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Anti-Diabetic and Antioxidant Effects of Methanolic Flower Head Extract of *Tagetes Patula* in Streptozotocin and Dexamethasone Induced Diabetic Rats

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Abstract: Diabetes is a chronic metabolic disorder characterized by a high blood glucose concentration, i.e., hyperglycemia or caused by insulin deficiency, often combined with insulin resistance. In the Present study, the antidiabetic and antioxidant activity of the methanolic extract of *Tagetes patula* flower heads was screened out in rodent models. The pharmacological evaluation was carried out using 200 and 400 mg/kg, b.w. The *in-vivo* antidiabetic activity was performed in streptozotocin, dexamethasone-induced diabetic models and oral glucose tolerance test. Metformin hydrochloride was used as a standard. The parameters like blood glucose levels, insulin levels, homeostasis assessment of insulin resistance, insulin sensitivity, and fasting glucose-insulin ratio were estimated in these models. The *in-vitro* antioxidant activity of methanolic extract of *Tagetes patula* was performed by using hydroxyl radical scavenging assay and Ascorbic acid was used as a standard. The preliminary phytochemical screening of *Tagetes patula* revealed the presence of phytoconstituents like alkaloids, flavonoids, saponins, steroids, phenols, carbohydrates, triterpenoids, and tannins. The extract showed a significant decrease in the elevated blood glucose levels in streptozotocin, dexamethasone-induced diabetic models, and also in the oral glucose tolerance test. The extract prevented insulin resistance and significantly increased insulin sensitivity in the dexamethasone-induced model. The various phytochemical constituents like alkaloids, flavonoids, saponins, steroids, and phenols might be responsible for lowering the blood glucose levels in the animal models mentioned above. The extract showed significant scavenging activity against hydroxyl free radicals. From the above outcomes, it is flawless that the methanolic extract of *Tagetes patula* has antidiabetic and antioxidant activities.

Keywords: Anti-diabetic activity, Dexamethasone, Hydroxyl radical, Streptozotocin, *Tagetes patula*

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1. INTRODUCTION

Diabetes is an enduring metabolic ailment categorized through a high blood glucose concentration, i.e., hyperglycemia (fasting plasma glucose >7.0 mmol/1, or plasma glucose >11.1 mmol/1, 2hr after meal) caused by insulin deficiency, often combined with insulin resistance. Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis.[1] Streptozotocin could be an intense poison to the islets of Langerhans and causes extreme diabetes. This condition is characterized by a noteworthy increment in serum glucose levels and a huge lessening in insulin discharge.[2] Glucocorticoids play a vital role in several metabolic pathways. They are helpful in several clinical applications. However, excess glucocorticoids of any origin either endogenous or exogenous, leads to metabolic disorders. They increase blood glucose, insulin levels and alter lipid metabolism. This further result in a state of insulin resistance. Dexamethasone (Dex) is a synthetic glucocorticoid. Dex, due to its anti-inflammatory and immunosuppressant properties has got wide therapeutic applications. But, the major drawback with this is, it may cause glucose intolerance and reduce insulin sensitivity in some vulnerable patients, depending upon the dose and frequency of administration which can cause diabetes mellitus.[3] Oxidative stress is involved in the development and progression of diabetes-associated complications. In hyperglycemic conditions, continuous generation of reactive oxygen species (ROS) occurs and the evidence shows diabetes induced changes in the activities of antioxidant enzymes in various tissues. Antioxidants play an important role in scavenging the free radicals and protect the human body from oxidative stress.[4] Hence, drugs with both antioxidant and antidiabetic property would be useful for the treatment of the diabetes mellitus. In recent times, many medicinal plants have been reported to cure diabetes worldwide and have been used widely as antidiabetic remedies. WHO has suggested the evaluation of traditional plant treatments for diabetes as they are effective, nontoxic, with less or no side effects, and are considered to be excellent candidates for oral therapy.[5] The antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibiting the intestinal absorption of glucose or to the facilitation of metabolites in insulin-dependent processes. Glycosides, alkaloids, terpenoids, flavonoids, carotenoids, and so forth, from the plants, are frequently implicated in having antidiabetic effect.[6] Hydroxyl radicals can be formed by the Fenton reaction in the presence of reduced transition metals (such as Fe^{2+}) and H_2O_2, which is known to be the most reactive of all the reduced forms of di-oxygen and is thought to initiate cell damage *in vivo*. Hydroxyl radical scavenging is an essential antioxidant activity because of the very high reactivity of the OH radical, enabling it to react with a wide range of molecules present in living cells. Hydroxyl radical removal is significant for the protection of living systems.[7] *Tagetes patula* belonging to the family *Asteraceae* is one plant that has the phytochemical constituents that are useful in treating diseases. Leaves and flowers are used for their diuretic, depurative, antiseptic, and insect repellent activities in folk medicine. Chemical investigations with leaves and flowers of *T. patula* acknowledged the presence of terpenes, alkaloids, carotenoids, thiophenes, fatty acids and flavonoids as constituents, some of which may elicit, the biological activities reported to date; these include insecticidal, nematicidal, larvicidal, antifungal and anti-inflammatory activities.[8] Therefore, the present study was carried out to evaluate the antidiabetic and antioxidant potential of *Tagetes patula* in streptozotocin and dexamethasone-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Chemicals, drugs, and instruments

Methanol, EDTA and ascorbic acid were purchased from SD fine chemicals, Mumbai. Thiobarbituric acid, 2-Deoxy d-ribose, and Trichloroacetic acid were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai. Streptozotocin (Sisco Research Laboratories Pvt. Ltd, Maharashtra), Dexamethasone (Zydus Alidac health care Ltd, Ahmedabad), Metformin hydrochloride (Cipla, Mumbai), Metrogyl gel (Lekar Pharma Ltd, Ahmedabad) and Glucose estimation kit (KK Diagnostics), Semi-auto analyzer (Tulip, India), UV spectrophotometer (Shimadzu Corporation, Japan), Centrifuge (Remi, India) are purchased and used in the present study.

2.2 Plant material

The flower heads of *Tagetes patula* were collected from Hyderabad (India) during 12th November 2018. They were identified and authenticated with voucher specimen no (TP 25022020) by P. Suresh Babu, Head, Department of Botany, New Government Degree College, Kukatpally.

2.3 Preparation of methanolic extract

The collected flower heads were air-dried under the shade, powdered mechanically, and stored in an airtight container. The powder was extracted using a soxhlet apparatus, and methanol was used as a solvent. 100 g of powder and 1000 ml of methanol were used for the preparation of the extract. The extract was dried and stored in a refrigerator for further use. [9]

2.4 Test animals

Sprague Dawley rats of either sex weighing (180–280 g) were used for the pharmacological activities. The animals were housed in polypropylene cages at 25 ± 2° C under standard conditions of relative humidity 45-55% and 12 h light and dark cycles, and they were acclimatized to the laboratory conditions before use. Standard animal feed and water *ad libitum* were fed for the animals. All the experimental pharmacological protocols were approved by the Institutional Animal Ethics Committee (IAEC)1175/PO/Re/S/08//CPCSEA.

2.5 Phytochemical screening

Preliminary phytochemical screening was performed for the evaluation of phytochemical constituents present in the plant extract. [9]

2.6 Acute oral toxicity studies

Acute toxicity training was carried out on female Wistar albino mice by the oral route at dose levels up to 2000 mg/kg of the methanolic extract of *Tagetes patula* flowers as per the OECD- guidelines 425. [10]

2.7 Streptozotocin-induced diabetic model

This study was carried out for 21 days by taking Sprague Dawley rats weighing 240-260 gm. Administration of streptozotocin (55 mg/kg, *i.p*) induced diabetes. The animals were confirmed with diabetes (after 72 h of streptozotocin injection) by elevated plasma glucose levels. Methanolic flower heads extract (200 & 400 mg/kg, *p.o*) and standard metformin hydrochloride (5 mg/kg, *p.o*) was administered for 21 days. On 0, 7th, 14th, and 21st days, blood will be withdrawn from retro-orbital sinus. Glucose levels in the blood were estimated by using a semi-auto analyser. [11]

2.8 Dexamethasone induced diabetic model

Sprague Dawley rats weighing 230-280 g was used for the study. This experiment was carried out for 12 days. Administration of dexamethasone (4 mg/kg, *i.p*) induced diabetes. Methanolic flower head extract (200 & 400 mg/kg, *p.o*) and standard metformin hydrochloride (5 mg/kg, *p.o*) was administered for 12 days to their respective groups. On treatment, for 12 days, the blood was withdrawn from retro-orbital sinus for the estimation of glucose levels. Glucose levels in the blood were estimated by glucose oxidase peroxidase method using an automated analyzer.[12]

Parameters to be analysed: Blood glucose levels
Insulin sensitivity indices:
Homeostasis model assessment for insulin resistance (IR) (HOMA-IR) = (Fasting insulin x fasting glucose)/ 405
Homeostasis model assessment for insulin sensitivity (IS) (HOMA-IS) = 1000/ (fasting insulin x and fasting glucose)

2.9 Oral glucose tolerance test

Sprague Dawley rats weighing 240-260 g was be used for the study. Methanolic flower head extract (200 & 400 mg/kg, *p.o*) and standard metformin hydrochloride (5 mg/kg, *p.o*) was administered to their respective groups. Glucose (2 g/kg, *p.o*) was given orally 30 min after the administration of extract and standard drug at 0, 30, 60, 90, and 120 min. Glucose levels in the blood was be estimated by glucose oxidase peroxidase method.[11]

2.10 In-vitro antioxidant activity

Hydroxy Radical scavenging assay: The reaction mixture (1.0 mL) contains 100 µL of 2-deoxy- D ribose (28 mM in 20 mM KH_2PO_4-KOH buffer, pH 7.4), 500 µL of the extract, 200 µL EDTA (1.04 mM) and 200 µM. $FeCl_3$ (1:1 v/v), 100 µL of H_2O_2 (1.0 mM) and 100 µL ascorbic acid (1.0 mM) which is incubated at 37 ℃ for 1 h. One milliliter of thiobarbituric acid (1%) and 1.0 mL of trichloroacetic acid (2.8%) were added and incubated at 100 ℃ for 20 min. Absorbance was measured at 532 nm after cooling, against a blank sample.[12]Percentage inhibition of scavenging activity of the above model was evaluated by using the following formula *In-vitro* antioxidant activity was performed using hydroxyl.

$$\text{Percentage inhibition (\%)} = (A_{\text{control}} - A_{\text{test}})/ A_{\text{control}} \times 100$$

$A_{control}$ is the absorbance of the control, and A_{test} is the absorbance of the test.

3. STATISTICAL ANALYSIS

Values were expressed as mean ± SEM. Statistical analyses were performed by using a one-way analysis of variance (ANOVA) followed by the Dunnett's test. The Graph Pad Prism software package, version 8 (Graph Pad Software, Inc., San Diego, CA, USA), was used to perform all statistical investigations.[9]

4. RESULTS AND DISCUSSION

4.1 Phytochemical screening

The percentage yield of extract was found to be 12.3 w/w. The preliminary phytochemical screening of METP showed the presence of alkaloids, flavonoids, saponins, steroids, phenols, triterpenoids, tannins, and carbohydrates.

4.2 Acute Oral Toxicity Studies

Methanolic extract of *Tagetes patula* was tested on female mice at the dose of 2000 mg/kg b.w. *p.o.* The extract did not exhibit any signs of toxicity and mortality, even up to 2000 mg/kg. b.w. All animals were found to be safe even after 14 days of observation. The pharmacological evaluation of the extract was done at 200 and 400 mg/kg b.w.

4.3 Streptozotocin-Induced Diabetic Model

The streptozotocin-induced diabetic group showed a significant increase in the blood glucose levels ($p<0.001$) when compared to the healthy control group. The Methanolic extract of *Tagetes patula* (METP) treated groups at doses (200 mg/kg and 400 mg/kg) showed a significant decrease in the blood glucose levels ($p<0.001$) when compared to streptozotocin-induced diabetic groups. The Metformin (5 mg/kg) treated group showed a significant decrease in the blood glucose levels ($p<0.001$) when compared to the streptozotocin-induced diabetic group. Result of the effect of METP on blood glucose levels is expressed in Table 1.

Table 1: Anti-diabetic activity of methanolic extract of Tagetes patula using streptozotocin induced diabetic model.

Treatment groups	Blood glucose levels (mg/dl)			
	0th day	7th day	14th day	21st day
Control group	98.16 ± 0.60	98 ± 0.49	98.5 ± 0.42	96 ± 0.85
Disease control group	262.5 ± 0.95*	277.5 ± 0.76*	289.6 ± 0.66*	299.6 ± 0.76*
METP (200 mg/kg)	251.6 ± 0.66* # $	219.5 ± 1.17* # $	156.3 ± 0.88* # $	109.3 ± 0.71* # $
METP (400 mg/kg)	257.1 ± 0.87* # $	206.8 ± 0.60* # $	126.8 ± 0.79* # $	104.5 ± 0.76* # $
Metformin (5 mg/kg)	263.16 ± 1.07* ns	172 ± 0.57* #	111.66 ± 0.88* #	92 ± 1.06** #

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (* = p < 0.001, ** = p < 0.01) when compared with control group, (# = p<0.001) when compared with disease control group and ($ = p < 0.001) when compared with standard, ns = non significant. METP (Methanolic extract of Tagetes patula), ANOVA (Analysis of Variance).

Streptozotocin is a naturally occurring alkylating antineoplastic agent that is particularly toxic to the insulin-producing beta cells of the pancreas. STZ enters the pancreatic beta-cell through GLUT-2 transporter and causes alkylation of the DNA. Subsequent activation of PARP leads to NAD+ depletion, a reduction in cellular ATP, and subsequent inhibition of insulin production.[13] Saponins can reduce the increase in blood glucose by inhibiting the enzymes that break down and divided disaccharides into monosaccharides due to their antioxidant.[14] Beta-sitosterol (steroid) decreases the altered levels of blood glucose, serum insulin, lipid profile, oxidative stress markers, antioxidant enzymes, insulin receptor (IR), and glucose transporter 4 (GLUT4) proteins and it improves glycaemic control through activation of insulin receptor (IR) and GLUT4 in the adipose tissue.[15]Beta-carbolines (Alkaloid) causes a decrease in the blood glucose levels by potentiating the insulin secretion from the islets.[16] Flavonoids animate glucose uptake in peripheral tissues, managed the activity and expression of the rate restricting enzymes in the carbohydrate metabolism pathway and act as insulin mimetics, by impacting the pleiotropic systems of insulin signaling, to enhance the diabetes status.[17] Quercetin brings about the regeneration of the pancreatic islets and probably increases insulin release in streptozotocin-induced diabetic rats, thus exerting its beneficial anti-diabetic effects. [18] Gallic acid (phenolic acid) causes a decrease in blood glucose levels in streptozotocin-induced diabetic rats. Gallic acid induced a significant reduction in blood glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol, urea, uric acid, and creatinine and, at the same time markedly increased plasma insulin, C-peptide, and glucose tolerance levels.[19] Limonene (triterpenoid) causes the stimulation of insulin secretion due to the regenerated cell, lower the blood glucose, and modulate the critical enzymes of carbohydrate metabolism (increased glycolysis and decreased gluconeogenesis).[20] Tannic acid causes a lowering of blood glucose levels. It enhances the glucose utilization by inducing glucose transporters and through activation of insulin-mediated signaling pathways.[21]

4.4 Dexamethasone induced diabetic model

The dexamethasone-induced diabetic group showed a significant increase in fasting blood glucose levels ($p<0.001$) and insulin levels ($p<0.001$) when compared to the normal control group. The METP treated groups at doses (200 mg/kg b.w. and 400 mg/kg b.w.) showed a significant decrease in the fasting blood glucose levels ($p<0.001$) and insulin levels ($p<0.001$) when compared to dexamethasone induce diabetic group. The metformin (5 mg/kg b.w.) treated group showed a significant decrease in the fasting blood glucose levels ($p<0.001$) and insulin levels ($p<0.001$) when compared to dexamethasone-induced diabetic group. The dexamethasone-induced diabetic group showed significantly influenced insulin resistance ($p<0.001$) by HOMA-IR when compared to the control group. The METP treated groups at doses (200 mg/kg b.w. and 400 mg/kg b.w.) significantly prevented the dexamethasone-induced insulin resistance. The dexamethasone-induced diabetic group showed a significant reduction in insulin sensitivity ($p<0.001$) by HOMA-IS and FGIR ($p<0.001$) when compared to the control group. The METP treated groups at doses (200 mg/kg b.w. and 400 mg/kg b.w.) significantly increased insulin sensitivity ($p<0.001$) and FGIR ($p<0.001$) when compared to the dexamethasone-induced diabetic group. Result of the effect of METP on blood glucose levels is expressed in Table 2.

Table 2: Anti-diabetic activity of methanolic extract of Tagetes patula using dexamethasone induced diabetic model.

Treatment	Fasting glucose (FG) mg/dL	Fasting insulin (FI) mU/L	HOMA-IR	HOMA-IS	FGIR (FG/FI)
Normal control	85.831±1.07	76.5±0.76	16.19±0.28	0.14±0.003	1.11±0.016
Disease control	143±0.89*	282.16±0.70*	99.62±0.69*	0.02±0.0003*	0.5±0.003*
METP (200 mg/kg)	117.16±0.87 * # $	144±0.63* # $	41.65±0.28* # $	0.05±0.0004* # $	0.81±0.008* # $
METP (400 mg/kg)	103.83±0.60* # $	116.6±0.91* # $$$	29.9±0.29* # $	0.08±0.0007* # $	0.88±0.008* # $
Metformin(5mg/kg)	93.5±0.76 * #	99.83±0.94* #	23.04±0.27* #	0.1±0.0012* #	0.93±0.012* #

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (* = p < 0.001) when compared with control group, (# = p<0.001) when compared with disease control group and ($ = p < 0.001) when compared with standard. HOMA-IR (Homeostasis model assessment for insulin resistance IR), HOMA-IS (Homeostasis model assessment for insulin sensitivity IS), FGIR (Fasting glucose insulin ratio), ANOVA (Analysis of Variance)

Dexamethasone is a type of corticosteroid that reduces the expression of Insulin Receptor Substrate-1, Phosphatidylinositol-3 Kinase, and protein kinase B in skeletal muscle, liver, and adipose tissues. This, in turn, leads to the reduced translocation of glucose transporters to the cell surface, which further leads to hyperglycemia and resistance to insulin action.[12] Flavonoids exert direct effects on insulin-signaling pathways, leading to an improvement of insulin sensitivity by inducing IR and IRS phosphorylation and activate the PI3K/Akt pathway and AMPK promoting GLUT4 expression and translocation in skeletal muscle.[22] Triterpenoids significantly prevented dexamethasone rise in serum insulin and glucose levels. Triterpenoid peroxisome proliferator-activated receptor - γ sensitizes the tissue to insulin contributes to insulin-sensitizing and anti-diabetic activity.[13] Tannic acid causes a decrease in the glucose and insulin levels by inducing glucose transport by directly or indirectly activating the IR.[23] Gymnemic acid (saponin) decreases the glucose and insulin levels by filling the receptors on absorptive layers of the intestine, reduces the absorption of glucose, resulting in low blood sugar levels.[24]

4.5 Oral Glucose Tolerance Test

The oral glucose tolerance test showed a significant increase in blood glucose levels ($p<0.001$) when compared to the normal control group. The METP treated groups at doses (200 mg/kg and 400 mg/kg) showed a significant decrease in the blood glucose levels ($p<0.001$) when compared to the disease control group. The Metformin (5 mg/kg) treated group showed a significant decrease in the blood glucose levels ($p<0.001$) when compared to the disease control group. Results of the effect of METP on blood glucose levels were expressed in Table 3.

Table 3: Anti-diabetic activity of methanolic extract of Tagetes patula using oral glucose tolerance test.

Treatment	Blood glucose levels (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Normal control	82.66±0.84	91.33±0.98	89.83±0.61	90.66±0.98	89±0.73
Disease control (Glucose 2 g/kg)	102.33±0.91*	131±0.96*	122.5±0.88*	116.16±0.60*	100.5±0.88*
METP (200 mg/kg)	92.16±0.83*$^{\#\$}$	118±0.89*$^{\#\$}$	111±0.63*$^{ns\$}$	90.33±0.95$^{ns\#\$}$	73.16±0.47*$^{\#\$\$}$
METP (400 mg/kg)	85.16±0.60$^{ns\#\$\$}$	111.66±0.66*$^{\#\$}$	105.16±0.79*$^{\#\#\$}$	83.16±0.87*$^{\#ns}$	71.66±0.84*$^{\#ns}$
Metformin (5mg/kg)	80.5±0.56$^{ns\#}$	104±0.68*$^{\#}$	90.5±0.56$^{ns\#}$	80.83±0.47*$^{\#}$	69.83±0.87*$^{\#}$

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (= p < 0.001) when compared with control group, ($^{\#}$ = p<0.001, $^{\#\#}$ = p < 0.01) when compared with disease control group and ($^{\$}$ = p < 0.001, $^{\$\$}$ = p < 0.01) when compared with standard group, ^{ns} = non significant. METP (Methanolic extract of Tagetes patula), ANOVA (Analysis of Variance)*

The Oral Glucose Tolerance Test (OGTT) processes the body's capacity to custom a kind of sugar called glucose, which is the body's primary source of energy. Owing to the deficit of the insulin, populace's anguish from diabetes has high blood glucose level. This assessment can be used to detect pre-diabetes and diabetes.[25] Flavonoids showed a significant decrease in the levels of blood glucose and an increase in the levels of serum insulin. These potentiate the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.[26] Phenols lowered blood glucose levels was due to insulin secretion from β-cells and increased glucose utilization by the tissues. Tannins show a significant decrease in serum glucose. The possible mechanism might be due to a reduction in intestinal glucose absorption, induction of glycogenic process, including a reduction of glycogenolysis and gluconeogenesis.[27] Saponins reduced the blood glucose levels by the restoration of insulin response and stimulating glycogen synthesis.[28] β-carboline (alkaloid) causes a decrease in the glucose levels by increasing insulin secretion from islets of Langerhans. β-sitosterol (steroid) normalizes the insulin levels and the blood sugar levels by inducing the secretion of insulin even in the absence of any stimulatory glucose concentration. This secretion, therefore, inhibits glucose-6- phosphatase enzyme.[29]

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4.6 In-Vitro Antioxidant Activity

The IC_{50} value of the METP was 40 µg/ml, and the standard drug ascorbic acid was 35 µg/ml. Results of the effect of METP on hydroxyl radical scavenging were expressed in Table 4

Table 4: Antioxidant activity of methanolic extract of Tagetes patula using hydroxyl radical scavenging assay.

S. No	Compounds	Concentration (µg/mL)	% inhibition	IC_{50} values
1	METP	10	13.9 ± 0.23	40
		20	26 ± 1.15	
		30	34.66 ± 0.02	
		40	50.28 ± 0.02	
		50	60.3 ± 0.24	
2	Ascorbic acid	10	14.56 ± 0.02	35
		20	26.73 ± 0.02	
		30	39.84 ± 0.01	
		40	55.69 ± 0.03	
		50	65.49 ± 0.01	

Values are expressed as Mean ± SEM, (n=3), METP (Methanolic extract of Tagetes patula), SEM (Standard Error Mean)

The OH radical is an exceptionally responsive free radical made in the biotic system. It has been implicated as a highly damaging species capable of damaging almost every molecule found in the living cell. These radicals can join the nucleotides in DNA and cause strand breakage. Besides, these species are considered to be rapid initiators of the lipid peroxidation process due to the abstraction of the hydrogen atom from unsaturated fatty acid.[8] The phenolic compounds may suppress lipid peroxidation through different chemical mechanisms, including free radical quenching, electron transfer, radical addition.[11] β-sitosterol (steroid) shows antioxidant property by its redox properties, which allow them to act as reducing agents, hydrogen donors, and single oxygen quenchers.[30]Kaempferol (flavonoid) shows reducing property by acting as the chelators of iron ions and thereby preventing the formation of radical, play a significant role in the inhibition of ribose fragmentation.[31] Tannic acid shows hydroxyl radical scavenging activity by inhibiting the reduction of iron and thus preventing the deoxyribose damage.[32] Hydroxyl radicals were effectively scavenged, and 2-deoxyribose was prevented from degradation by lupeol (triterpenoid).[33] β- carboline scavenged hydroxyl radical by reacting with hydroxyl radical and prevents DNA damage.[34] Saponins scavenge the hydroxyl radical by inhibiting the hydroxyl radical generation, thus preventing the degradation of deoxyribose of DNA.

5. CONCLUSION

The METP was screened for its anti-diabetic activity by using streptozotocin, dexamethasone models, and oral glucose tolerance tests. In conclusion, the present findings demonstrated that the flower heads extract of *Tagetes patula* is capable of exhibiting significant antidiabetic activities in STZ-induced and dexamethasone induced diabetic rats. The methanolic extract of *Tagetes patula* also showed improvement in parameters such as oral glucose tolerance, hypoglycemic and antioxidant activity. On the other hand, the presence of flavonoids, alkaloids, phenols, saponins, triterpenoids, steroids, carbohydrates, tannins present in *Tagetes patula* flower heads concludes that this herb has multiple biological properties. Further studies to find out the exact mechanism of this plant for its antidiabetogenic effect and to isolate and identify the bioactive compounds responsible for this effect are necessary.

6. AUTHORS CONTRIBUTION STATEMENT

The authors Dr. M. Ganga Raju, Anusha, Gayathri and Pavani conceived of the present idea. Anusha, Gayathri and Pavani designed and performed the experiments. Dr. M. Ganga Raju and Dr. Suvarchala analyzed the data and wrote the paper with input from all authors.

7. ACKNOWLEDGEMENTS

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8. CONFLICT OF INTEREST

Conflict of interest declared none.

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