to diabetic control, administration of 10 mg/kg of atorvastatin exhibited a substantial ($p<0.01$) reduction in SGOT.

When dexamethasone (10 mg/kg, s.c.) was administered, there was a substantial ($P<0.01$) increase in SGPT when compared to the normal group. When glibenclamide (500 u/kg, p.o.) was administered, there was a substantial ($P<0.01$) reduction in SGPT as compared to the diabetic control group. When compared to a diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) resulted in a substantial ($P<0.05$ and $P<0.01$) reduction in the SGPT. When compared to diabetic control, administration of 10 mg/kg of atorvastatin exhibited a substantial ($p<0.01$) reduction in SGPT.

Table 6: Effect of EEEJ on Total bilirubin, Direct bilirubin and Creatinine in dexamethasone induced diabetes mellitus

Group No.	Groups	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Creatinine (mg/dl)
I	Normal Control (Normal Saline)	0.895±0.089	0.364±0.032	0.756±0.048
II	Diabetic control	1.513±0.163 ##	0.901±0.084 ##	1.567±0.141 ##
III	HFD + Dexamethasone 10 mg/kg	1.096±0.069*	0.875±0.116	1.117±0.105*
IV	EEEJ 200 mg/kg	0.955±0.059**	0.659±0.05	1.057±0.087**
V	EEEJ 400 mg/kg	0.868±0.085**	0.457±0.052**	0.872±0.049**
VI	Glibenclamide 0.5 mg/kg	0.88±0.080**	0.483±0.042**	0.746±0.065**
VII	Atorvastatin 10 mg/kg	0.85±0.076**	0.392±0.053**	0.769±0.032**

The data are presented as mean±standard error (n = 6). Compared it to the normal group (Students' test) with ##p<0.01, to the diabetic control group (One way ANOVA followed by Dunnett's test) with *p<0.05 and **p<0.01.

When compared to the normal group, the administration of dexamethasone (10 mg/kg, is.c.) resulted in a statistically significant rise (P<0.01) in total bilirubin levels. As glibenclamide (500 u/kg, p.o.) was administered, there was a substantial (P<0.01) drop in total bilirubin levels as compared to the diabetic control group. When compared to a diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) resulted in a substantial (P<0.05 and P<0.01) reduction in total bilirubin. When compared to a diabetic control, the administration of 10 mg/kg of atorvastatin demonstrated a substantial (p<0.01) reduction in total bilirubin levels.
When compared to the normal group, the administration of dexamethasone (10 mg/kg, s.c.) resulted in a substantial (P<0.01) rise in direct bilirubin levels. As glibenclamide (500 u/kg, p.o.) was administered, there was a substantial (P<0.01) drop in direct bilirubin levels as compared to the diabetic control group. When compared to a diabetic control, the administration of EEEJ (200, 400 mg/kg, p.o.) showed a substantial (P<0.01) reduction in direct bilirubin, although HFD + Dexamethasone (10 mg/kg) exhibited a negligible impact. When compared to a diabetic control, the administration of 10 mg/kg of atorvastatin demonstrated a substantial (p<0.01) reduction in direct bilirubin levels. When dexamethasone (10 mg/kg, s.c.) was administered, creatinine levels increased significantly (P<0.01) in comparison to the normal group. When glibenclamide (500 u/kg, p.o.) was administered, there was a substantial (P<0.01) reduction in creatinine when compared to the diabetic control group. When compared to a diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) demonstrated a substantial (P<0.01) reduction in creatinine, while HFD + dexamethasone (10 mg/kg) exhibited a negligible impact. When compared to the diabetic control, the administration of 10 mg/kg of atorvastatin revealed a substantial (p<0.01) reduction in creatinine.

Table 7: Effect of EERH on Cholesterol and Triglycerides in dexamethasone induced diabetes mellitus

Group No.	Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)
I	Normal Control	90.878±4.726	145.1±50.252
II	Diabetic control	176.5±20.038 ##	180.53±20.733 ##
III	DHFD+ Dexamethasone 10 mg/kg	136.07±13.437	170.18±20.575
IV	EEEJ 200 mg/kg	120.91±9.955*	136.58 i±15.526*
V	EEEJ 400 mg/kg	91.09±9.889**	147.3±19.029**
VI	Glibenclamide 0.5mg/kg	92.707±9.548**	135.82±17.151**
VII	Atorvastatin10 mg/kg	92.754±8.966**	102.754±8.966**

The data are presented as mean±standard error (n = 6). ##p<0.01, *p<0.05, **p<0.01, compared it to the diabetic control group (one-way ANOVA followed by Dunnett's test) and the normal group (Students't' test).

When dexamethasone (10 mg/kg, s.c.) was administered, there was a substantial (P<0.01) rise in cholesterol as compared to the normal group. When glibenclamide (500 mg/kg, p.o.) was administered, there was a substantial (P<0.01) reduction in the amount of cholesterol as compared to the diabetic control group. When compared to a diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) resulted in a statistically significant reduction in cholesterol (P<0.05 and P<0.01). When compared to a diabetic control, the administration of 10 mg/kg of atorvastatin demonstrated a substantial (p<0.01) drop in cholesterol. When dexamethasone (10 mg/kg, s.c.) was administered, there was a substantial (P<0.01) rise in triglycerides when compared to the normal group. When glibenclamide (500 iu/kg, p.o.) was administered, there was a substantial (P<0.01) drop in triglycerides when compared to the diabetic control group. When compared to a diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) resulted in a substantial (P<0.05 and P<0.01) reduction in triglycerides, respectively. When compared to a diabetic control group, the administration of 10 mg/kg of atorvastatin demonstrated a substantial (p<0.01) reduction in triglycerides.

Table 8: Effect of EERH on MDA and GSH of liver in dexamethasone induced diabetes mellitus

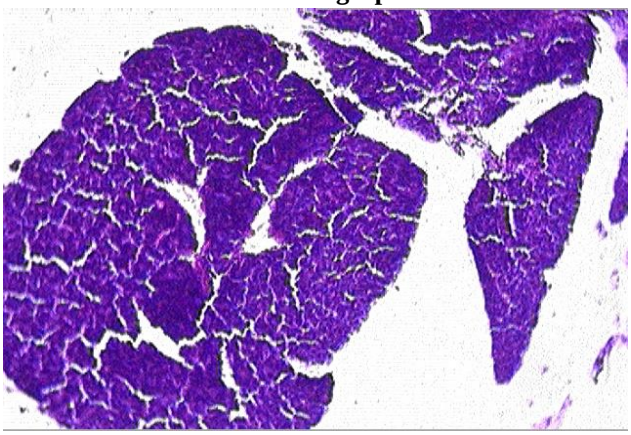
Group No.	Groups	MDA (ng/ml)	GSH (ng/mg protein)
I	Normal Control	5.739± 0.271	25.191±0.269
II	Diabetic control	8.588±0.521##	14.835±0.199##
III	HFD + Dexamethasone 10 mg/kg	8.294±0.348	15.655±0.267
IV	EEEJ 200 mg/kg	7.436±0.401	18.036±0.178**
V	EEEJ 400 mg/kg	5.89±0.369**	21.254±0.153**
VI	Glibenclamide 0.5 mg/kg	5.528±0.363**	22.513±0.425**
VII	Atorvastatin 10 mg/kg	5.234±0.335**	24.178±0.378**

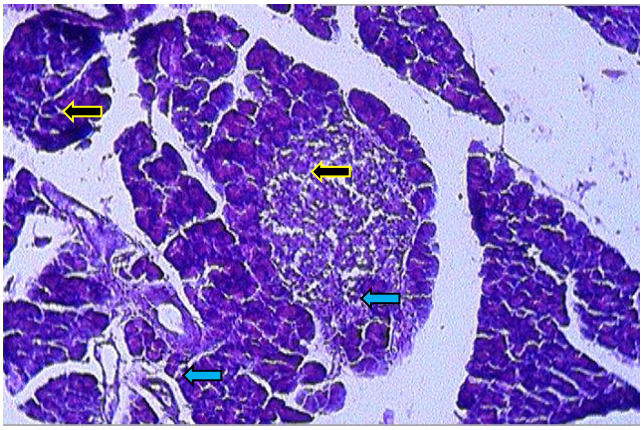
The data are presented as mean±standard error (n = 6). Compared it to the normal group (Students' test) with ##p<0.01, to the diabetic control group (One way ANOVA followed by Dunnet's test) with *p<0.05 and **p<0.01.

When compared to the normal group, the administration of dexamethasone (10 mg/kg, s.c.) demonstrated a substantial (P<0.01) rise in malondialdehyde. When glibenclamide (500 u/kg, p.o.) was administered, there was a substantial (P<0.01) reduction in aldehyde when compared to the diabetic control group. When compared to a diabetic control, the administration of EEEJ (200 and 400 mg/kg, p.o.) showed a substantial increase (P<0.01 and P<0.01) in malondialdehyde, while HFD + dexamethasone (10 mg/kg) exhibited a negligible impact. When compared to a diabetic control, the administration of 10 mg/kg of atorvastatin demonstrated a substantial (p<0.01) reduction in malondialdehyde.

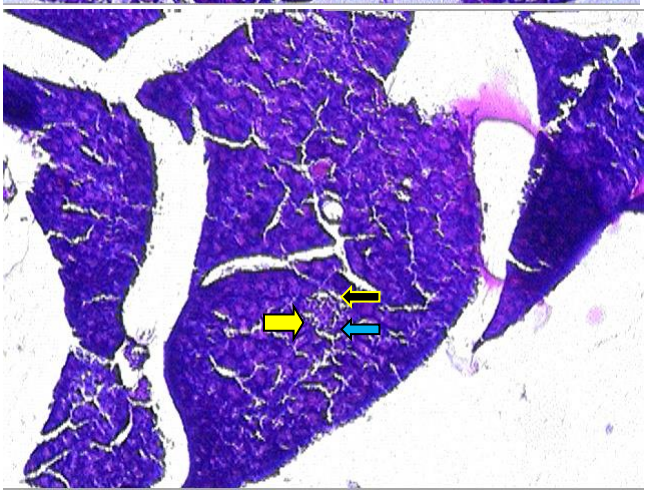
When dexamethasone (10 mg/kg, s.c.) was administered, there was a substantial (P<0.01) drop in glutathione when compared to the normal group. As glibenclamide (500 iu/kg, p.o.) was administered, glutathione levels increased significantly (P<0.01) as compared to the diabetic control group. When compared to a diabetic control, the administration of EERH (400 mg/kg, p.o.) showed a substantial increase (P<0.01) in glutathione, while EEEJ (200 mg/kg, p.o.) and HFD + Dexamethasone (10 mg/kg) exhibited a negligible impact. When compared to the diabetic control group, the administration of 10 mg/kg of atorvastatin demonstrated a substantial (p<0.01) increase in glutathione levels.

Histopathological observation of pancreas tissue

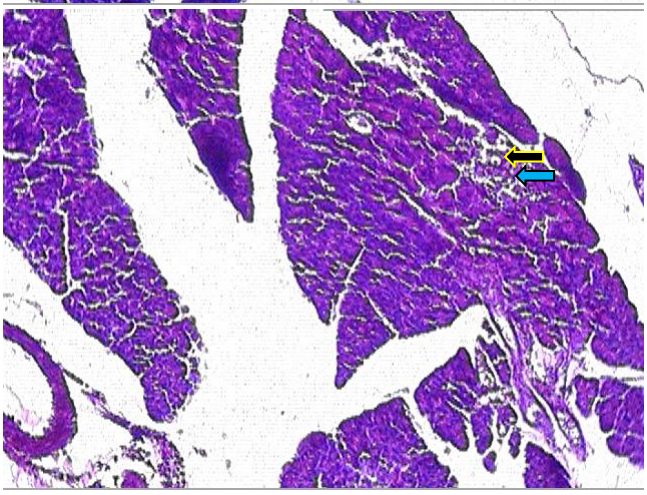
Photographs	Descriptions
	**Normal pancreas**



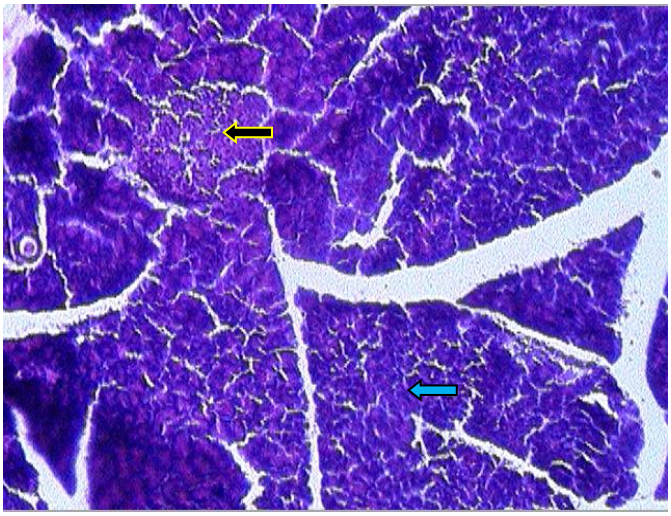
Diabetic control – 10X photograph showing necrosis of islets (black arrow), cellular infiltration (blue arrow) H&E stain 100X



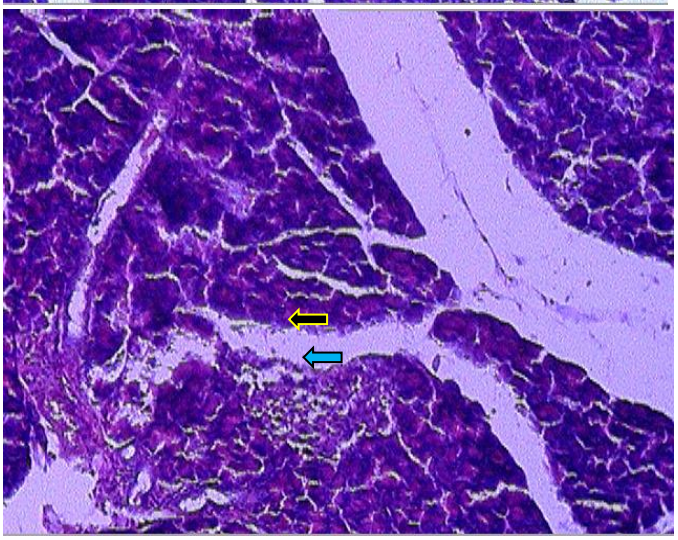
HFD 100 - 10X photograph showing necrosis of islets (black arrow), cellular infiltration (blue arrow) and small sized islet H&E stain 100X



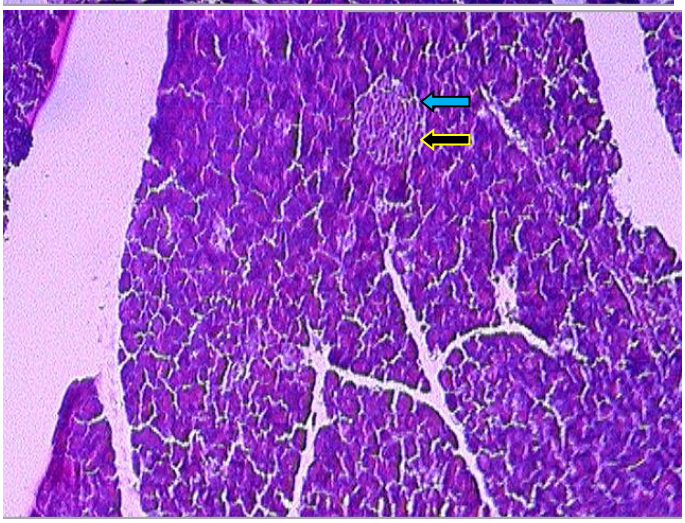
EEEJ 200 mg/kg- 10X photograph showing necrosis of islets (black arrow), cellular infiltration (blue arrow) H&E stain 100X



EEEJ 400 mg kg - 10X photograph showing necrosis of islets (black arrow), cellular infiltration (blue arrow) H&E stain 100X



Glibenclamide - 10X photograph showing necrosis of islets (black arrow), cellular infiltration (blue arrow) H&E stain 100X



Atorvastatin 10 mg/kg – 10X photograph showing necrosis of islets (black arrow), cellular infiltration (blue arrow) H&E stain 100X

Figure 1: Histopathological analysis of pancreatic tissue in insulin-induced diabetes n diabetic rabbits

Conclusion

This study has assessed the phytochemical and pharmacological screening of the antidiabetic activities of *Eugenia jambolana*. It has also evaluated the plant's various parameters, including pharmacognostic and physicochemical, which offer the quickest, easiest, and least expensive ways to determine the identity and purity of the drug. There is evidence to suggest that Eugenia jambolana contains potentially beneficial chemicals. The various extracts were produced and assessed for their potential to prevent diabetes in vivo using the Dextamethsone-induced diabetes paradigm. Following therapy with *Eugenia jambolana*, there was a reduction in the weight of the liver, kidney, and pancreas. This might be attributed to the avoidance of inflammation, necrosis, and MNC infiltration as a result of the oxidative stress caused by dexamethasone. The reduction in inflammation, necrosis, and MNC infiltration in pancreatic tissue, as well as the antioxidant capability of EEEJ, are indicative of the protection against histopathological harm to the pancreas. Moreover, it has been claimed that lowered levels of bilirubin, SGOT, and SGPT successfully protected against liver impairment caused by oxidative stress caused by dexamethasone.

References

[1]. Berton O, Nestler EJ, "New approaches to antidepressant drug discovery: Beyond monoamines." Nat Rev Neurosci. 2006, 7, 137–151.
[2]. Sullivan PF, Neale MC, Kendler KS, "Genetic epidemiology of major depression: Review and meta-analysis." Am. J. Psychiatry. 2000, 157, 1552–1562.
[3]. Arban R, Maraia G, Brackenborough K, "Evaluation of the effects of lamotrigine, valproate and carbamazepine in a rodent model of mania." Behav Brain Res. 2005, 158, 123–132.
[4]. Telner JI, Singhal RL. "Psychiatric progress: The learned helplessness model of depression." J Psychiatr Res. 1984, 18, 207–215.
[5]. Cryan JF, Slattery DA. "Animal models of mood disorders: Recent developments." Curr. Opin. Psychiatry. 2007, 20, 1–7.
[6]. Slattery DA, Markou A, Cryan JF. "Evaluation of reward processes in an animal model of depression." Psychopharmacology (Berl.), 2007, 190, 555–568.
[7]. O'Donnell JM, Marek GJ, Seiden LS, "Antidepressant effects assessed using behavior maintained under a differential-reinforcement-of-low-rate (DRL) operant schedule." Neurosci Biobehav Rev. 2005, 29, 785–798.
[8]. Brigitta B, Pathophysiology of depression and mechanisms of treatment. Dialogues in clinical neuroscience, 2002, 4, 1, 7–20.
[9]. Tripathi KD. In Essentials of Medical Pharmacology, 13th Edn.; Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 2013, pp. 454.
[10]. Gihyun Lee, Hyunsu Bae, "Therapeutic Effects of Phytochemicals and Medicinal Herbs on Depression", BioMed Research International, 2017, 1- 11.
[11]. Jeanette M, Brijesh S., "Phytochemistry and pharmacology of anti-depressant medicinal plants: A revie', Biomedicine & Pharmacotherapy, 2018, 104, 343-365.
[12]. Nakazawa T, Yasuda T, Ohsawa K, "Metabolites of orally administered Magnolia officinalis extract in rats and man and its antidepressant-like effects in mice", J Pharm Pharmacol., 2003, 55, 11, 1583-91.
[13]. Yankelevitch-Yahav R, Franko M, Huly A, Doron R, "The forced swim test as a model of depressive-like behavior", *J Vis Exp*. 2015, 97, 52587.
[14]. Chokpaisarn J, Chusri S, Voravuthikunchai SP, "Clinical randomized trial of topical Quercus infectoria ethanolic extract for the treatment of chronic diabetic ulcers." Journal of Herbal Medicine, 21, 2020, 100301.
[15]. Elham A, Miradel Arken, Gulina Kalimanjan, Abudulaziz Arkin, Mubarak Iminjan, "A Review of the Phytochemical, Pharmacological, Pharmacokinetic, and Toxicological Evaluation of Quercus Infectoria Galls." Journal of Ethnopharmacology, 2020, 113592.
[16]. Arina MZI, Harisun Y, "Effect of extraction temperatures on tannin content and antioxidant activity of Quercus infectoria (Manjakani)." Biocatalysis and Agricultural Biotechnology, 2019, 19, 101104,