International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319-6718, ISSN (Print): 2319- 670X
www.ijpsi.org Volume 12 Issue 3 ǁ May –June. 2023 ǁ PP. 01-14

Evaluation of Antidiabetic Activity of Flowers of *Gaillardia Pulchella* in Alloxan Induced Diabetes in Wistar Rats

Tushar Jadhav*
[1]*Anuradha College of Pharmacy, Chikhli, Maharashtra, India.*
B.Pharm
M.Pharm Student
Department of Pharmacology, Anuradha Colllege of Pharmacy, Chikhli-443201, Dist. Bulldhana. (MS)
Corresponding Author: Dr. G.V. Bihani, Dr. K.R.Biyani

ABSTRACT*: This study aimed at Antidiabetic activity of flowers of Gaillardia Pulchella was evaluation in alloxan induced diabetes in Wistar rats. The hypoglycemic activity of ethanolic (70%) extract of flowers of Gaillardia pulchella was evaluated at different dosage of 100 mg/kg and 200 mg/kg. Administration of this extract caused significantly increased body weight, decreased blood glucose levels, increased glucose tolerance, improved hematological parameters and improved lipid metabolism in diabetic rats.These are indications of antidiabetic property of ethanolic extract of flowers of Gaillardia pulchella proving hypoglycemic action by comparing favorably well with Glibenclamide, a standard hypoglycemic drug. The ethanolic extract of flowers of Gaillardia Pulchella restored liver function and haematological parameters to normal control levels in diabetic rats. The investigational process and results showed in addition to its hypoglycemic activity Gaillardia Pulchella may also protect the blood against impairment due to diabetes. But, some function of kidney may be compromised at 200 mg/kg of dose.*

KEYWORDS: *Diabetes, Alloxan induced Diabetes, Oral glucose tolerance, Blood glucose levels, kidney function, Gaillardia Pulchella, ethanolic extract.*

Date of Submission: 01-05-2023 Date of acceptance: 12-05-2023

I. INTRODUCTION

Diabetes mellitus (DM), characterized by hyperglycemia is the world's largest metabolic disorder. "Mellitus" which means "from honey" was included by the Briton John Rolle in late 1700s, which separates it from Diabetes Insipidus, which occurs when body cannot regulate how to handles fluids.

This condition is caused by pituitary hormone Vasopressin abnormality and does not relate to Diabetes. Diabetes mellitus is a fast growing global problem. Estimated people suffering from the disease are up to 430 million. Diabetes mellitus is a chronic metabolic disease that is caused by partial or complete insulin deficiency or insulin resistance. It is usually accompanied by renal failure, coronary artery disease, blurred vision, neuropathy and impaired wound healing that may predispose to limb amputation. These diabetes complications are attributed to hyperglycemia, hyperlipidemia and oxidative stress which often characterize diabetes mellitus. Type 1 diabetes mellitus: It appears when more than 90 percent of beta cells of pancreas are destroyed by an autoimmune process. The onset is acute and the peak incidence is around fifteen years. In type 1 diabetes mellitus, there is insulin deficiency. Insulin is essential with type 1 DM all patients. Type 2 diabetes mellitus: Genetic influence is much more powerful in type 2 DM. It is the most common form of diabetes. Overeating, obesity, under activity and ageing are the main risk factors. Type 2 DM associates with increased hepatic glucose and resistance of target tissues to the action of insulin.

II. MATERIALS AND METHOD

Animals:-

Animals were housed under standard laboratory conditions of temperature 25 ± 1°C with free access to food and water. The experiments were performed during the light cycle (12 12 h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee. Proposal no. **751/PO/Re/S/03/CPCSEA/2023/1-4.**

Plant Material:-
The *Gaillardia pulchella* plant was collected from the available source and authenticated by botanist of an institute's botany department.

Extraction:-
The fresh flowers of *Gaillardia pulchella* was shade dried and coarsely powdered. The powder was filled in to filter paper bag and placed in the Soxhlet apparatus for extraction.

The Soxhlet apparatus was connected to round bottom flask which was filled by ethanol (70%) solvent and water bath to maintain temperature. The ethanol was boiled for over a period to obtain the extract which was further stored at room temperature.

Preliminary Phytochemical Evaluation of *Gaillardia Pulchella* Flowers Extract

Test for saponins
A. Foam test - Drug extract or dry powder was wobbled vigorously with water. If persistent foam observed, shows presence of saponins**.**
B. Haemolytic test -Added drug extract or dry powder to one drop of blood placed on glass slide. If hemolytic zone is found indicates presence of saponins.
Test for alkaloids
1) **Dragendorff's test: -** The extract was treated with Dragendorff's reagent (potassium bismuth iodine solution), formation of reddish brown precipitate indicates the presence of alkaloids.
2) **Mayer's test: -** The extract was treated with Mayer's reagent (potassium mercuric iodide solution) formation of creamy colour precipitate, indicate the presence of alkaloids**.**
3) **Wagner's test: -** The extract was treated with Wagner's reagent (iodine potassium iodide solution) reddish brown precipitate indicated the presence of alkaloids.
4) **Hager's test**: - The extract was treated with Hager's reagent (saturated solution of picric acid) formation of yellow precipitate indicated the presence of alkaloids.
Test for flavonoids
1) **Shinoda test: -** to the extract was add few magnesium turnings and concentrated Hcl drop wise , pink scarlet ,crimson red, or occasionally green to blue appears after few Minutes.
2) **Alkaline reagent test: -** to the extract was add few drops of NaOH solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicated the presence of flavonoids
3) **Zinchydrochloride test: -** to the extract add a mixture of zinc dust and concentrated Hcl. It gives red colour after few minutes**.**
Test for phenols
2ml of extract was added to 2ml of ferric chloride solution a deep bluish green colour indicated the presence of phenol**.**
Test for Triterpenoids
1) Libermann-Burchard test: - treated the extract with few drops of acetic anhydride, boiled and cooled the extract, then added 1ml of conc. H_2SO_4 along the side of test tube. Formation of deep red colour ring indicated the presence of triterpenoids**.**
2) Sulfur powder test: - to the extract small amounts of Sulphur powder was added, it sinks at the bottom, indicate the presence of triterpenoids
Test for lignins
Extract was treated with concentrated HCL and phloroglucinol solution, pink colour is indicate the presence of lignin.
Thionine test for lignin
Extract was treated with thionine solution, after 15 min. wash with alcohol, bluish violet colour is formed.

Experimental Design for Anti-Diabetic Activity:-
For Anti-diabetic study, the fasted diabetic rats were divided in to 5 groups of 6 animals each.
· Group I- Non diabetic control group: This group received 0.5ml of NS daily for 30 days.
· Group II- Diabetic control group: This group received 0.5ml of NS daily for 30 days.
· Group III - Standard control: This group received glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.
· Group IV - Diabetic test group I: Received 100mg/kg of ethanolic extract of flowers of *Gaillardia pulchella* daily for 30 days.
· Group V- Diabetic test group II: Received 200mg/kg of ethanolic extract of flowers of *Gaillardia pulchella* daily for 30 days.

Method of Collection of Blood:-
Blood sample for glucose estimation was collected from rat tail vein. Fasting blood glucose readings were recorded in all rats after an overnight fasting. Blood samples were obtained from rat tail vein, after applying xylene to make vein prominent. Blood glucose was estimated by a glucometer.

ALLOXAN INDUCED DIABETES MODEL:-
Prepared a 2% solution of Alloxan monohydrate in 0.9% normal saline.
Ensured the solution is freshly prepared before injection.
Selected the rats for the study and make sure they were kept fasting overnight before the injection.
Injected Alloxan monohydrate solution into the rats intra-peritoneally at a dose of 150mg/kg body weight.
Observed the rats for 24-48 hours for any signs of allergic reactions, behavioral changes, and convulsions.
Measured fasting blood glucose levels daily around 9:30 AM until stable hyperglycemia was established (FBS>200mg/dl).
Selected the rats that developed stable hyperglycemia for the anti-diabetic study.

Body weight, water intake, and food intake:-
Body weight, water intake, and food intake were measured as additional evaluation parameters for this study. Body weight was measured at the beginning of the study and end of the study for all groups and the change in body weight was noted. Water intake and food intake were measured daily for all groups. The data obtained was analyzed to determine any changes water intake, and food intake, which may indicate the overall health of the rats and the effect of *Gaillardia pulchella* extract on their metabolism.

Blood glucose levels:-
Blood glucose levels were measured using a glucometer as the evaluation parameter for this study. Glucometers were portable devices that measured glucose levels in whole blood and were commonly used for self-monitoring by diabetic patients. Blood samples were collected by pricking the tail vein of the rats and measuring glucose levels using a glucometer.
Fasting blood glucose levels were measured at around 9:30 AM daily until stable hyperglycemia was established. Blood glucose levels were also measured on days 0, 10, 20 and 30. of the study for all groups. The data obtained was analyzed to determine the changes in blood glucose levels and evaluate the anti-diabetic activity of *Gaillardia pulchella* extract.

Serum Lipid Profile:-
Serum Lipid Profile was measured as an additional evaluation parameter for this study. Blood samples were collected at the end of the study 30th day, and serum cholesterol, triglycerides, HDL, and LDL levels were measured using spectrophotometer of pathological laboratories. The data obtained was analyzed to determine any changes in Serum lipid profile, whichmay indicate the effect of *Gaillardia pulchella* extract on lipid metabolism.

Oral glucose tolerance test:-
An oral glucose tolerance test was performed as an additional evaluation parameter for this study. On day 30, the rats in groups 1 to 6 were given glucose (2 g/kg body weight saline solution); 30 min after administration of the extract/drug. Blood samples were collected from the tail vein prior to glucose administration and at 30, 60, and 90min after glucose loading for immediate measurement of blood glucose levels.

Kidney function parameters:-
Kidney function parameters were measured as an additional evaluation parameter for this study. Blood samples were collected at the end of the study 30th day, and serum levels of creatinine and blood urea, uric acid were measured using spectrophotometer of pathological laboratories. The data obtained was analyzed to determine any changes in kidney function parameters, which may indicate the effect of *Gaillardia pulchella* extract on renal function.

Haematological parameters:-
Haematological parameters were measured as an additional evaluation parameter for this study. Blood samples were collected at the end of the study 30th day, and complete blood count (CBC) was performed using an automated hematology analyzer. The CBC included the measurement of hemoglobin (Hb) level, red blood cell (RBC) count, white blood cell (WBC) count, platelet count, and other parameters. The data obtained was analyzed to determine any changes in haematological parameters, which may indicate the effect of *Gaillardia pulchella* extract on blood cells and blood-forming tissues.

III. RESULTS:-

Phytochemical investigation:

Table 01. The phytochemical investigation for various chemical constituents in ethanolic extract of flowers of *Gaillardia Pulchella* is given below.

Chemical constituents	Name of the test	Procedure	Observation	Inference
Flavonoids	Shinoda test	2-3 ml of Extract + few drops of cone. HCI+0.5 gm magnesium turnings	Slight pink color	Flavonoids present
Flavonoids	Lead acetate test	2-3 ml of Extract lead acetate	Yellow color ppt	Flavonoids present
Flavonoids	NaOH test	2-3 ml of Extract + increasing amt. of NaOH	Yellow color which decolourized on addition of acid	Flavonoids present
Tannins & phenolic comp.	$FeCl_3$ test	2-3 ml of extract+5% $FeCl_3$ solution	Deep blue color	Tannins & phenolic comp. present
Tannins & phenolic comp.	HNO_3TEST	2-3 ml of Extract +Dil. HNO_3	Reddish yellow color	Tannins & phenolic comp. present
Tannins & phenolic comp.	Acetic acid test	2-3 ml of Extract+ Acetic acid solution	Red color	Tannins & phenolic comp. present
Tannins & phenolic comp.	$KMnO_4$	2-3 ml of Extract+ $KMnO_4$ solution	Decolourisation $KMnO_4$	Tannins & phenolic comp. present
Alkaloids	Mayer's test	2-3 ml of Extract+ Mayer's solution	Cream color ppt	Alkaloids present
Alkaloids	Dragondroff's test	2-3 ml of Extract+ Dragondroff's reagent	Orange color ppt	Alkaloids present
Alkaloids	Wagner's test	2-3 ml of Extract+ Wagner's reagent	Reddish brown ppt	Alkaloids present
Alkaloids	Hager's test	2-3 ml of Extract+ Hager's reagent	Yellow color ppt	Alkaloids present

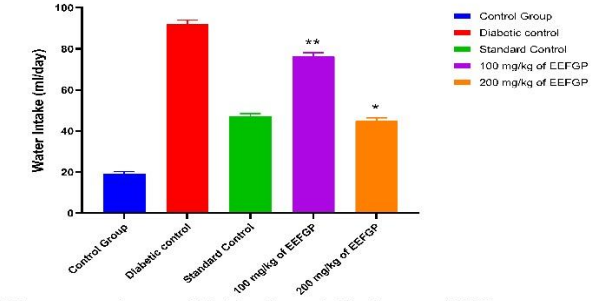
Water Intake:-

While water consumption increased in the untreated diabetic rats (Group 2), the administration of ethanolic extract of flowers of *Gaillardia Pulchella* significantly reduced the quantity of water intake in diabetic animals.

Table 02. Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on water intake. (n = 6, mean ± SEM).

Group	Treatment	Water intake (ml/day)
Group I [Non diabetic control group]	0.5ml of NS daily for 30 days.	19.09 ± 1.08
Group II [Diabetic control group]	0.5ml of NS daily for 30 days.	91.90 ± 2.12
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.	47.05 ± 1.48
Group IV [Diabetic test group I]	100mg/kg ethanolic extract of flowers of *Gaillardia pulchella* daily for 30 days.	76.55 ± 1.53**
Group V [Diabetic test group II]	200mg/kg ethanolic extract of flowers of *Gaillardia pulchella* daily for 30 days	44.94 ± 1.49*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at $^{**}p < 0.01$ and $^{*}p < 0.05$



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

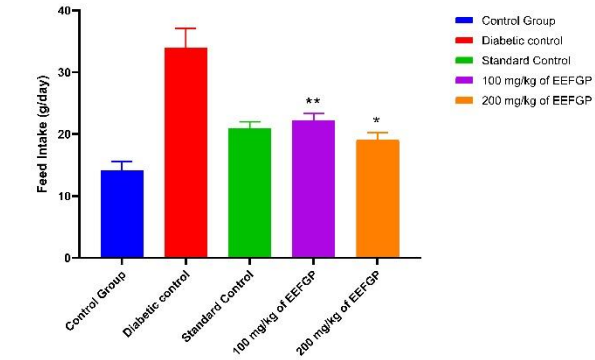
Feed Intake:-

While food consumption increased in the untreated diabetic rats (Group 2), the administration of ethanolic extract of flowers of *Gaillardia Pulchella* significantly reduced the quantity of feed intake in diabetic animals. Similarly, the untreated diabetic rats showed polyphagic condition and consumed higher quantity of feed compared to the control and treatment groups.

Table 03. Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on feed intake. (n = 6, mean ± SEM).

Group	Treatment	Feed intake (g/day)
Group I [Non diabetic control group]	0.5ml of NS daily for 30 days.	14.08 ± 1.48
Group II [Diabetic control group]	0.5ml of NS daily for 30 days.	33.92 ± 3.13
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.	20.98 ± 1.02
Group IV [Diabetic test group I]	100mg/kg ethanolic extract of flowers of *Gaillardia pulchella* daily for 30 days.	22.24 ± 1.06**
Group V [Diabetic test group II]	200mg/kg ethanolic extract of flowers of *Gaillardia pulchella* daily for 30 days	19.05 ± 1.18*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

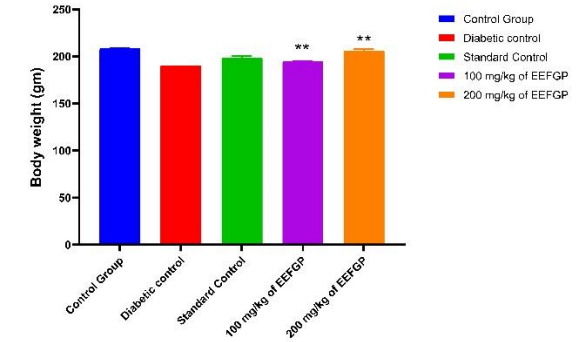
Body Weight:-

The ethanolic extract of flowers of *Gaillardia Pulchella* significantly increased the body weight of diabetic animal at higher dose. Generally, body weights are reduced in diabetic animals, but in this study, the decrease in body weight was diminished by the extract treatment.

Table 04. Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on Body Weight. (n = 6, mean ± SEM).

Group	Treatment	Body Weight (g)
Group I [Non diabetic control group]	0.5ml of NS daily for 30 days.	208.4 ± 0.8
Group II [Diabetic control group]	0.5ml of NS daily for 30 days.	190.4 ± 0.02
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.	198.9 ± 1.8
Group IV [Diabetic test group I]	100mg/kg ethanolic extract of flowers of *Gaillardia pulchella* daily for 30 days.	194.6 ± 0.8**
Group V [Diabetic test group II]	200mg/kg ethanolic extract of flowers of *Gaillardia pulchella* daily for 30 days	206.3 ± 1.6**

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01

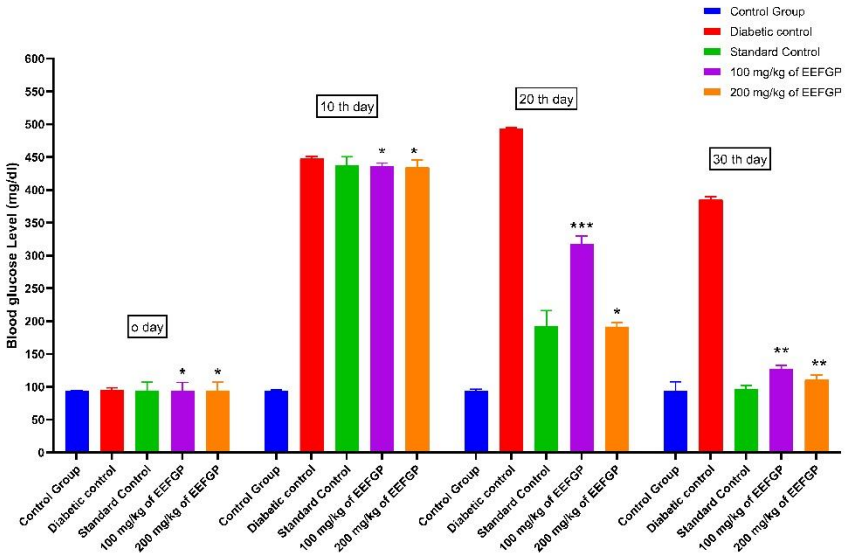
Blood Glucose Levels:-

The continuous administration of ethanolic extract of flowers of *Gaillardia Pulchella* was found to significantly reduce the blood glucose level in diabetic rats at the end of the experiment. Again, the effect was more pronounced in the rats treated with 200mg/kg of the extract and it compared favorably well with Glibenclamide treated rats.

Table 05. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on blood glucose levels In Alloxan Induced Diabetic Wistar Rats. (n = 6, mean ± SEM).

Group	Treatment	0 th day (mg/dl)	10th day (mg/dl)	20 th day (mg/dl)	30 th day (mg/dl)
Group I [Non diabetic control group]	0.5ml of NS daily for 30 days.	94.1 ± 0.5	94.5 ± 1.1	94.5 ± 1.6	94.3 ± 13.5
Group II [Diabetic control group]	0.5ml of NS daily for 30 days.	94.7 ± 3.8	448 ± 2.7	493 ± 2.2	386.1 ± 3.2
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.	94.5 ± 12.4	437.4 ± 13	192.6 ± 23.4	97.2 ± 4.3
Group IV [Diabetic test group I]	100mg/kg EEFGP daily for 30 days.	94.3 ± 12.4*	435.6 ± 5.4*	318 ± 11.3***	127.8 ± 4.9**
Group V [Diabetic test group II]	200mg/kg EEFGP daily for 30 days	94.5 ± 12.4*	435 ± 10.8*	191.7 ± 6.5*	111.1 ± 7**

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at ***p < 0.001, **p < 0.01 and *p < 0.05



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at ***p < 0.001, **p < 0.01 and *p < 0.05

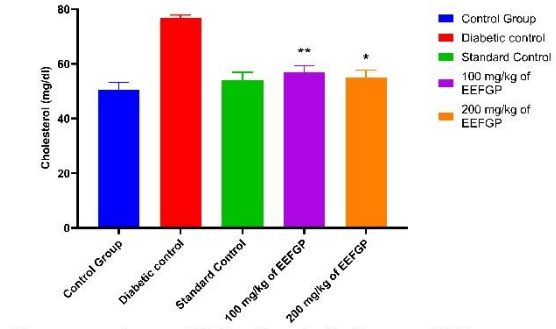
Serum Lipid Profile:-

There was significant elevation in the levels of serum cholesterol, triglycerides, and LDL and reduced HDL in diabetic rats when compared with the control group. The extract of *Gaillardia Pulchella* and glibenclamide significantly reduced the levels of serum cholesterol, triglycerides, and LDL and increased HDL to near normalcy as observed in the control after 30 days of treatment.

Table 06. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on Cholesterol In Alloxan Induced Diabetic Wistar Rats. (n =6, mean ± SEM).

Group	Treatment	Cholesterol (mg/dl)
Group I [Non diabetic control group]	0.5ml of NS daily for 30 days.	50.40 ± 2.80
Group II [Diabetic control group]	0.5ml of NS daily for 30 days.	76.63 ± 1.21
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.	53.96 ± 2.90
Group IV [Diabetic test group I]	100mg/kg EEFGP daily for 30 days.	56.86 ± 2.41**
Group V [Diabetic test group II]	200mg/kg EEFGP daily for 30 days	54.95 ± 2.80*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

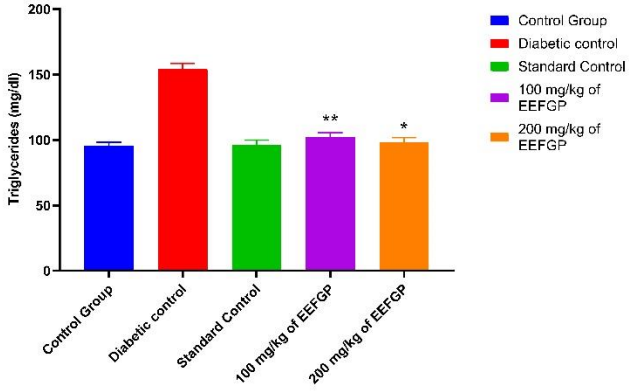


Values were expressed as mean + SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

Table 07. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on Triglycerides In Alloxan Induced Diabetic Wistar Rats. (n =6, mean ± SEM).

Group	**Treatment**	**Triglycerides (mg/dl)**
Group I [Non diabetic control group]	0.5ml of NS daily for 30 days.	95.72 ± 2.50
Group II [Diabetic control group]	0.5ml of NS daily for 30 days.	154.3 ± 4.32
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.	96.20 ± 3.68
Group IV [Diabetic test group I]	100mg/kg EEFGP daily for 30 days.	101.89 ± 3.44**
Group V [Diabetic test group II]	200mg/kg EEFGP daily for 30 days	98.30 ± 3.56*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05



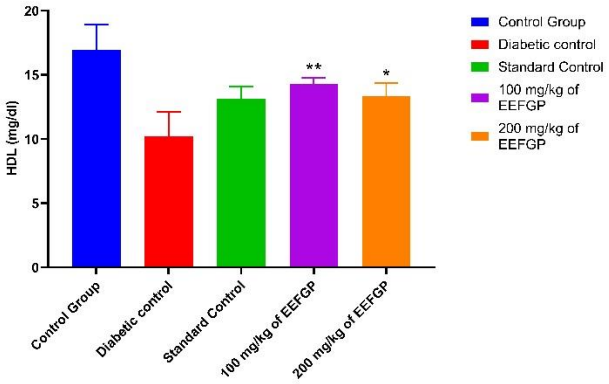
Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

Table 08. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on HDL In Alloxan Induced Diabetic Wistar Rats. (n =6, mean ± SEM).

Group	**Treatment**	**HDL (mg/dl)**
Group I [Non diabetic control group]	0.5ml of NS daily for 30 days.	16.93 ± 1.98
Group II [Diabetic control group]	0.5ml of NS daily for 30 days.	10.23 ± 1.9
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.	13.11 ± 0.99
Group IV [Diabetic test group I]	100mg/kg EEFGP daily for 30 days.	14.28 ± 0.48**

Group V [Diabetic test group II]	200mg/kg EEFGP daily for 30 days	13.36 ± 1*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

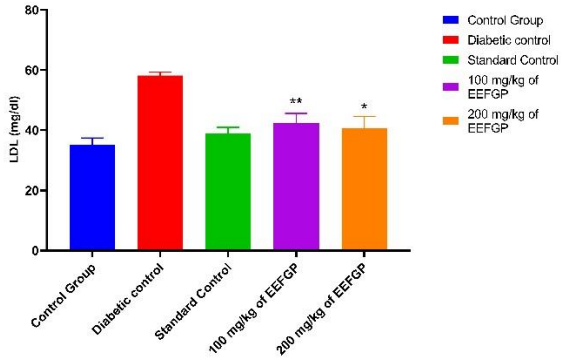


Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

Table 09. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on LDL In Alloxan Induced Diabetic Wistar Rats. (*n* =6, mean ± SEM)

Group	**Treatment**	**LDL (mg/dl)**
Group I [Non diabetic control group]	0.5ml of NS daily for 30 days.	35.19 ± 2.20
Group II [Diabetic control group]	0.5ml of NS daily for 30 days.	58.18 ± 1.14
Group III [Standard control]	Glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.	38.96 ± 1.98
Group IV [Diabetic test group I]	100mg/kg EEFGP daily for 30 days.	42.32 ± 3.18**
Group V [Diabetic test group II]	200mg/kg EEFGP daily for 30 days	40.66 ± 3.92*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

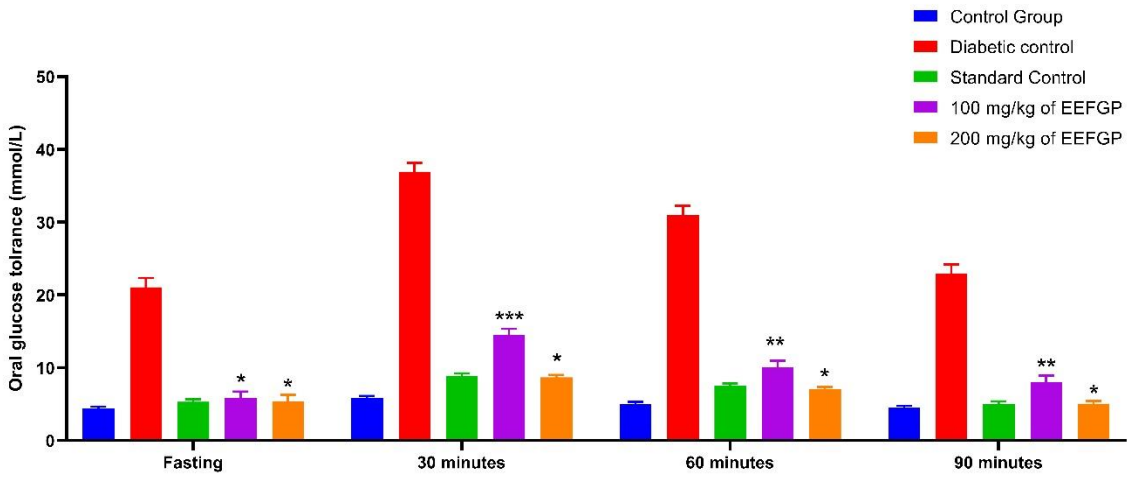
ORAL GLUCOSE TOLERENCE TEST:

Shows the blood glucose levels of the rats after oral administration of glucose. The level in the control rats rose to the peak 30 min after glucose load and decreased to near normal levels at 90min. In the untreated diabetic rats, the peak increase in blood glucose concentration was observed after 30 min and remained high over the next 60 min. *Gaillardia Pulchella* and glibenclamide-treated diabetic rats showed significant decrease in blood glucose concentration at 60 and 90 min compared with diabetic control rats.

Table 10. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on blood sugar levels in glucose loaded diabetic Wistar rats. (OGTT) (n =6, mean ± SEM).

Group	**Fasting (mmol/L)**	**30 minutes (mmol/L)**	**60 minutes (mmol/L)**	**90 minutes (mmol/L)**
Group I [Non diabetic control group]	4.44 ± 0.23	5.85 ± 0.27	5.03 ± 0.27	4.53 ± 0.24
Group II [Diabetic control group]	21.06 ± 1.27	36.92 ± 1.23	30.94 ± 1.29	22.93 ± 1.24
Group III [Standard control] glibenclamide 0.5mg/kg	5.31 ± 0.36	8.91 ± 0.33	7.50 ± 0.34	5.02 ± 0.36
Group IV [Diabetic test group I] 100mg/kg EEFGP	5.90 ± 0.82*	14.52 ± 0.84***	10.05 ± 0.90**	8.06 ± 0.84**
Group V [Diabetic test group II] 200mg/kg EEFGP	5.40 ± 0.86*	8.67 ± 0.34*	7.02 ± 0.32*	5.06 ± 0.34*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at ***p < 0.001, **p < 0.01 and *p < 0.05



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at ***p < 0.001, **p < 0.01 and *p < 0.05

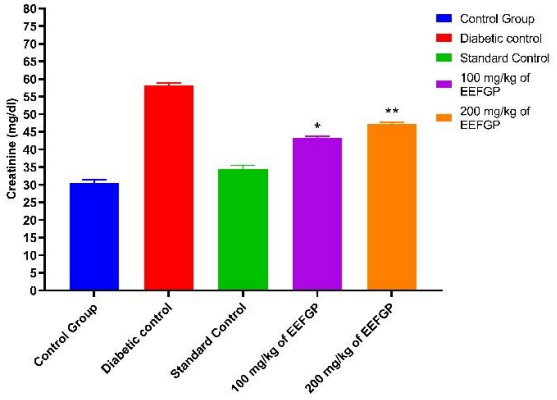
KIDNEY FUNCTION PARAMETERS:

A significant increase was observed in all the kidney function parameters examined in the untreated diabetic rats when compared with the control. The ethanolic extract of this herb had a positive impact on the kidney function reducing creatinine, urea and uric acid.

Table 11. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on creatinine of diabetic Wistar rats (n = 6 ± SEM).

Group	**Group I**	**Group II**	**Group III**	***Gaillardia pulchella* extract**	
	Control group	**Diabetic group**	**Standard control**	**100mg/kg**	**200mg/kg**
Creatinine (mg/dL)	30.52 ± 0.85	58.25 ± 0.60	34.49 ±1.02	43.35 ± 0.48*	47.28 ± 0.38**

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

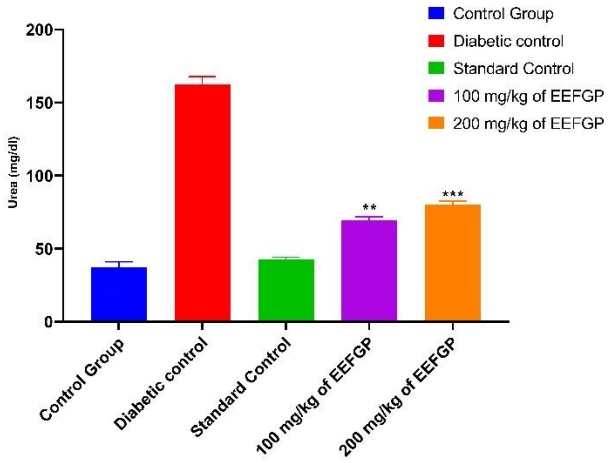


Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

Table 12. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on Urea of diabetic Wistar rats (n = 6 ± SEM)

Group	**Group I** **Control group**	**Group II** **Diabetic group**	**Group III**	***Gaillardia pulchella* extract**	
			Standard control	**100mg/kg**	**200mg/kg**
Urea (mg/dL)	37.42 ± 3.60	162.32 ± 5.30	42.72 ±1.35	69.60 ± 2.07**	80.29 ± 2.29***

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at ***p < 0.001 and **p < 0.01

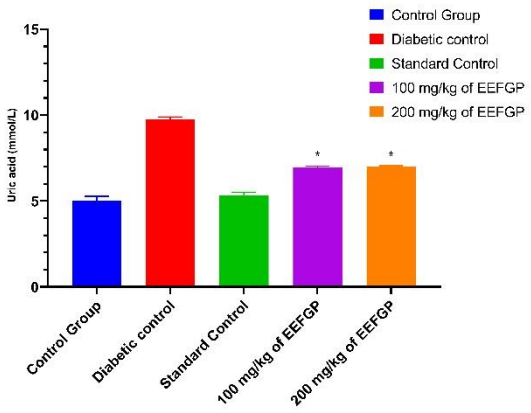


Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at ***p < 0.001 and **p < 0.01

Table 13. Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on Uric Acid of diabetic Wistar rats (n = 6 ± SEM).

Group	**Group I** **Control group**	**Group II** **Diabetic group**	**Group III**	***Gaillardia pulchella* extract**	
			Standard control	**100mg/kg**	**200mg/kg**
Uric acid (mmol/L)	5.04 ± 0.23	9.75 ± 0.14	5.32 ±0.19	6.93 ± 0.09*	6.98 ± 0.08*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at *p < 0.05



Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at *p < 0.05

HEMATOLOGICAL PARAMETERS:

In addition, the diabetic rats exhibited significantly reduced levels in all the haematological parameters with the exception of white blood cell count and lymphocytes which were significantly increased. Oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* in diabetic rats for 30 days, however, restored the haematological parameters to normalcy with the exception of platelets and neutrophils which were significantly increased but not to the control levels.

Table 14. Effect of *Gaillardia Pulchella* extract on some haematological parameters of diabetic rats ($n = 6 \pm$ SEM).

Group	**Group I**	**Group II**	**Group III**	**Gaillardia pulchella extract**	
	control group	**Diabetic group**	**Standard Control**	**Group IV 100mg/kg**	**Group V 200mg/kg**
White blood cells ($\times 10^9$ /L)	7.76 ± 0.96	13.16 ± 1.03	8.63 ± 0.66	8.71 ± 0.1*	8.80 ± 0.90*
Red blood cells ($\times 10^{12}$/L)	8.29 ± 0.27	6.56 ± 0.20	8.09 ± 0.37	7.94 ± 0.37*	7.92 ± 0.26*
Haemoglobin (g/dL)	15.43 ± 0.30	12.55 ± 0.35	15.36 ± 0.47	15.30 ± 0.42*	15.07 ± 0.46*
Platelets ($\times 10^9$ /L)	925 ± 11.36	638 ±12.85	766.1 ± 14.12	746.33 ± 15.8**	746.01 ± 13.16**
Neutrophils (%)	13.20 ± 0.27	4.54 ± 0.23	8.11 ± 0.42	7.84 ± 0.35*	7.89 ± 0.31*
Lymphocytes (%)	60.23 ± 1.94	68.69 ± 1.08	61.33 ± 1.44	62.34 ± 1.47*	62.41 ± 1.25*
Eosinophils (%)	3.03 ± 0.50	1.16 ± 0.17	2.66 ± 0.48	2.51 ± 0.18*	2.47 ± 0.32*

Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

IV. CONCLUSION

Oral administration of Ethanolic extract of flowers of *Gaillardia Pulchella* showed hypoglycemic activity in Alloxan-induced diabetes in experimental Wistar rats. The results also revealed the beneficial effects of this herb in improving the imbalance in lipid metabolism experienced during diabetes. It can, therefore, be concluded from this study that the ethanolic extract of flowers of *Gaillardia Pulchella*, besides its hypoglycemic action, could protect the liver, kidney, and blood against impairment due to diabetes. However, some renal functions may be compromised at higher dosages of the extract.

REFERENCES:

[1]. Tara V Shanbhag, Smita Shenoy, Pharmacology for medical graduates, Third Edition, ELSEVIER, 374-389
[2]. M Abhijith & Sori, Ravi. (2018). Evaluation of antidiabetic activity of *Tinospora cardifolia* in alloxan induced diabetes in albino wistar rats. International Journal of Basic & Clinical Pharmacology. 7. 1382.

[3]. Akhtar N, Akram M, Daniyal M, Ahmad S. Evaluation of antidiabetic activity of *Ipomoea batatas L*. extract in alloxan-induced diabetic rats. *Int J Immunopathol Pharmacol*. 2018;32:2058738418814678.
[4]. Fatma A. Moharram, Rabab Abd El Moneim El Dib1, New Apigenin Glycoside, Polyphenolic Constituents, Anti-inflammatory and Hepatoprotective Activities of *Gaillardia grandiflora* and *Gaillardia pulchella* Aerial Parts, Pharmacogn. Mag.,A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products 2016.
[5]. Amira Barky, Amany abdel hamid ezz, The potential role of apigenin in diabetes mellitus, international journal of clinical case reports and reviews, 2020.
[6]. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina (Kaunas). 2017;53(6):365-374.
[7]. IP Dmitry, Simila LA, NM Chervonnaya. Anti-stress activity of some plant extracts of the north *Caucasus flora*, Indonesian Journal Of Pharmacy, 2020: 131-143.
[8]. Taofik OS and Anthony JA. Evaluation of antidiabetic activity and associated toxicity *Artemisia afra* aqueous extract in wistar rats. Hindawi publishing corporation, 2013, 929074.
[9]. Mayuren Candasamy, TE Gopala Krishna Murthy, Kumar Shiva Gubiyappa, Dinesh and Gaurav Gupta. Alteration of glucose lowering effect of glibenclamide on single and multipletreatments with fenofibrate in experimental rats and rabbit models. J Basic Clin Pharm., June 2014-August 2014; 5(3): 62–67.
[10]. Xiao TY, Pei XL, Analysis of the essential oil from *Gaillardia Pulchella* Foug. And its antioxidant activity, Journal of Oleo science 329-333 (2013).
[11]. IACUC Guideline, Blood Collection The Rat, UCSF office of research, Instituional Animal Care and Use Program, April 2022.
[12]. Dmitry IP, Anastasiya Ep, Antihypoxic and anti-ischemic properties of the north Caucaus flora plant extracts, Blacpma, 504-517 (2019).
[13]. M. Shankar, R. Suthakaran, Antidiabetic activity of *Eugenia Jambolana* leaves in alloxan induced diabetic rats, 11 Aug 2014.
[14]. Joyeeta Bhattacharya, evaluation of antidiabetic activity of *Vitis Pedata* in alloxan induced diabetic rats Adamas university, 2020.
[15]. Naseer Ali shah, Muhammad Rashid Khan, Antidiabetic Effect of *Sida Cordata* in Alloxan induced Diabetic rats, Hindawi publishing corporation vol 2014.
[16]. Manivannan R, Shopna R, Antidiabetic activity of *CALOTROPIS GIGANTEA* white flower extracts in alloxan induced diabetic rats, Journal od drug delivery, 2017.
[17]. Augustine I Airaodion , Emmanuel O Ogbuagu, Antidiabetic activity of Ethanolic extract of *Carica Papaya* Leaves in Alloxan induced Diabetic rats, American Journal of Science & Research, 2019.