Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 1 Year 2024 Page 1201-1213

Anti-Diabetic Study of Flower Extract of Eugenia Jambolona In Rats

Deepika Devnani[1], Pradeep C. Dave[2*], Aparna Subhash Acharya[3], Pankaj Mohan Pimpalshende[4], Manoj Kumar Katual[5], Falgunee D Ghadi[6], Sachin Shankar Rahate[7]

[1] School of Pharmacy, G H Raisoni University, Saikheda, Chhindwara, M.P
[2] Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Navi Mumbai, Kharghar*
[3]Tatyasaheb Kore College of Pharmacy Diploma, Warananagar.
[4]Hi Tech College of Pharmacy, Chandrapur, Maharashtra, India
[5]University College of Pharmacy, Guru Kashi University, Sardulgarh Road, Talwandi Sabo, Bhatinda.
[6]St. Wilfred Institute of Pharmacy, Panvel, Shedung, Panvel, Raigad
[7]Department of Endocrinology, T.N.Medical College & B.Y.L.Nair Ch. Hospital, Mumbai Central, Mumbai

***Corresponding Author:** Pradeep C. Dave*
** Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Navi Mumbai, Kharghar*
Email: davepradeep.dp@gmail.com

Article History:
Received: 05/01/2024
Revised: 20/01/2024
Accepted: 31/01/2024

Abstract

We are learning more about the role that integrated medicine plays in treating metabolic illnesses. This is partially based on an abundance of scientific data regarding therapeutic herbs, including those with promise to treat diabetes, and in part on the WHO's and other governmental organizations' support for the practise. The anti-diabetic effect of number of flowers of Eugenia jambolana (EJA) was evaluated in the current study on streptozotocin-induced diabetic rats. The The study's primary goal was to look into the potential antidiabetic effects of sub-chronic oral administration of E. jambolana flowers infusion in rats with STZ-induced diabetes. In diabetic experimental rats, the floral extract considerably reduced blood sugar and cholesterol levels when given 400 mg/kg of body weight are the dosage. It also increased liver glycogen and total protein levels, enhanced reduced Hb1AC, serum level, and antioxidant levels, as well as impaired glucose tolerance. Additionally, it raised liver glycogen and total protein levels as well as glucose tolerance. A common hypoglycemic medication called Glibenclamide was used to compare the hypoglycemic efficacy.The preclinical investigation's results could be useful for phase 2 clinical studies that aim to reduce the morbidity and mortality of diabetes mellitus exacerbated by drug-induced hypoglycemia by implementing integrated medicine.

INTRODUCTION

There is a dearth of scientific information on E. jambolana grown in the Western Ghats of coastal India, despite the fact that the blossoms are a popular remedy and are advised in certain parts of India by Ayurvedic medicine for diabetes mellitus [1]. It is also generally recognized that soil, climate, location, and humidity can all affect the chemical components of plants that have been isolated [2].A phase 2 clinical study that combines the Ayurvedic herb Eugenia jambolana with a recognized oral hypoglycemic medication, also known by the names Syzygium cumini and Syzygium jambolanum, family Myrtaceae, has been urgently needed among a particular subset of type 2 diabetes patients [3]. Evidence from preclinical animal research and acute tests for sub-acute toxicity are required for the institutional ethics committee to approve clinical trials [1]. Based on contradictory

reports regarding the application of a tea derived from E. jambolana and its inability to prevent diabetes in rats treated with streptozotocin (STZ)-induced diabetes, the researchers determined that the necessary preclinical data should be gathered from locally grown plants [4]. Limited scientific information is available about E. jambolana, which is grown in the coastal Western Ghats of India, despite the fact that the blossoms are a frequent remedy and are advised in these locations by Ayurvedic medicine for diabetes mellitus [5]. It is also generally recognised that soil, climate, location, and humidity can all affect the chemical components of plants that have been isolated. The study's main goal was to find out whether giving rats with STZ-induced diabetes sub-chronic oral infusions of E. jambolana flowers could have any antidiabetic effects [6].

It has been demonstrated that Eugenia jambolana, which is frequently used to lower blood glucose levels and treat diabetes among diabetic animals by tincturing and administering aqueous extracts to its bark, fruits, seeds, or flowers, which have been collected from various parts of the world [2]. Furthermore, traditional medicine has employed E. jambolana infusions and decoctions—basic aqueous extracts that are heated but not boiled —to treat diabetes mellitus [3].
Many impoverished and emerging nations have turned to plant infusions and decoctions as common medications as an alternate form of treatment for a variety of ailments, including diabetes [1]. There are 14 species in the genus Eugenia (synonym: Syzygium, family: Myrtaceae), including Syzygium jambos (L.) Alst., Eugenia uniflora, Eugenia punissifolia, and Eugenia jambolana Lam. (syzygium cumini (L.) skeels, sometimes called Syzygium jambolanum). All of these species have been reported to have physiological effects in a variety of species, including humans [7]. E. uniflora flowers, for instance, have been said to possess anti-inflammatory and diuretic qualities, antihypertensive effects, anti-gout effects, hypoglycemic and hypo-triglyceride effects, and antihypertensive effects [8].

There have also been claims that this plant's fruit possesses antihypertensive properties. In both healthy and pancreatectomized dogs, the roots of E. punissifolia have been demonstrated to produce a hypoglycemic effect [9]. Both Syzygium jambos and E. jambolana are commonly referred to as Jambolo in Brazil. S. jambos flowers have been described as having a hypotensive effect, and E. jambolana flowers and flower buds are thought to have diuretic properties [10]. This plant's seeds have shown to have anticonvulsant qualities, and E. jambolana bark has shown to have antidiarrheal and HIV-1 protease activity inhibitory effects [4].

STUDY DESIGN:

Study duration for study design
The rats reported to have the glucose levels of > 135 mg/dl in plasma after two days of being injected with streptozotocin were covered by the research. The therapy with the Eugenia Jambolana flower extract was initiated only after 72 hours passed by injecting with streptozotocin. Up to the completion of the trial (i.e., six weeks), blood samples were taken once a week. On days 1, 7, 14, 21, and 28 of the trial, body weight was measured and fasting blood glucose levels were estimated [11].

Group	No. of animals	Drug/Dose (mg/kg)	Duration
Group-1 Control	6	Normal Saline	45 Days
Group-2 Test Control	6	STZ (55)	45 Days
Group-3	6	STZ (55) + EJA (100)	45 Days
Group-4	6	STZ (55) + EJA (200)	45 Days
Group-5	6	STZ (55) + EJA (400)	45 Days
Group-6	6	STZ(55)+Glibenclamide(5)	45 Days
Group-7	6	EJA (400)	45 Days

Reagents and chemicals:
Dimethyl sulfoxide (DMSO), streptozotocin, and insulin assay kits were purchased from Sigma Chemicals in the United States. Analytical grade materials and reagents were all employed in the tests.

Preparation of the Eugenia Jambolana flower ethanolic extracts:
The ethanolic extracts from Eugenia Jambolana flowers were prepared by cleaning the flowers with distilled water, letting them air dry in the shade for two days at 4°C, and then roughly pulverizing them. Following homogenization, ethanol in the concentration of 70 (%) v/v in ten volumes was used to extract the powdered Jambolana flowers. At -20°C, the supernatant was gathered and kept cold after being subjected to 12,000 g for

five minutes at 4°C after centrifugation. Next, Whattman filter paper was used to filter the supernatant component of the E. jambolana flower extract[12].

Animals used in experiments
Male Wistar albino rats of the weight 150-250 grams were the experimental rodents used in this inquiry. The animals were kept in a variety of groups that were each well-ventilated. Water and regular food were given to the animals without restriction.

Acute oral toxicity study
The requirements of OECD (2001) were followed when doing research on acute toxicity. Following an overnight fast, six animals were split up into groups of six healthy male Wistar albino rats for each dosage. Aqueous extracts of E. Jambonia flower in suspension at doses of 10, 20, 100, 500 and 1000 mg/kg/kg body weight were given to the group undergoing acute toxicity testing. All of the aforementioned doses were administered by gastric gavage. After receiving the treatment, the animals were watched every hour for the first six hours, then every day for 14 days [13].

Induction of Diabetes Mellitus
The experimental animals were fed high-fat diets with an estimated energy content of 5 kcal/g, consisting of 60% calories from fat, 35% calories from protein, and 5% calories from carbohydrates. The next step was administering a single intraperitoneal dosage of 30 mg/kg of streptozotocin diluted in 0.1 mol/L of pH 4.5 citrate buffer's. Three days following treatment, the type 2 diabetes model was successfully created in rats given streptozotocin; nonetheless, the level of fasting blood glucose had to be higher than 300 mg/100 ml [14].

Glutathione: Hydrogen Peroxide Oxido-reductase (GPx) Assay
Using the Rotruck method, glutathione peroxidase activity was measured.

Reagents
- 7.0 pH, 0.32 M phosphate buffer, and EDTA of concentration 0.8 mM
- Sodium azide of concentration 10 mM
- A drop in glutathione (three millimolar)
- H_2O_2: (2.5 mM)
- 10% TCA
- Dihydrophosphate of sodium: (0.3 M)
- 40 mg of DTNB in 100 ml of 1% sodium citrate is the DTNB solution.
- In 100 cc of distilled water, 10 mg of reduced glutathione was dissolved.

Procedure
There are the following ingredients in the reaction mixture: 0.1 ml homogenate, 0.4 ml phosphate buffer, 0.5 ml sodium azide, 0.5 ml EDTA, and 0.1 ml H_2O_2. Various amounts of time were spent incubating this at 37°C. Once the reaction was stopped by adding 0.5 ml of TCA, the tubes were centrifuged at 2000 rpm. After adding 0.5 milliliters of DTNB and 4 milliliters of disodium hydrogen phosphate to 0.5 milliliters of supernatant, the color was measured at 420 nanometers. GPx activity was assessed in terms of moles of glutathione oxidized/mg protein/min [15].

Catalase (Hydrogen-Peroxide): Oxido-reductase Assay
The Sinha (1972) approach was used to measure the activity of catalase.
Reagents

- Reagent of dichromate and acetic acid
- Acetic acid was combined with 5% potassium dichromate in distilled water in a 1:3 (v/v) ratio.A further 1 :5 dilution of the solution in water was performed.
- A pH 7 phosphate buffer (0.1M)3. 0.2 M Hydrogen peroxide

Procedure
In order to make hydrogen peroxide (0.2M), the homogenate was combined with 0.5 cc of phosphate buffer. The reaction was halted by adding 2.0 ml of the dichromate acetic acid reagent. With regular hydrogen

peroxide, with handling in the range of 4 to 20 M in a similar manner. Ten minutes were spent heating the tubes in a bath of hot water. The green color that had appeared was measured at 570 nm in a spectrophotometer. The quantity of H2O2 ingested per milligrams of protein per minute was used to calculate the catalase activity [16].

Glutathione -s-transferase estimation:
The following reaction is catalyzed by glutathione-s-transferase.
RX+GSH ⟶ HX+R-SG

The enzyme was measured using the procedure given by the researcher Habig et al.

Principle: The increase in CDNB-GSH conjugate absorbance at 340 nm, which is caused by GST catalyzing the reaction between glutathione and 1-chloro-2,4,-dinitrobenzene (CDNB), is used to quantify the enzyme activity.
CDNB+GSH ⟶ CDNB-GSH conjugate

Assay: The enzyme sample, the required quantity of distilled water, pH 6.5, ethanol with 1 mM CDNB, and the ultimate concentration of 0.1M sodium phosphate buffer were all present in the 3 ml reaction container. This mixture underwent a 5-minute 37°C pre-incubation period. One milligram was added to begin the reaction GSH, and 340 nm absorbance was measured. The enzyme activity was determined using the mill molar extinction coefficient between CDNB- GSH conjugate and CDNB. It was determined that the enzyme has a specific activity of n moles of GSH-CDNB conjugate per minute per mg protein [17].

Nitric Oxide scavenging activity
Reagents
- 10 mM sodium nitroprusside
- Phosphate buffer saline having pH 7.4 of concentration 0.1 M
- 1 mM of the sulfanilic acid reagent
- 0.1% w/v concentration of naphthyl ethylene diamine dihydrochloride
- 20% acetic acid

Procedure
In an aqueous solution with a physiological pH, sodium nitroprusside produces nitric oxide on its own. The Griess Illosvoy reaction can be used to measure the amount of nitrite ions that are created when nitric oxide reacts with oxygen. Instead of naphthyl ethylene diamine dihydrochloride (0.1% w/v), 1-napthylamine (5%) was utilized as the Griess-Illosvoy reagent in this experiment. Nitric oxide synthesis is reduced as a result of the conflict between oxygen and nitric oxide scavengers. Different amounts of water, phosphate buffer saline (0.5 ml), and sodium nitroprusside are present in the reaction mixture (3 ml) (10 mM, 2 ml, 100, 200, 300, 400, and 500 mM). Kaempferol (4 g/ml) 0.5 ml was incubated at 25°C for 150 minutes.

0.5 ml of the nitrite-based solution and 1 ml of the sulfanilic acid reagent (0.33% in 20% glacial acetic acid) containing reaction mixture were pipetted into a mixture to complete the diazotization procedure. It was combined with 1 milliliter of naphthyl ethylene diamine dihydrochloride, followed by 30 minutes of standing at 25 °C. When nitrite and naphthyl ethylene diamine were mixed after being diazotized with sulphanilamide, a pink chromophore resulted. 540 nm was used to measure the absorbance [18].

Superoxide: Oxido-reductase (SOD) (EC 1.15.1.1) assay
The Marklund and Marklund (1974) approach was used to assess the superoxide dismutase's activity.

Reagents
- 0.1 M Tris-HCl buffer with pH 8.2;
- 0.06 M Tris-HCl buffer with pH 7.9; and
- 2 mM Pyrogallol stock solution in 0.05 M buffer Tris-HCl
- 100% ethanol
- Chloroform 5

Procedure
To make 1 ml, the material was mixed with 0.25 ml of 100% ethanol and 0.15 ml of chloroform. The suspension was centrifuged and the resulting supernatant was utilized as the source of enzymes after it had been shaken for 15 minutes in a mechanical shaker. 1.5 ml of distilled water, 0.5 ml of (2 mM) pyrogallol, and the buffer (Tris-HCl, pH 8.2) made up the reaction mixture for auto-oxidation.First, during three minutes, the rate of auto-oxidation of pyrogallol was recorded at one-minute intervals. 4 ml of water made up the final volume of the enzyme assay combination, which also included 2 ml of 0.1 M Tris-HCl buffer and 0.5 ml of pyrogallol aliquots of the enzyme preparation. Following the addition of the enzyme, the rate of inhibition of pyrogallol auto-oxidation was observed. An enzyme's activity is expressed in units/mg protein/min, where one unit is the quantity of the enzyme needed to 50% inhibit the auto-oxidation reaction [19].

Calculating the amount of glycosylated hemoglobin
Reagents necessary:
- NaClO-9%
- 0.3M of oxalic acid.
- TCA40%
- 0.05M thiobarbituric acid.
- Standard fructose, 10 to 40 grams.

Procedure:
The mixture of 0.2ml hemolysate and 1.8ml of 0.3M oxalic acid was hydrolyzed for two hours, cooled, and then added 1.0ml of 40% TCA. After 20 minutes of centrifugation at 1400 Xg. 0.5ml of 0.05M thiobarbituric acids were added to 1.5ml of the supernatant for treatment. The colour obtained after 40 minutes of incubation at 37°C was measured at 443nm. The processing of common fructose in the range of 10 to 40 ig was similar. Values were given as a percentage of glycosylated Hb [20].

Estimating serum insulin levels
The specific hormone or the method used to separate the free hormone from the hormone-antibody complex will determine if there are many radioimmunoassay methods for peptide hormones. Of course, there are additional minor characteristics that differ from one laboratory to the next, such as reagents, temperature, and incubation time. Herbert's coated charcoal method for insulin was modified and used as the basis for the research to develop a radioimmunoassay for glucagon.

In the modified approach, antibodies against bovine insulin made in guinea pigs are incubated using either plasma samples or normal bovine insulin solutions that have been 125 tested prior to the addition of I-insulin for five days at 4°C in sodium barbital buffer. 7.5 g Norit A, 0.75 g dextran-80, and 3 g bovine serum albumin per 100 ml of the pH 7.4 acetate-barbital buffer made by Herbert et al., 1965 is the amount of coated charcoal slurry that is used in 1.5 ml of the buffer. Then added after the tubes have been incubated at 4°C for two additional days. The tubes are centrifuged at a speed of 2000 g for twelve minutes at 4 °C after one to two hours of adsorption [21-56].

Calculating Glutathione
We computed glutathione using the Moron et al. (1979) approach.

Ingredients
- 5.33 g of disodium hydrogen phosphate solution were dissolved in 100 milliliters of clean water**.**
- TCA 10%
- DTNB: 0.6mM.
- Standard: Using distilled water, a 100 ml solution containing 10 mg of reduced glutathione was created.

Procedure
To precipitate one milliliter of tissue homogenate, one milliliter of 10% TCA was employed. By using centrifugation, the precipitate was eliminated. A part of the supernatant was mixed with 4.0 ml of phosphate solution and 0.5 ml of DTNB reagent. A 420 nm reading of the color developed was made. To express the glutathione concentration in the tissue, g/mg protein/min was utilized [22].

Result

	Day 1	Day 7	Day 14	Day 21	Day 28
Group I control	3.5 ± 0.2	3.2 ± 0.1	3.9 ± 0.51	4.5 ± 0.32	4.2 ± 0.34
Group II STZ (55 mg/kg)	17.8 ± 0.5	6.5 ± 0.1	15.4 ± 0.22	15.5 ± 0.14	13.2 ± 0.66
Group III STZ (55 mg/kg) + EJA (100 mg/kg)	14.6 ± 0.1	12.9 ± 0.4	12.2 ± 0.40	11.5 ± 0.30	11.0 ± 0.75
Group IV STZ (55 mg/kg) + EJA (200 mg/kg)	12.7 ± 0.4	11.5 ± 0.5	11.1 ± 0.72	10.8 ± 0.53	10.1 ± 0.15
Group V STZ (55 mg/kg) + EJA (400 mg/kg)	9.4 ± 0.1	8.6 ± 0.1	7.4 ± 0.70	6.5 ± 0.42	5.8 ± 0.85
Group VI STZ (55 mg/kg) + Glibenclamide (5mg/kg)	7.4 ± 0.7	6.5 ± 0.2	5.4 ± 0.52	5.0 ± 0.25	4.8 ± 0.75
Group VII EJA (400 mg/kg)	3.8 ± 0.6	3.4 ± 0.3	3.2 ± 0.60	4.7 ± 0.35	3.6 ± 0.60

Table1: Impact of extract from EJA flowers on the levels of glucose in blood during fasting in streptozotocin-induced diabetic rats

Table.1: The levels of blood glucose levels in control, streptozotocin, EJA and Glibenclamide treated animals Table 1 indicates the impact of repeated EJA injection on the blood glucose levels of mice with STZ-induced diabetes. The group that was administered STZ had significantly greater blood glucose levels than the control group. Doses of 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.01$), and 400 mg/kg ($p<0.001$) of the EJA extract significantly lowered blood glucose levels in comparison to STZ therapy. Group VII, who received EJA extract treatment, did not show any discernible differences in performance from the control group.

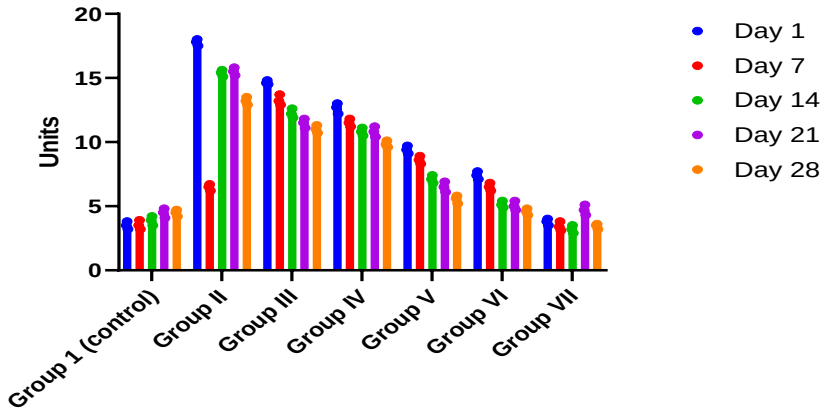


Fig 1: Impact of extract from EJA flowers on the blood glucose levels during fasting in streptozotocin-induced diabetic rats

Table 2: Effect of EJA flower extract on STZ-induced diabetic rats' body weight STZ-induced animals had considerably lower body weights than control rats ($p< 0.001$) (Table 2). Body weight rose considerably in response to EJA extract doses of 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.01$), and 400 mg/kg ($p<0.001$) relative to the other groups. The group's creatures given EJA flower extract carried on gaining weight until the study's conclusion. The EJA extract treated group (group VII) did not show any noteworthy outcomes in comparison to the control group.

	Day 1	Day 7	Day 14	Day 21	Day 28
Group I control	261 ± 5.30	266.00±1.00	265.67±1.53	269.33±2.08	269.33±1.53
Group II STZ (55 mg/kg)	$261.67 \pm 1.53^{***}$	$254.00\pm1.00^{***}$	$252.00\pm1.00^{***}$	$249.00\pm1.00^{***}$	$247.00\pm1.00^{***}$
Group III STZ (55 mg/kg) + EJA (100 mg/kg)	261.43 ± 1.53^{a}	259.00 ± 1.00^{a}	259.00 ± 1.00^{a}	259.00 ± 1.00^{a}	257.00 ± 1.00^{a}
Group IV STZ (55 mg/kg) + EJA (200 mg/kg)	260.33 ± 1.53^{b}	260.00 ± 1.00^{b}	260.33 ± 1.53^{b}	262.00 ± 2.00^{b}	263.00 ± 1.00^{b}
Group V STZ (55 mg/kg) + EJA (400 mg/kg)	258 ± 1.00^{c}	262.00 ± 1.00^{c}	269.67 ± 2.08^{c}	264.00 ± 1.00^{c}	265.00 ± 1.00^{c}
Group VI STZ (55 mg/kg) + Glibenclamide (5mg/kg)	261.67 ± 1.53^{c}	262.33 ± 1.53^{c}	264.00 ± 1.00^{c}	266.00 ± 2.00^{c}	267.00 ± 1.00^{c}
Group VII EJA (400 mg/kg)	263.67 ± 0.58^{NS}	264.00 ± 1.00^{NS}	266.00 ± 1.00^{NS}	270.00 ± 1.00^{NS}	270.00 ± 1.00^{NS}

Table 2: The periodic body weight of control, streptozotoc in, E. Jambolona (EJA) flower extract and glibenclamide treated animals

The values indicate the mean ± standard deviation for six animals in each group. * p<0.05, **p<0.01, ***p<0.001 compared to the control group; a p<0.05, b p<0.01, c p<0.001 compared to group II are the significant changes.
Non-significant results as compared to group I are denoted by NS.

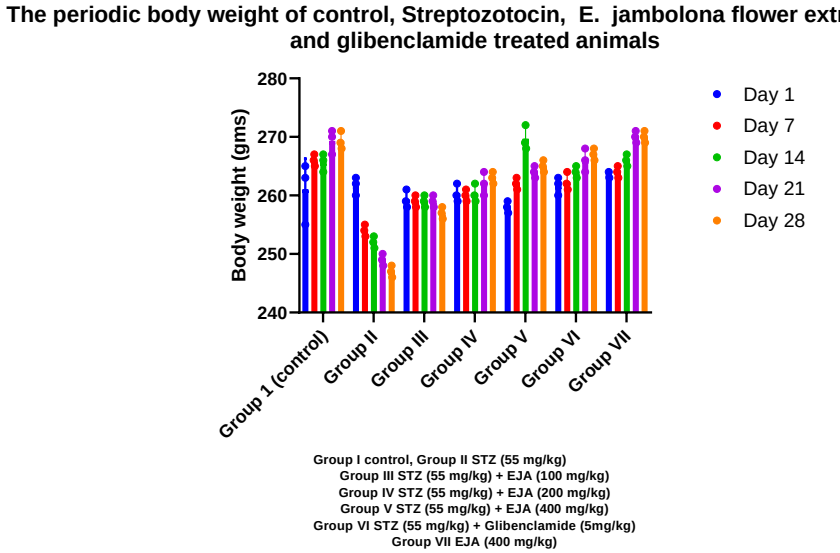


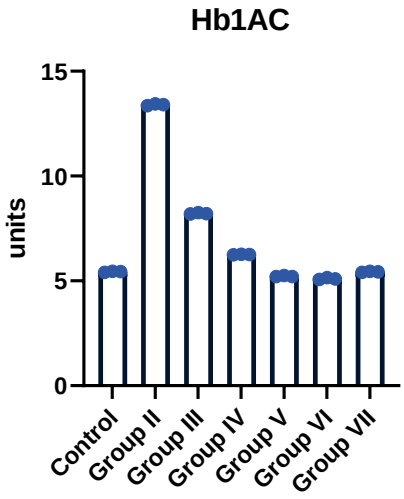
Fig 2: Effect of EJA flower extract on the body weight of STZ- induced diabetic rats

Table 3: The HbA1 C and serum glucose levels of control, streptozotoc in, E. Jambolona (EJA) flower extract and Gliben clamide treated animals
Table 3 shows how different experimental rat groups' blood glucose and HbA1C levels were impacted by EJA flower extract. Comparing the other groups to the control group, it was discovered that their serum glucose and HbA1C levels were greater. Rats with diabetes caused by streptozotocin had elevated hemoglobin A1C levels. At 100 mg/kg, 200 mg/kg, and 400 mg/kg of the EJA extract, respectively, the levels of HbA1C and blood glucose were dramatically (p<0.001) reduced. In contrast to the control group, the EJA-treated group (group VII) did not exhibit any discernible results.

Table3: Hb A1 C and serum glucose levels of control, streptozotocin, EJA flower extract streptozotocin, EJA flower extract and Gliben clamide treated animals

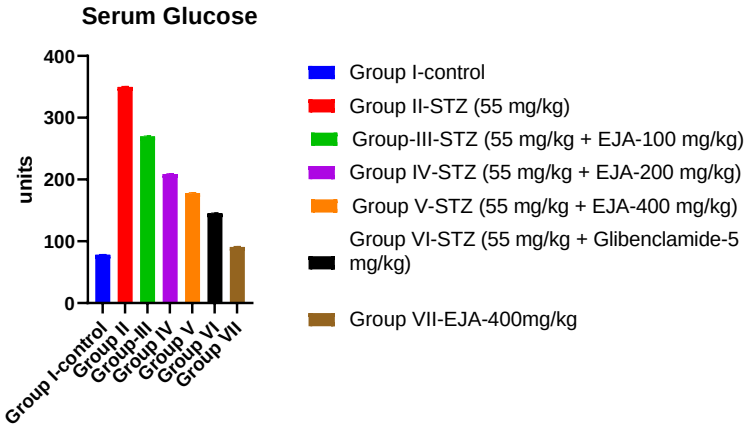
	HbA1C	**Serum Glucose**
Group I control	5.46 ± 1.1	78.17 ± 0.25
Group II STZ (55 mg/kg)	13.45 ± 0.35***	349.83 ± 0.76***
Group III STZ (55 mg/kg) + EJA (100 mg/kg)	8.26 ± 0.16^{a}	269.60 ± 0.58^{a}
Group IV STZ (55 mg/kg) + EJA (200 mg/kg)	6.24 ± 0.24^{b}	208.90 ± 0.36^{b}
Group V STZ (55 mg/kg) + EJA (400 mg/kg)	5.25 ± 0.42^{c}	177.67 ± 0.76^{c}
Group VI STZ (55 mg/kg) + Glibenclamide (5mg/kg)	5.07 ± 1.02^{c}	145.00 ± 0.50^{c}
Group VII EJA (400 mg/kg)	5.45 ± 1.54^{NS}	90.97 ± 0.45^{NS}

Each value represents as mean ± SD for six animals in each group. Significant changes are calculated as * p<0.05, **p<0.01, ***p<0.001 compared to control group; ^{a} p<0.05, ^{b} p<0.01, ^{c} p<0.001 compared to group II.
NS stands for non-significant results compared to group I.



Each value represents as mean ± SD for six animals in each group. Significant changes are calculated as * p<0.05, **p<0.01, ***p<0.001 compared to control group; [a] p<0.05, [b] p<0.01, [c] p<0.001 compared to group II.
NS stands for non-significant results compared to group I.

Fig 3: Hb A1 C levels of control, streptozotocin, EJA flower extract streptozotocin, EJA flower extract and Glibenclamide treated animals



Each value represents as mean ± SD for six animals in each group. Significant changes are calculated as * p<0.05, **p<0.01, ***p<0.001 compared to control group; [a] p<0.05, [b] p<0.01, [c] p<0.001 compared to group II.
NS stands for non-significant results compared to group I.

Fig 4: Serum levels of control, streptozotocin, EJA flower extract streptozotocin, EJA flower extract and Glibenclamide treated animals

Table 4: The levels of antioxidants in control, streptozotocin, EJA flower extract and Glibenclamide treated animals

Table 4 reveals the approximate antioxidant enzyme concentrations in the rats' experimental groups that received Glibenclamide, STZ, and controls. Up until the trial's end, the mice administered streptozotocin showed a statistically significant reduction when compared to control animals (P<0.001). Following the injection of EJA extract, levels of catalase (CAT), superoxide dismutase (SOD), reduced glutathione, and glutathione peroxidase (GPx) all restored to nearly normal levels in a dose-dependent manner. The EJA extract significantly reduced the levels of antioxidant enzymes at doses of 100 mg/kg (p<0.05), 200 mg/kg (p<0.01), and 400 mg/kg (p<0.001). Group VII, which was administered with EJA extract, did not exhibit any noteworthy outcomes when compared to the control group.

	GST (U/Gram Protein)	Glutathione Peroxidase (U/Gram tissue)	CAT (U/Gram Protein)	SOD (units/mg protein)
Group I control	2.52 ± 0.04	5.2±0.1	2.50±0.3	72.5±3.9
Group II STZ (55 mg/kg)	0.48± 0.1	2.7±0.6	1.21±0.1	67.7±1.4
Group III STZ (55 mg/kg) + EJA (100 mg/kg)	0.77±0.1	3.6±0.2	1.53±0.1	52.5±1.5
Group IV STZ (55 mg/kg) + EJA (200 mg/kg)	0.84±0.2	3.9±0.4	2.0±0.3	57.4±2.6
Group V STZ (55 mg/kg) + EJA (400 mg/kg)	1.36±0.4	4.2±0.5	2.4±0.1	63.2±2.8
Group VI STZ (55 mg/kg) + Glibenclamide (5mg/kg)	2.65±0.8	4.6±0.2	1.6±0.3	67.5±3.6
Group VII EJA (400 mg/kg)	3.64±0.7	4.9±0.2	1.9±0.2	70.3±2.7

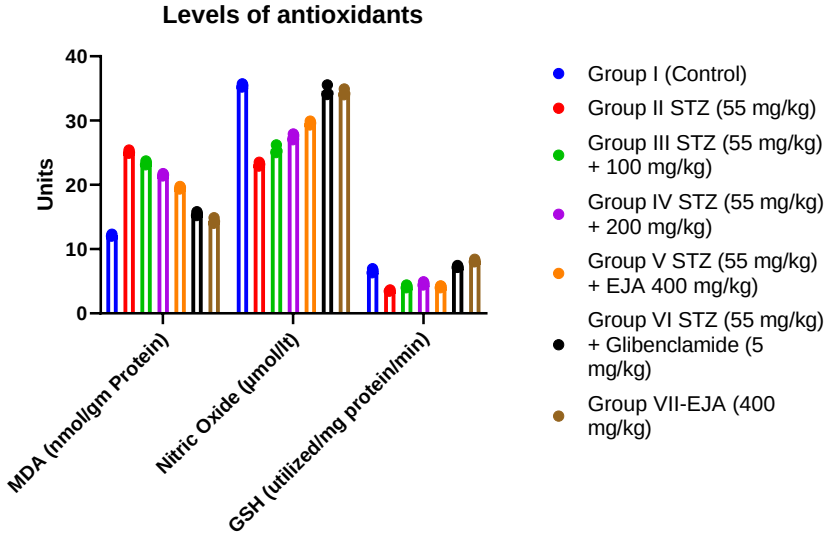
Table 4: The levels of GST, G Px, C A T and SOD of control, streptozotocin, EJA flower extract and Glibenclamide

Table 5: The levels of MDA, NO, and GSH of control, streptozotocin, and Glibenclamide treated animals
Table 5 shows the MDA, NO, and GSH concentrations in control, streptozotocin-, EJA flower-, and Glibenclamide-treated rats. While NO and GSH levels were decreased The MDA levels in the mice treated with Glibenclamide were found to increase (P<0.001) in these animals. The EJA extract markedly lowered MDA, NO, and GSH levels at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg (p<0.001), respectively. The EJA extract-treated group (group VII) did not show any discernible differences from the control group.

	MDA (nmol/gm Protein)	Nitric Oxide (µmol/lt)	GSH (utilized/mg protein/min)
Group I (Control)	12.2 ± 0.14	35.47 ± 0.25	6.66 ± 0.33
Group II STZ (55 mg/kg)	25.1 ± 0.27***	23.20 ± 0.3***	3.62 ± 0.15***
Group III STZ (55 mg/kg) + 100 mg/kg)	23.4 ± 0.3[a]	25.5 ± 0.62[a]	3.98±0.35[a]
Group IV STZ (55 mg/kg) + 200 mg/kg)	21.56 ± 0.19[b]	27.53 ± 0.47[b]	4.42±0.46[b]
Group V STZ (55 mg/kg) + EJA 400 mg/kg)	19.55±0.44[c]	29.6 ± 0.3[c]	4.15 ± 0.15[c]
Group VI STZ (55 mg/kg) + Glibenclamide (5 mg/kg)	15.50 ± 0.30	34.6 ± 0.82	7.27 ± 0.16
Group VII-EJA(400 mg/kg)	14.3 ± 0.45	34.38 ± 0.48	8.18 ± 0.8

Table5: The levels of MDA, NO and GSH of control, streptozotocin, EJA flower extract and Glibenclamide treated animals

Each value represents as mean ± SD for six animals in each group. Significant changes are calculated as * p<0.05, **p<0.01, ***p<0.001 compared to control group; [a] p<0.05, [b] p<0.01, [c] p<0.001 compared to group II.



Each value represents as mean ± SD for six animals in each group. Significant changes are calculated as * p<0.05, **p<0.01, ***p<0.001 compared to control group; a p<0.05, b p<0.01, c p<0.001 compared to group II.

Discussion

The most essential therapeutic agents available to humans to treat a wide range of human health issues have historically been plants with medicinal characteristics. Up until the advent of modern synthetic medicines, herbal treatments were still occasionally used to treat and manage medical conditions. According to Ogbonnia et al. (2008), the use of plant-based products declined from roughly the turn of the 20th century until the 1970s as a result of the introduction of synthetic medicines into the health care system, industrialization, development in the most developed countries, and adoption of western culture by developing countries.
According to Jabbar et al. (2014), the diabetic rat group had less body weight. This might be brought on by a delay in growth, which might be brought on by a blockage of glucose uptake resulting from a shortage of insulin and then treated with streptozotocin. After receiving thymoquinone treatment, the body weight of the impacted group dramatically rose. Compared to rats without diabetes, diabetic rats suffered a substantial loss in body weight, according to Eman et al. (2006).They mentioned that this loss may be due to inhibition of synthesis of DNA and RNA in the diabetic animals and/or it is recognized to diverse side effects on the capability to utilization of carbohydrates together with acidosis glycogenolysis and lipolysis. This outcome is generally due to damage of β -cells which may lead to rapid reduction in secretion of insulin. Thanaa and Ibrahiem (2016) revealed that diabetic rats grew less body weight and exhibited low feed effectiveness ratio as compared to control rats. Diets added with Nigella sativa enhanced body weight gain and improved feed efficiency ratio against the diabetes control group.

The glucose levels in the diabetic animals were higher. When diabetic rats produced by streptozotocin were matched with control rats, the treatment with EJA flower extract significantly affected their glycemic state. Compared to the non-diabetic control group and the group of animals given only EJA flower extract, all streptozotocin-induced diabetic rats displayed hyperglycemia four to five days after the streptozotocin therapy. The injection of 100, 200, and 400 mg/kg body weight of EJA flower extract significantly decreased fasting blood glucose levels as compared to the diabetic control group.
We measured the levels of serum insulin in experimental and control mice. The serum insulin levels of the streptozotocin-induced diabetic animals decreased when compared to control rats. However, insulin concentrations in the streptozotocin-treated animal group exhibited notable changes in rats given EJA in a dose-dependent manner. However, when comparing the serum insulin levels of the rats treated with EJA alone to the control group, no discernible alterations were found. HbA1c has commonly served as a sign of glycemic control. Sanjay Kumar Karan et al, (2013) demonstrated that Streblusasper and α-amyrin acetate, efficiently control glycemic condition as it is apparent from the considerably decreased level of glycosylated hemoglobin (HbA1c) in the treated rats.The animals in the experimental and control groups had their levels of antioxidant enzymes assessed. After the trial was over, animals given streptozotocin showed a statistically significant drop in antioxidant enzyme levels in comparison to control animals. A dose-dependent return to almost normal levels of glutathione peroxidase (GPx), reduced glutathione, catalase (CAT), and superoxide dismutase (SOD) was seen after the injection of ethanolic flower extract of EJA. The ethanolic flower extract of the EJA alone treated group did not exhibit any statistically substantial deviations from the control group in any of the measures.
Numerous antioxidant systems both enzymatic and non-enzymatic have been established by cellular systems, and these systems may work in concert to protect the organism from the harm that free radicals can do. Many antioxidant systems were depleted as diabetes mellitus progressed, including specific antioxidants like levels of vitamin C and E in the serum and plasma. In addition, a higher plasma oxi- disability was noted in the case of type 2 diabetes.

REFERENCES

1. Baliga, M. S., Bhat, H. P., Baliga, B. R. V., Wilson, R., &Palatty, P. L. (2011). Phytochemistry, traditional uses and pharmacology of Eugenia jambolana Lam.(black plum): a review. Food Research International, 44(7), 1776-1789.
2. Ayyanar, M., Subash-Babu, P., &Ignacimuthu, S. (2013). Syzygium cumini (L.) Skeels., a novel therapeutic agent for diabetes: folk medicinal and pharmacological evidences. Complementary Therapies in Medicine, 21(3), 232-243.
3. Arumugam, G., Manjula, P., &Paari, N. (2013). A review: Anti diabetic medicinal plants used for diabetes mellitus. Journal of Acute Disease, 2(3), 196-200.
4. Ayyanar, M., &Subash-Babu, P. (2012). Syzygium cumini (L.) Skeels: A review of its phytochemical

constituents and traditional uses. Asian Pacific journal of tropical biomedicine, 2(3), 240-246.
5. Yadav, M., Yadav, A., Kumar, S., &Yadav, J. P. (2016). Spatial and seasonal influences on culturableendophyticmycobiota associated with different tissues of Eugenia jambolana Lam. and their antibacterial activity against MDR strains. BMC microbiology, 16(1), 1-12.
6. Patil, R., Patil, R., Ahirwar, B., &Ahirwar, D. (2011). Current status of Indian medicinal plants with antidiabetic potential: a review. Asian Pacific Journal of Tropical Biomedicine, 1(2), S291-S298.
7. Dametto, A. C., Agustoni, D., Moreira, T. F., Plaza, C. V., Prieto, A. M., Silva, T. G., ... &Soares, C. P. (2017). Chemical composition and in vitro chemoprevention assessment of Eugenia jambolana Lam.(Myrtaceae) fruits and flowers. Journal of Functional Foods, 36, 490-502.
8. Sangeetha, S., Archit, R., &SathiaVelu, A. (2014). Phytochemical testing, antioxidant activity, HPTLC and FTIR analysis of antidiabetic plants Nigella sativa, Eugenia jambolana, Andrographispaniculata and Gymnemasylvestre. J. Biotechnol, 9, 1-9.
9. Jayaraju, K. J., &Ishaq, B. M. (2021). Evaluation of antidiabetic activity of a novel polyherbalpreparationagainst streptozotocin induced diabetes rat model. Journal of Pharmaceutical Research International, 33(26A), 42-54.
10. Akbar, S., & Akbar, S. (2020). Syzygium cumini (L.) Skeels (Myrtaceae) (Syns.: Eugenia cumini (L.) Druce; E. jambolana Lam.). Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications, 1715-1728.
11. Ogato, D. M., Mauti, E. M., Mauti, G. O., Kowanga, D. K., &Ouno, G. A. (2015). Antimicrobial activity of Eugenia jambolana seeds against foodborne isolates.
12. Singh, A., &Waldia, A. (2015). Acute toxicity study and antidiabetic activity of poly herbal hydro ethanolic extracts [phe-a, b, c, d and e] of individual plants in different ratios.
13. Ebaid, H., Al-Tamimi, J., Hassan, I., Alhazza, I., & Al-Khalifa, M. (2014). Antioxidant bioactivity of Samsum ant (Pachycondylasennaarensis) venom protects against CCL 4-induced nephrotoxicity in mice. Oxidative Medicine and Cellular Longevity, 2014.
14. Chiu, D. T., Stults, F. H., &Tappel, A. L. (1976). Purification and properties of rat lung soluble glutathione peroxidase. BiochimicaetBiophysicaActa (BBA)-Enzymology, 445(3), 558-566.
15. Shangari, N., & O'Brien, P. J. (2006). Catalase activity assays. Current protocols in toxicology, 27(1), 7-7.
16. Pabst, M. J., Habig, W. H., &Jakoby, W. B. (1974). Glutathione S-transferase A: a novel kinetic mechanism in which the major reaction pathway depends on substrate concentration. Journal of Biological Chemistry, 249(22), 7140-7148.
17. Jagetia, G. C., &Baliga, M. S. (2004). The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: a preliminary study. Journal of Medicinal Food, 7(3), 343-348.
18. Kumar, A., Khushboo, Pandey, R., & Sharma, B. (2020). Modulation of superoxide dismutase activity by mercury, lead, and arsenic. Biological trace element research, 196, 654-661.
19. Huang, Z., Liu, Y., Mao, Y., Chen, W., Xiao, Z., & Yu, Y. (2018). Relationship between glycatedhaemoglobin concentration and erythrocyte survival in type 2 diabetes mellitus determined by a modified carbon monoxide breath test. Journal of Breath Research, 12(2), 026004.
20. Younis, H. Y., Thanoon, I. A., Fadhil, N. N., &Merkhan, M. M. (2022). Effect of zinc as an add-on to metformin therapy on glycemic control, serum insulin, and c-peptide levels and insulin resistance in type 2 diabetes mellitus patient. Research Journal of Pharmacy and Technology, 15(3), 1184-1188.
21. Akhtar, M. F. (2021). Anti-Parkinson's evaluation of Brassica juncea leaf extract and underlying mechanism of its phytochemicals. Frontiers in bioscience-Landmark.
22. L. D. Devhare, A. P. Ghugare, B. P. Hatwar, D. C. Goupale. Method development for determination of water content from various materials by spectrophotometry and it's validation. International journal of drug delivery, 2015, 7(4), 233-240.
23. L. D. Devhare, P. K. Kore. A Recent Review On Bioavailability And Solubility Enhancement Of Poorly Soluble Drugs By Physical And Chemical Modifications. Research chronicle in health sciences. 2016, 2(5), 299-308.
24. T. U. Tonde, R. H. Kasliwal, L. D. Devhare. Quantitative estimation of bacoside a in polyherbal memory enhancer syrup for memory boosting activity using hptlc method. Research chronicle in health sciences. 2016, 2(6), 315-320.
25. A. P. Ghugare, L. D. Devhare, B. P. Hatwar. Development and validation of analytical methods for the simultaneous estimation of Nimorazole and Ofloxacin in tablet dosage form. International journal of drug delivery. 2016,8(3), 96-98.

26. Harshal. G. Salpe, L. D. Devhare, Anjali Ghugre, & Navjot Singh. Formulation and evaluation of hpmc coated diltiazem hcl tablet and its comparison with other marketed preparation. Research chronicle in health sciences. 2016, 3(1), 11-17.
27. A. A. Makhani and L. D. Devhare. Development and validation of vierordt's spectrophotometric method for simultaneous estimation of drotaverine and nimesulide combination. Research chronicle in health sciences. 2017, 3(2), 22-28.
28. A. A. Makhani and L. D. Devhare. Development and validation of analytical methods for drotaverine and nimesulide combination. Research chronicle in health sciences. 2017, 3(3), 40-44.
29. Gayatri Katole, L. D. Devhare et al. Recent insight into some emerging natural resources with remarkable hepatoprotective potentials. International journal of pharmaceutical science and research. 2020, 5(1),41-47.
30. Vaibhav P Uplanchiwar, Sushil Yadaorao Raut, Lalchand D Devhare, et al. Pharmacological assessment of antiulcer activity of gloriosa superba linn tubers in experimentally induced gastric ulcers. Journal of medical pharmaceutical and allied science. 2021, 10(3), 2852-2856.
31. Lalchand D. Devhare and Niharika Gokhale. Acid Neutralizing capacity and antimicrobial potential of selected solvent extract from various indigenous plant. Journal of Advanced Scientific Research (JASR). 2021, 12(4), 175-179.
32. Lalchand D. Devhare and Niharika Gokhale. Antioxidant and antiulcer property of different solvent extracts of cassia tora linn. Research journal of pharmacy and technology. 2022, 15(3), 1109-1113.
33. Lalchand D. Devhare and Niharika Gokhale. In silico anti-ulcerative activity evaluation of some bioactive compound from cassia tora and butea monosperma through moleculer docking approach. International journal of pharmaceutical sciences and research. 2023, 14(2), 1000-08.
34. Lalchand D. Devhare and Niharika Gokhale. A brief review on: phytochemical and antiulcer properties of plants (fabaceae family) used by tribal people of gadchiroli maharashtra. International journal of pharmaceutical sciences and research. 2023, 14(4), 1572-1593
35. Nikam Nikita R, Akotkar Vaishnavi, Devhare Lalchand, et al. Parenteral drug delivery approach: an overview. Journal of xidian university. 2023, 17(1), 386-400.
36. Shubham M. Shende , Pooja Bhandare, Lalchand Devhare, et al. In-vitro: micropropagation of mint and investigate the antibacterial activity of mint extract. Eur. Chem. Bull. 2023, 12(Special Issue 5), 780 – 784
37. Shishupal S. Bodhankar, Lalchand D.Devhare, et al. Formulation and in vitro evaluation of dental gel containing ethanglic extract of Mimosa Pudica. Eur. Chem. Bull. 2023, 12(5), 1293-1299
38. Lalchand D. Devhare, Shishupal S. Bodhankar, et al. Important role of food and nutritional security during Covid-19: A survey. Eur. Chem. Bull. 2023, 12(5), 1363-1374
39. Neha R. Pathak, Lalchand D. Devhare, et al. Aclinial reveiew on pharmacological evaluation of Thiazolidine and Isatin in the new millenium as magic moieties. Eur. Chem. Bull. 2023, 12(5), 3410-3417.
40. Shailja Singh, Kimee Hiuna Minj, Lalchand D Devhare, et al. An update on morphology, mechanism, lethality, and management of dhatura poisoning. Eur. Chem. Bull. 2023, 12(5), 3418-3426.
41. Pravin B. Suruse, B. A. Jadhav, Lokesh G. Barde, Lalchand D Devhare et al. Exploring the potential of Aerva Lanata extract in a herbal ointment for fungal infection treatment. Journal of Survey in Fisheries Sciences. 2023, 10(1), 1922-1932
42. Shubham Shende, Bhagyashri Meshram, Heera Karemore, Lalchand D Devhare. et al. Development And Characterization of Glycerogelatin Suppositories For Enhanced Efficacy. European Journal of Pharmaceutical and Medical Research. 2023, 10(6), 522-528
43. Vinod M. Thakare, Shraddha A. Umare, Lalchand D Devhare et al. Separation and purification of carboxymethyl cellulose from Spinacia Oleracea for use in pharmaceutical dosage form. Eur. Chem. Bull. 2023, 12(5), 4062-4080.
44. Pravin B. Suruse, Aakansha P. Deshmukh, Lokesh G. Barde, Lalchand D Devhare et al. Rimegepant-embedded fast dissolving films: A novel approach for enhanced migraine relief. Journal of Survey in Fisheries Sciences. 2023, 10(1), 2071-2084
45. Mahesh Prasad, Abhishek Suman, Swati Srivastava, Lalchand D Devhare et al. Butea monosperma stem bark extract partially reverses high fat diet-induced obesity in rats. Eur. Chem. Bull. 2023, 12(5), 4267 - 4273
46. Akshaykumar B Kadam, Lalchand D Devhare, Smita T Kumbhar, et al. In-Silico Molecular Docking Study of Substituted Imidazo 1,3,4 Thiadiazole Derivatives: Synthesis, Characterization, And Investigation of Their Anti-Cancer Activity. Journal of Coastal Life Medicine. 2023, 2(11), 1237-1245.

47. Mamta Shukla, Shital A Tiware, Sanjeevani R Desai, Lalchand D Devhare. et al. Pharmacological Evaluation of Gloriosa Superba Linn Flower Extract For Antiulcer Activity. Journal of Survey in Fisheries Sciences. 2023, 10(2) 463-470.
48. Papagatla Polireddy, Vedanshu Malviya, Lalchand D Devhare. et al. Assessment of Hepatoprotective Potential of Ecbolium Linneanum Extract on Experimental Animals. Journal of Coastal Life Medicine. 2023, 2(11) 884-890
49. Ruchi Tiwari, Juhi Mishra, Lalchand Devhare, Gaurav Tiwari. An updated review on recent developments and applications of fish collagen. Pharma Times. 2023, 55(6), 28-36
50. Konda VVS Krishna, Pushpendra Kumar Jain, Lalchand D Devhare. et al. A Study on Antidiabetic Potential of Dried Fruits Extract of Eucalyptus Globulus in Experimental Animals. Journal of Biomedical Engineering. 2023, 40(3), 99-110
51. Tiwari, G., Gupta, M., Devhare, L. D., & Tiwari, R. (2023). Therapeutic and Phytochemical Properties of Thymoquinone Derived from Nigella Sativa. Current Drug Research Reviews.
52. Anil K. Adimulapu, Lalchand D. Devhare, Anasuya Patil, Nilesh O. Chachda, G. Dharmamoorthy(2023). Design and Development of Novel Mini Tablet Cap Technology for the Treatment of Cardiovascular Diseases. International Journal of Drug Delivery.Technology. 2023;13(3):801-806
53. Chawla A, Devhare LD, Dharmamoorthy G, Ritika, Tyagi S. Synthesis and In-vivo Anticancer Evaluation of N-(4-oxo-2- (4-((5-aryl-1,3,4 thiadiazole-2yl) amino) Phenyl thiazolidine-3-yl) Benzamide derivative. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):470-474.
54. Gnana RPM, Devhare LD, Dharmamoorthy G, Khairnar MV, Prasidha R. Synthesis, Characterisation, Molecular Docking Studies and Biological Evaluation of Novel Benzothiazole Derivatives as EGFR Inhibitors for Anti-breast Cancer Agents. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):475-480.
55. Sonule M, Devhare LD, Babu MN, Gunjal SD, Varalaxmi S. Microemulgel-based Hydrogel of Diclofenac Sodium using Lipidium sativum as a Gelling Agent. International Journal of Drug Delivery Technology. 2023;13(4):1235-1239.
56. Shriram BK, Devhare LD, Mehrotra A, Deokar SS, Singh SP. Formulation and Evaluation of Mosquito Repellent Stick. International Journal of Drug Delivery Technology. 2023;13(4):1283-1286.