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ORIGINAL ARTICLE

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Multiherbal Formulation Effect on Blood Sugar and Body Weight in Diabetic Albino Rats

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Background: Therapeutic plants are an important source of medicine, and various active compounds were isolated from the plants. As per the Indian traditional system of medicine, many medicinal plants have been used for the management of various health disorders, including *diabetes mellitus*. *Diabetes mellitus* is a non-communicable disease that is also referred to as a lifestyle disorder that requires modifications in diet, exercise, and behavior along with medication. The present research work was designed to evaluate the impact of multiherbal formulations on blood sugar and body weight in albino rats. Three different formulations were prepared from five plant extracts (*Allium sativum, Azadirachta indica*, *Phyllanthus emblica, Tamarindus indica,* and *Zingiber officinale*). After daily administration of multiherbal formulations at a dose of 300 mg/kg b.wt. and the standard drug (Glibenclamide) at a dose of 5 mg/kg b.wt. for 15, 30, and 45 days, respectively, blood samples were collected from each rat and analyzed according to standard techniques (parameters). Blood sugar and body weight were measured.

Results: There was a significant ($p<0.01$) increase in the blood sugar levels and a significant ($p<0.01$) decrease in the body weight in diabetic rats when compared with normal control. However, treatment of alloxan-injected rats with three different formulations significantly ($p<0.01$) decreased blood sugar levels and significantly ($p<0.01$) increased body weight as compared to the standard group and normal control group.

Conclusion: The present study made us conclude that among the three different formulations, formulation-2 (*Allium sativum, Azadirachta indica,* and *Zingiber officinale*) was more effective as compared to the other two formulations, exhibited an antihyperglycemic effect, and also possessed beneficial effects in diabetic rats.

Key words: active compounds, antidiabetic property, formulations, body weight

Diabetes mellitus is defined as a group of chronic metabolic disorders characterized by persistent hyperglycemia resulting from a complete or relative lack of insulin secretion or action. It is a dominant disease in the world, and it has no total treatment. The prevalence of diabetes and its complications is increasing worldwide and has become an important cause of morbidity and mortality. Based on earlier studies, we find that single plants or extracted phytochemical constituents of these plants collectively contain antidiabetic activity (useful in lowering blood sugar levels) and also help in the prevention of different diseases due to the presence of certain active phytochemical constituents like alkaloids, flavonoids, tannins, phenols, saponins, etc. A combination of herbs is believed to work synergistically and may have a more beneficial effect than a single preparation.

Garlic (*Allium sativum*) is a member of the Alliaceae family, and all parts of the plant (inflorescence, leaves, and cloves) have been used in science since ancient times. The antidiabetic effects of garlic are especially due to the volatile sulfur compounds. Garlic is effective in decreasing insulin resistance as well **(Padiya and Banerjee, 2013**). Neem belongs to the family Meliaceae and exhibits many naturally active compounds in almost every part of the plant (bark, branches, fruit, leaves, oil, roots, seeds, and trunk) with many curative activities (**Singh *et al.,* 2015**). In Ayurveda, different parts of Neem are used in analgesics, diabetes, eye problems, intestinal worms, leprosy, piles, skin ulcers, urinary disorders, and wounds (**Mishra *et al.,* 2016**). Amla belongs to the family Euphorbiaceae, and its fruits are widely used in Ayurveda. The ancient system of drugs used almost all of its parts, i.e., root, leaf, and stem, and was mostly known for the immense activities of fruit. It is regularly used in antioxidants, chronic diseases, diabetes, ophthalmic disorders, ulcers, graying of the hair, and to increase defense against many other diseases (**Jain *et al.,* 2015**). Tamarind belongs to the family Caesalpiniaceae, which is a sub-family in Leguminosae. Hyperglycemia, hyperlipidemia, and overweight or obesity are the main causes of *diabetes mellitus*, metabolic syndrome, and cardiovascular problems. These metabolism abnormalities are controlled by Tamarind (**Yerima *et al.,* 2014**). Ginger belongs to the family Zingiberaceae and is used worldwide as a spice, flavoring agent, and herbal remedy. The main constituent of ginger is [6]-gingerol, which showed hypoglycemic activity when administered to diabetic mice and enhanced weakened insulin signaling in arsenic-intoxicated mice. Plasma insulin levels increased with the help of [6]-gingerol, which decreased raised blood glucose levels and oxidative stress by increasing the activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione (**Chakraborty *et al.,* 2012**). Different herbal formulations are preferred due to their maximum therapeutic efficacy, low cost, and lesser side effects. They are not addictive or habit-forming, and they are powerful nutritional agents that support the body naturally.

MATERIALS AND METHODS

Collection of Plant Materials

The bulbs of garlic (*Allium sativum*), the fruits of amla (*Phyllanthus emblica*), and the rhizome of ginger (*Zingiber officinale*) were purchased from the local market of Jhansi (Uttar Pradesh). The seeds of neem seeds (*Azadirachta indica*) and leaves of imli (*Tamarindus indica*) were collected from adjacent areas of Jhansi. These were subsequently authenticated and identified in the NISCAIR Authentication No.: NISCAIR/RHMD/Consult/2019/3448-49-1, NISCAIR/RHMD/Consult/2019/3448-49-2, NISCAIR/RHMD/Consult/2019/3448-49-3, NISCAIR/RHMD/Consult/2019/3448-50-1, and NISCAIR/RHMD/Consult/2019/3448-50-2. The samples were air-dried in the shade at room temperature (25$\pm$5°C), which took about 1 week to 1 month to dry until total moisture was removed from the plant. These were ground into fine powder using an electric blender and stored at room temperature.

Preparation of plant extract

The preparation of plant extracts involves the separation of the required constituents from plant materials. Extracts of garlic, neem, and amla (ethanolic extracts) and imli (hydro-alcoholic extract-ethanol:water

[80:20]) were extracted through the Soxhlet apparatus, and an aqueous extract of ginger was extracted through the triple maceration process.

Extraction through the Soxhlet apparatus process

The plant material is packed in a cylinder of the Soxhlet extractor. The solvent, *i.e.*, ethanol, is placed in the flask. When a solvent is boiled on heating, it gets converted into vapors. These vapors move into the condenser through a side tube and get condensed into a liquid, which falls on the column of the crude drug. When the extractor gets filled with the solvent, the level of the siphon tube also rises to its top. The solvent containing the active constituents of the drug in the siphon tube runs into the flask, thus emptying the body extractor continuously. The soluble active components of the drug stay in the flask while the solvent is frequently volatilized. The process of filling and emptying the extractor is repeated until the drug is drained. Usually, the process is repeated about 15 times for complete exhaustion of the crude drug.

Triple Maceration Process

In this process, the drug is concentrated three times by using the menstruum, which is divided into three parts in such a manner that the same volume is used for maceration. The whole drug is macerated for one hour, with a portion of the menstruum required for the first maceration and strained. Maceration again for one hour with a part of the menstruum required for the second maceration and strained. Maceration again for one hour with a part of the menstruum required for the third maceration and strained. Press the marc lightly. Then combine the liquids obtained from the second and third macerations and evaporate them to a specified extent. Mix it with the liquid attained from the first maceration. Add absolute alcohol to 1/4th of the volume of the finished product. Adjust the volume with water to allow it to stand for 14 days, and filter through a silk cloth or Whatman filter paper no. 1.

Preparation of the dose

Formulations were prepared in gum acacia and physiological saline (0.9% NaCl) in a ratio of 1:1 of various herbs. Three different herbal formulations **(Table 1)** were used at a dose level of 300 mg/kg b.wt. Then it was given orally (1 mL/day) to diabetic rats for different durations, and their effects were studied after 15, 30, and 45 days of chronic treatment.

Test animal

The present study was carried out at the Department of Zoology, Institute of Basic Science, Bundelkhand University Campus, Jhansi (UP), India. For experimentation, sexually mature adult female Albino rats of the Wistar strain (200±10 gm) of about 3 months were purchased from DRDE (Defense Research and Development Establishment) in Gwalior. Before the study, ethical clearance was obtained from the Institutional Animal Ethical Committee (CPCSEA) of the Government of India with approval No. BU/Pharm/IAEC/a/17/09, New Delhi. All the experiments and protocols were conducted in strict agreement with the guidelines and ethical principles provided by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA). The animals were acclimatized to the experimental room at a temperature of 25-30˚C, controlled humidity conditions (50-55%), and a 12-hour light and 12-hour dark cycle. They were fed a rat's pellet diet (Amrut Feeds, Pranav Agro Ltd., Sangli) and water *ad-libtum*.

Induction of diabetes

Diabetes was induced in rats by a single intraperitoneal injection of alloxan monohydrate (CDH, Bombay Ltd.). Alloxan monohydrate was dissolved in ice-cold physiological saline (0.9% NaCl) to constitute a 10% (w/v) solution, and a dose of 100 mg/kg b.wt. of the rat was selected to induce diabetes. The fasting blood glucose level of rats was measured after 72 hours of alloxan injection. The rats with effective and permanent elevated blood glucose levels (above 150 mg/dl) were selected for the study.

Normal Level of Blood Sugar

The normal levels of blood sugar in humans and albino rats are 70-110 mg/dl, and after meals, blood sugar is elevated, *i.e.,* 115-135 mg/dl.

Standard Drug

The standard drug (Glibenclamide) was administered to the animals *via* oral route at 5 mg/kg b.wt. with the help of a gastric feeding needle.

Experimental designs

The research work was carried out for 45 days and 1 week before the experiment. Diabetes was induced in rats, and rats were allowed to acclimatize to the laboratory environment. Thirty-six rats were grouped into six groups of six rats each, following the experimental design.

Group I: Normal Control

Group II: Diabetic Control

Group III: Diabetic; will receive the standard drug (Glibenclamide) at 5 mg/kg b.wt.

Group IV: Diabetic; will receive formulation 1 extract at 300 mg/kg b.wt.

Group V: Diabetic; will receive formulation 2 extract at 300 mg/kg b.wt.

Group VI: Diabetic; will receive formulation 3 extract at 300 mg/kg b.wt.

After daily administration of the dose for intervals of 15, 30, and 45 days, an autopsy of the animal was also performed. This was done by giving anesthesia with chloroform. Blood samples were collected from each rat by puncturing the tail veins and optical veins of the rat eye, i.e., the retro-orbital plexus, with the help of capillaries. The collected blood samples were analyzed according to standard techniques (parameters). Blood sugar and body weight were measured.

Blood sugar analysis (Asatoor and King, 1969)

Principle: The proteins are precipitated by the twist method, followed by the reduction of the alkaline copper. The amount of cuprous copper formed is estimated colorimetrically by reacting with phosphomolybdic acid. The cuprous copper is oxidized to cupric acid. The phosphomolybdic acid is reduced to molybdenum.

Reagents required

Sodium Sulfate and Copper Sulfate (Isotonic Solution): 1.32 gm of sodium sulfate (anhydrous) and 0.6 gm of copper sulfate were dissolved in 100 ml of distilled water.

Sodium Tungstate: 10% Solution.

Reagent A: 1.32 gm of $CuSO_4$ was dissolved in 100 ml of distilled water.

Reagent B: 5.0 gm of sodium bicarbonate and 4.0 gm of anhydrous sodium carbonate were dissolved in 70 ml of distilled water at room temperature. Subsequently, 3.68 gm of potassium oxalate was dissolved in a small quantity of water and mixed with the above solution. 2.4 gm of sodium potassium tartrate was dissolved separately in a beaker and added to the above mixture. The final volume was made up of 100 ml of distilled water.

Phosphomolybdic acid: 35.0 gm of molybdic acid and 5.0 gm of sodium tungstate were dissolved in 200 ml of 10% NaOH and 200 ml of distilled water. The solution was boiled for 30-40 minutes to remove the ammonia present in the molybdic acid. The remaining was cooled, and diluted by adding 125 ml of phosphoric acid, and the final volume was made up to 500 ml with distilled water.

Stock Solution of Glucose (Standard Solution): 100 mg of glucose was dissolved in 100 ml of an isotonic solution of sodium sulfate and copper sulfate.

Working Standard Glucose Solution: 2.5 ml of stock glucose solution was made up to 100 ml with isotonic solution.

Estimation of blood sugar

0.1 ml of blood was taken in 3.8 ml of isotonic solution in a test tube. To it, 0.1 ml of a 10% sodium tungstate solution was added. This solution was centrifuged after 10 minutes. 1 ml of supernatant was taken in another test tube, and to it was added 1 ml of Regent C (Regent A and Regent B in the ratio of 1:1). Tubes were plugged with cotton and heated in a boiling water bath for 10 minutes. The mixture was cooled, and 3 ml of phosphomolybdic acid and 3 ml of distilled water were added. The contents were mixed well, and readings were taken after 5 minutes at 680nm. Simultaneously, a standard and a blank tube were also made by taking 1 ml of working glucose solution and isotonic solution, respectively.

The level of blood sugar was estimated by the following formula:

$$\text{Blood Sugar} = \frac{\text{O.D. of Unknown}}{\text{O.D. of Known}} \times 100$$

It was expressed as mg glucose/100 ml of blood.

Statistical analyses

The data were expressed as the Mean ± SEM obtained from the number of experiments (n). A one-way ANOVA followed by Dunnett's posttest was performed using GraphPad software. Differences between groups were considered. Set the p-value at $p<0.05$, the minimum level of significance.

RESULTS

Blood sugar level

In the present study, blood sugar levels increased significantly ($p<0.01$) due to the administration of alloxan in albino Wistar rats as compared to the normal control group. During the daily administration of multiherbal formulations **(Table 1)** at a dose of 300 mg/kg b.wt. and the standard drug (Glibenclamide) at a dose of 5 mg/kg b.wt. for 15, 30, and 45 days, respectively, the blood sugar level decreased significantly ($p<0.01$) as compared to the normal control group and standard group and recouped towards the normal group **(Table 2)**. The most significant decrease in blood sugar levels was obtained after 30 and 45 days of three formulations dose administration, especially in formulation-2, which was more effective in comparison to the other two formulations and exhibited an antihyperglycemic effect.

Table 1: Ratio of three multi-herbal formulations

S.No.	Multiherbal Formulation	Plant Extract	Ratio
1.	Formulation 1 (F1)	*Azadirachta indica* (Neem seed), *Phyllanthus emblica* (Amla fruit), and *Tamarindus indica* (Imli leaf).	1:1:1
2.	Formulation 2 (F2)	*Allium sativum* (Garlic bulb), *Zingiber officinale* (Ginger rhizome), and *Azadirachta indica* (Neem seed).	1:1:1
3.	Formulation 3 (F3)	*Allium sativum* (Garlic bulb), *Azadirachta indica* (Neem seed), *Phyllanthus emblica* (Amla fruit), *Tamarindus indica* (Imli leaf), and *Zingiber officinale* (Ginger rhizome).	1:1:1:1:1

Table 2: Showing variations in Blood Sugar (mg/dl) among different experimental groups due to daily administration of different formulations (values are expressed as Mean ± SEM, where N = 6).

		BLOOD SUGAR (mg/dl)					
S. No.	Experimental group	Days					
		0 Day	15 Days	0 Day	30 Days	0 day	45 Days
1.	Group I (Normal Control)	98.16 ±2.15	96.00 ±2.88	94.33 ±3.97	94.33 ±2.14	94.33 ±2.49	96.00 ±2.85
2.	Group II (Diabetic Control)	244.66 ±2.092	296.00 ±2.01[ab]	243.00 ±2.67	322.00 ±2.47[ab]	245 ±3.19	360.50 ±3.97[ab]
3.	Group III (Diabetic + Glibenclamide)	259.16 ±3.57	196.83 ±3.14[a]	247.50 ±2.83	170.16 ±1.24[a]	246.83 ±2.31	150.00 ±2.32[a]
4.	Group IV (Diabetic + Formulation-1)	244.83 ±2.91	219.00 ±1.94[ab]	252.50 ±6.43	203.66 ±5.54[ab]	248.33 ±3.55	180.33 ±2.67[ab]
5.	Group V (Diabetic + Formulation-2)	242.66 ±4.77	199.33 ±4.65[a•]	238.16 ±3.51	170.83 ±2.58[a•]	256.16 ±5.06	144.83 ±4.18[a•]
6.	Group VI (Diabetic + Formulation-3)	248.66 ±3.64	214.16 ±3.74[ab]	252.66 ±4.81	194.00 ±5.16[ab]	255.33 ±3.80	170.16 ±2.88[ab]

a = $p<0.01$ vs. Group I; b = $p<0.01$ vs. Group III; ● = $p>0.01$ vs. Group III. The data are expressed as the mean ± SEM for six rats each.

Table 3: Showing variations in Weight (gm) among different experimental groups due to daily administration of different formulations (values are expressed as Mean ± SEM, where N = 6).

S. No.	Experimental group	WEIGHT (gm)					
		Days					
		0 Day	15 Days	0 Day	30 Days	0 Day	45 Days
1.	Group I (Control)	202.50 ±1.11	224.16 ±1.53	200.00 ±1.29	229.16 ±2.38	200.00 ±1.29	242.50 ±2.14
2.	Group II (Diabetic Control)	190.00 ±1.82	174.16 ±2.00^{ab}	190.83 ±1.53	160.00 ±1.82^{ab}	190.83 ±1.53	143.33 ±2.47^{ab}
3.	Group III (Diabetic + Glibenclamide)	190.83 ±1.53	200.00 ±1.82^{a}	191.66 ±1.66	210.00 ±2.58^{a}	191.66 ±1.66	219.16 ±2.38^{a}
4.	Group IV (Diabetic + Formulation-1)	190.00 ±1.29	186.66 ±2.78^{ab}	190.83 ±1.53	193.33 ±2.47^{ab}	190.83 ±1.53	212.50 ±2.14$^{a•}$
5.	Group V (Diabetic + Formulation-2)	190.83 ±1.53	194.16 ±3.51$^{a•}$	191.66 ±1.05	207.50 ±3.09$^{a•}$	192.50 ±1.11	223.33 ±3.07$^{a•}$
6.	Group VI (Diabetic + Formulation-3)	191.66 ±1.05	191.66 ±2.78^{ad}	190.00 ±1.82	195.00 ±3.41^{ab}	191.66 ±1.66	214.16 ±4.90$^{a•}$

a = p<0.01 vs. Group I; b = p<0.01 vs. Group III; d = p<0.05 vs. Group III; ● = p>0.01 vs. Group III. The data are expressed as the mean ± SEM for six rats each.

Body Weight

In the present study, weight decreased significantly (p<0.01) due to the administration of alloxan in albino Wistar rats as compared to the normal control group. During the daily administration of multiherbal formulations **(Table 1)** at a dose of 300 mg/kg b.wt. and the standard drug (Glibenclamide) at a dose of 5 mg/kg b.wt. for 15, 30, and 45 days, respectively, the weight increased significantly (p<0.01) as compared to the normal control group and standard group and recouped towards the normal group **(Table 3)**. The most significant increase in weights was obtained after 30 and 45 days of three formulations dose administration, especially in formulation-2, which was more effective in comparison to the other two formulations and exhibited an antihyperglycemic effect, and its values are comparable to the standard drug.

DISCUSSION

Dietary carbohydrates are digested in the gastrointestinal tract into simple monosaccharides, which later get absorbed. Though starch provides glucose directly, fructose and galactose get converted into glucose in the liver. Various metabolic processes, *viz.,* glycogenolysis and glyconeogenesis, affect the blood glucose concentration. The level of glucose is always balanced between input, output, synthesis, and catabolism. Insulin is the principal hormone affecting blood glucose levels. Diabetes mellitus is a common endocrine disorder in which hyperglycemia occurs due to insulin resistance or an absolute or relative lack of insulin. An increase in the level of glucose in the blood is known as hyperglycemia. It occurs due to a disturbance in insulin hormone secretion. The abnormalities in blood glucose levels reflect disturbances in carbohydrate metabolism. Increased free radical generation and oxidative stress are hypothesized to play an important role in the pathogenesis of diabetes and its late complications. A constant rise in blood glucose causes glucose toxicity, which adds to cell dysfunction.

In the present study, the daily administration of formulations of plants significantly reduced the level of blood sugar as compared to their normal control group at various durations. Among the three formulations, formulation-2 which contains *Allium sativum, Azadirachta indica,* and *Zingiber officinale*, was more effective as compared to the other two formulations. It may be due to the protective effect of the plant and the presence of certain phytochemical constituents (like saponins, flavonoids, alkaloids, tannins, etc.), which may be helpful in maintaining the function of certain

enzymes. Several previous studies show that various herbs and medicinal plants possess medicinal properties and can overcome toxicity due to certain external agents.

Garlic is effective in lowering serum glucose levels in STZ-induced as well as alloxan-induced diabetic mice, rats, and rabbits. The hypoglycemic activity of garlic could be due to an increase in pancreatic secretion of insulin from β-cells, the release of bound insulin, or an enhancement of insulin sensitivity. Garlic (allicin) can increase serum insulin by effectively combining with compounds like cysteine, which would substitute insulin from SH group reactions, which are a common reason for insulin inactivation. The antioxidant effect of S-allyl cysteine sulfoxide (Allin), an isolated product from garlic, may contribute to its beneficial effect on diabetes. The hypoglycemic effect of allin was shown to be similar to that of glibenclamide **(Londhe *et al.,* 2011).** The hypoglycemic evaluation of neem root-bark extract was done by giving glucose *p.o.* 60 minutes after administering the standard drug (Glibenclamide). It was administered in doses of 200, 400, and 800 mg/kg in rats, and blood glucose levels were checked after each 30 minutes up to 4 hours. The hypoglycemic activity was observed in alloxan-induced diabetic rats with continuous dosing for 15 days. It showed a statistically significant effect at a dose of 800 mg/kg **(Patil *et al.,* 2013).** Aqueous fruit extract of amla (200 and 400 mg/kg doses) showed an anti-hyperglycemic effect in streptozotocin-induced diabetic obese rats. Both extract-treated groups exhibited a significant ($p \leq 0.001$) decrease in blood glucose levels in the fifth and sixth weeks compared to the metformin-treated group. All the treated rat groups showed significantly ($p \leq 0.001$) decreased blood glucose levels in comparison to diabetic control; though, there were no significant changes detected between the amla-treated groups **(Elobeid and Ahmed, 2015].** In STZ-diabetic rats, the effect of continuous oral administration of hydroalcoholic extract of *Tamarindus indica* leaves and aqueous extract of *Tamarindus indica* leaves at doses of 100 and 200 mg/kg on blood glucose levels were determined at 0, 7, and 14 days of treatment. Blood glucose levels significantly ($P<0.05$) decreased after 14 days of treatment. The hydroalcoholic extract of *Tamarindus indica* leaves at a dose of 200 mg/kg significantly increased the serum insulin level **(Meher and Dash, 2013).** Diabetes induced by streptozotocin significantly ($P<0.05$) increased blood glucose levels in rats. Oral administration of *Tamarindus indica* extract to diabetic rats at three dose levels (100, 200, and 300 mg/kg b.wt.) for 4 weeks significantly ($P<0.05$) decreased blood glucose, and the percentages of the decrease in blood glucose level were 48.25, 59.27, and 66.32, respectively **(Al-Ahdab, 2015).** Ginger has a remarkable effect on serum glucose concentration and insulin level in alloxan-diabetic rats. Administration of ginger extract at 500 and 1000 mg/kg of diabetic female rats for 21 days reduced blood glucose levels very significantly ($P<0.001$) in rats with 500 mg ginger/kg b.wt. as well as 1000 mg ginger/kg b.wt. (**Al-qudah *et al.,* 2016**). Type 1 diabetic mice showed significantly decreased blood glucose when treated with 10 mg/kg of 6-shogaol (the most abundant bioactive compound in ginger). 6-Shogaol therapy prevented the diabetes-induced changes in the area of the islet, or whole pancreas, composed of insulin-positive cells. 6-shogaol treatment was slightly more effective than insulin **(Yi *et al.,* 2016).**

Oral administration of swertiamarin (an active compound of *Enicostemma littorale*) at 50 mg/kg b.wt. showed a highly significant ($p<0.01$) dose-dependent fall in blood glucose levels of 79.76%, and glibenclamide (2.5 mg/kg b.wt.) showed a fall of 77.14% in comparison with diabetic rats after 28 days **(Dhanavanthy, 2015).** 400 mg/kg of polyherbal antidiabetic tablets (*Gymnema sylvestre, Momordica charantia, Phyllanthus amarus, Ocimum sanctum, Trigonella foenum-graecum,* and *Allium sativum*) treated diabetic rats showed a significant reduction in blood sugar **(Suman *et al.,* 2016)**. The increased serum glucose level was significantly decreased by treatment with Ojamin (aqueous extracts of fourteen herbs), Metformin, and Ojamin-Metformin. Ojamin-Metformin treatment was found to be less effective than Ojamin and Metformin alone in this regard **(Chaudhari *et al.,* 2017)**. Oral administration of polyherbal formulation (aqueous extracts of *Momordica charantia, Syzygium cumini, Acacia nilotica, Elettaria cardamomum, Cicer arietinum,*

Foeniculum vulgare, and *Gymnema sylvestre*) at doses of 200, 400, and 600 mg/kg in treated groups significantly (P≤0.01) reduced the serum glucose after 8 weeks. This decrease in glycemia could be associated with an enhancement in the insulin level because of the positive impact of the flavonoids present in the formulation **(Majeed *et al.,* 2018).** Treatment with a polyherbal extract mixture (*Allium cepa, Trigonella foenum-graecum, Tinospora cordifolia, Gymnema sylvestre, Syzygium cumini,* and *Momordica charantia*) at doses of 100 and 200 mg/kg for 28 days significantly ($p<0.05$) reduced blood glucose levels, especially at the 4^{th} week. Blood glucose levels were reduced up to 41.97 and 35.48% on day 29 compared to those values on day 22 in diabetic rats treated with a polyherbal extract mixture at 100 and 200 mg/kg, respectively. A polyherbal extract mixture with alkaloids, flavonoids, gallic acid, saponins, steroids, tannins, and insulin-like peptides might be responsible for such properties **(Ahmed *et al.,* 2018).** Graded doses (200, 400, and 600 mg/kg) of herbal formulation extract (*Artemisia absinthium, Bunium persicum, Caesalpinia bonduc, Citrullus colocynthis, Cuminum cyminum, Gymnema sylvestre, Sphaeranthus indicus,* and *Swertia chirata*) in diabetic-treated rats significantly (P≤0.01) recovered the fasting and serum glucose levels in a dose-dependent manner. The antihyperglycemic potential of the formulation may be due to the presence of phytoconstituents like quercetin, kampherol, and gallic acid **(Iftikar *et al.,* 2019).** Blood glucose was significantly (P<0.05) decreased in diabetic rats treated with either metformin or a turmeric, ginger, and cinnamon combination (300 mg/kg b.wt. for 6 weeks). No significant differences were seen between metformin and turmeric, ginger, and cinnamon combination-treated rats. These spices may increase pancreatic β-cell viability and protect them by reactivating the antioxidant defense system (**Moosavi *et al.,* 2020).** In a study, when diabetic rats were treated with oral gavage of 6-gingerol (10 mg/kg b.w.) for 8 weeks, the fasting blood glucose levels were significantly decreased ($p<0.05$). Treatment with 6-gingerol restored the fasting blood glucose level of diabetic rats **(Almatroodi *et al.,* 2021).** The blood glucose of the diabetic-treated group with an alcoholic extract of ginger and garlic (a dose of 500 mg/kg b.wt. for 10 days) together reduced the blood glucose of mice by 35.75%. While comparing with Glibenclamide, it was found that there were no significant differences in the blood glucose because it was lower than the blood glucose when treated with Glibenclamide **(Fadil *et al.,* 2021).** The PHF contains the extracts of *Syzygium cumini, Annona squamosa, Momordica charantia, Tinospora cordifolia, Gymnema sylvestre,* and *Curcuma longa,* which showed antidiabetic activity. Treatment of PHF, metformin, and a combination of PHF and metformin showed sustained and significant (P<0.05) lowers in blood glucose levels of diabetic rats at doses of 225 mg/kg and 450 mg/kg of PHF, respectively, as compared with the diabetic control group **(Gawali and Vyawahare, 2021).**

Daily treatment with diaronil for 3 weeks results in a fall in blood glucose levels. Diaronil at a dose of 400 mg/kg showed more significant antihyperglycemic effects as compared to the 200 mg/kg dose. The maximum antidiabetic effect was seen after the third week of treatment. Both doses of diaronil are effective over hyperglycemia **(Amrutha Raj *et al.,* 2022).** The methanol stem bark extract of *Azadirachta indica* was tested for its potency against hyperglycemia by assaying its α-amylase inhibitory potency. The obtained results exhibited positive inhibitory effects, and at a concentration of 0.1 mg/mL, the extract showed its highest value of inhibition (87.61%) when compared to acarbose (56.0%), used as the standard or control drug. Analysis results from *A. indica* showed crude fiber and carbohydrate came in at the highest concentrations of 33.75% and 32.49%, respectively. This high content of fiber has been opined to be associated with a decreased risk of diabetes. Fiber may also guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus **(Anarado *et al.,* 2022).** In a study, the eight different plant formulations (F1 to F8) were formed by the mixtures of three plants: *Azadirachta indica* leaves, *Tinospora cordifolia* stem, and *Ocimum sanctum* leaf extracts. When F1 to F8 formulations were administered at a dose of 20 ml/kg body weight to the diabetic rats, a significant decrease in blood glucose level was observed at the end of the study. The polyherbal

formulation showed an anti-diabetic effect in the following order: F7 > F4 > F8 > F5 > F6 > F1 > F2 > F3. The F7 has higher anti-diabetic activity compared to the other polyherbal formulations. It was also found that the formulations (F1 to F3) containing a single plant extract exhibited lower therapeutic efficacy than the polyherbal formulations (F4 to F8). The higher therapeutic efficacy of the polyherbal formulation is due to the synergistic effect of the different phytoconstituents in the plant mixtures (**Bhaskarrao *et al.,* 2022).** Treatment with poly-herbal powder [*Eugenia jambolana* (seeds), *Trigonella foenum-graecum* (whole plant), *Aegle marmelos* (leaves), *Cassia auriculata* (flowers), *Marsilea quadrifolia* (whole plant), *Mangifera indica* (leaves), and *Musa paradisiaca* (flower)] at a dose of 100 mg/kg and 200 mg/kg for diabetic rats significantly ($p<0.001$) reduces blood glucose levels. The effect of PHP was found to be maximum at the end of the 28th day and also on par with that of standard glibenclamide, respectively **(Manorama *et al.,* 2022).** In an in-vivo study, dried raw powders of *Cassia auriculata* leaf, *Centella asiatica* leaf, and *Zingiber officinale* rhizome were combined in three different ratios (a dose of 200-400 mg/kg b.wt.) to make polyherbal formulation (PHF), and allopolyherbal formulation (APHF) was prepared by combining PHF with metformin in three different ratios (200+22.5, 200+45, and 200+67.5 mg/kg b.wt). PHF and APHF were administered to albino rats for 21 consecutive days. PHF and APHF showed a significant ($p<0.05$) reduction in blood glucose levels in a dose- and time-dependent manner. PHF and APHF both showed synergistic anti-diabetic efficacy. Among all PHFs, PHF B had the most noticeable outcomes, while among APHFs, APHF C had the most prominent activity. As compared to metformin, APHF C was considerably more effective in lowering blood glucose levels (**Alhamhoom *et al.,* 2023).** The rats groups that were treated with 100 mg/kg and 200 mg/kg b.wt. of a combination of *A. paniculata* and *A. sativum* showed the highest significant reduction ($p<0.05$) in the fasting blood glucose concentration on days 20 and 28, which is 50.58% and 52.62%, respectively (**Nonso *et al.,* 2023).** The glutathione-enriched polyherbal formulation (GEF) was prepared by combining glutathione (100mg), leaf powder of *Murraya koenigii* (85 mg), grape seed extract (50 mg), *Capsicum frutescens* powder (30 mg), fresh turmeric powder (25 mg), and vitamin C (10 mg). The rise in blood glucose was significantly ($p<0.05$) controlled by GEF (a dose of 400 mg/kg b.wt. orally) supplementation to be near normal as compared to the diabetic control group. Glutathione-enriched polyherbal formulations have a powerful anti-diabetic effect by inhibiting oxidative stress and thus blocking PKC activation (**Sheethal *et al.,* 2023).** Blood glucose levels were significantly and gradually decreased after administration of Insuwin and Insuwin forte to diabetic rats for up to 21 days. Treatment with Insuwin and Insuwin forte significantly lowered blood glucose by 248.5 mg/dL (8%) with Insuwin and 213.5 mg/dL (21%) in Insuwin forte on day 14 and 210.5 mg/dL (22%) in Insuwin and 163.0 mg/dL (40%) in Insuwin forte on day 21. The hypoglycemic effect of Insuwin forte is almost equal to that of glimepiride. These beneficial effects of Insuwin forte might be due to its potential polyherbal combinations **(Thangavel *et al.,* 2024).** All of the above studies are in favor of the results revealed by our study, which found that chosen formulations have antihyperglycemic and antioxidant effects in alloxan-induced diabetic rats.

In this present study, during the daily administration of formulations of plants, weight increased significantly as compared to their normal control group at various durations. In three different formulations, formulation-2 which contains *Allium sativum, Azadirachta indica,* and *Zingiber officinale*, was more effective than the other two formulations.

Similarly, in a study, one group received a 1 ml aqueous extract of garlic (500 mg/kg b.wt.) seven days before alloxan-induction and 14 days after the induction, and another group received normal saline before alloxan-induction and garlic extract after induction. The result showed that pre- and post-administration of garlic extract before alloxan induction and post-administration of garlic extract resulted in a significant increase in body weight in rats. Diabetic rats pre-treated with garlic extract were more effective **(Ojo *et al.,* 2012).** Daily oral administration of aqueous *Allium sativum* extract to diabetic rats for 21 days at doses of 250 and 500 mg/kg significantly improves body weight in diabetic rats. A

high dose (500 mg/kg) was highly significant for diabetic rats **(Douaouya and Bouzerna, 2016).** In a research study, weaner rabbits fed with 3 different diets of neem leaf meal (5%, 10%, and 15%) for 10 weeks increased their body weight significantly ($p<0.05$). The results showed that the average total body weight was gained, and among these doses of neem leaf meal, 10% neem leaf meal was effective **(Unigwe *et al.,* 2016).** When the animals were treated with *Phyllanthus embilica* fruit powder at oral dosages of 200 and 400 mg/kg body weight for 28 days, the body weight of diabetic control rats treated with Amla powder significantly increased and was carried back almost to normal **(Menaga and Jegatheesan, 2015).** Oral administration of *Tamarindus indica* extract to diabetic rats at three dosage levels (100, 200, and 300 mg/kg) for 4 weeks significantly ($P<0.05$) increased body weight. Improvement in body weight was more effective with increasing the dose of *Tamarindus indica* extract. The increase in body weight in the treated groups was dose-dependent **(Al-Ahdab, 2015).** Bodyweight was re-established in albino rats fed with dietary inclusions of ginger rhizome (2% and 4%) for 27 days. Bodyweight was significantly ($p<0.05$) increased in gentamycin-administered rats fed diets supplemented with ginger rhizome (2% and 4%), and the high dose level (4% ginger) was more effective **(Ademiluyi *et al.,* 2012).** Animals treated for 28 days orally with swertiamarin (an active compound of *Enicostemma littorale*) at 50 mg/kg b.wt. and the standard drug glibenclamide showed a significant ($p<0.05$) increase in body weight by 33.49 and 30.08%, respectively, which indicated the reversal of diabetic rats to a normoglycemic condition on treatment with swertiamarin, which was almost similar to the synthetic drug glibenclamide **(Dhanavathy, 2015)**. In a study, a 400 mg/kg dose of polyherbal antidiabetic tablets (*Gymnema sylvestre, Momordica charantia, Phyllanthus amarus, Ocimum sanctum, Trigonella foenum-graecum,* and *Allium sativum*) treated diabetic rats showed a significant increase in body weight (**Suman *et al.,* 2016).** Decreased body weight was significantly improved by treatment with ojamin (an aqueous extract of fourteen herbs) and metformin, but administration of ojamin-metformin significantly ($p<0.001$) decreased body weight (**Choudhari *et al.,* 2017).** Body weight was a significant ($p<0.05$) decrease in diabetic rats in comparison to the normal control, and it was about 51.85%. The weight loss was reduced in metformin-treated rats by approximately 7%, whereas in the groups treated with a therapeutic dose and a 2X therapeutic dose of a polyherbal formulation (*Tinospora cardifolia, Cinnamomum zeylanicum, Curcuma longa, Trigonella foenum-graecum, Azadirachta indica,* and *Piper nigrum*), the weight loss was reduced by 2 and 3%, respectively **(Mali *et al.,* 2019).** Weight reduction was significantly ($P<0.05$) increased in the turmeric, ginger, and cinnamon combination (300 mg/kg b.wt. for 6 weeks)-treated diabetic rats. The reduction in body weight in diabetic rats is partly associated with increased catabolic reactions during diabetes (dehydration, fat and muscle cell degeneration, and frequent conversion of glycogen to glucose). It seems that these spices reduce protein and fat catabolism by improving glucose metabolism and reversing proteinuria (**Moosavi *et al.,* 2020).**

In a study, when diabetic rats were treated with oral gavage of 6-gingerol (10 mg/kg b.w.) for 8 weeks, this procedure attenuated the weight loss. As compared with the diabetes control rats, the surge in body weight was nearly 26.22% in 6-gingerol-treated diabetic rats **(Almatroodi *et al.,* 2021).** A significant increase in body weight was observed after treatment with alcoholic ginger and garlic extract (a dose of 500 mg/kg b.wt. for 10 days), and it increased animal body weight by 29%. While comparing with glibenclamide, it was found that there were no significant differences in body weight because it was higher than the body weight when treated with glibenclamide **(Fadil *et al.,* 2021).** The PHF contains the extracts of *Syzygium cumini, Annona squamosa, Momordica charantia, Tinospora cordifolia, Gymnema sylvestre,* and *Curcuma longa,* which showed antidiabetic activity. Treatment of PHF alone and in combination with metformin sustained improved body weight as compared to the diabetic control group, whereas oral administration of metformin 500 mg/kg did not improve body weight significantly as compared to the diabetic control group. The PHF-treated diabetic rats with 450 mg/kg and 225 mg/kg prevented the loss of body weight significantly ($P<0.01$) **(Gawali and**

Vyawahare, 2021). In alloxan-induced diabetic rats, body weight gain was recovered by both doses of diaronil and standard glibenclamide (0.6 mg/kg) at 3 weeks. The diaronil dose of 400 mg/kg showed a more significant effect as compared to the dose of 200 mg/kg **(Amrutha Raj *et al.,* 2022).** In a study, eight different plant formulations (F1 to F8) were formed by the mixtures of three plants: *Azadirachta indica* leaves*, Tinospora cordifolia* stem, and *Ocimum sanctum* leaf extracts. The body weight of rats recovered after administration of the polyherbal formulation (F1 to F8) at a dose of 20 ml/kg b.wt. for 28 days (**Bhaskarrao *et al.,* 2022).** Treatment with poly-herbal powder [*Eugenia jambolana* (seeds), *Trigonella foenum-graecum* (whole plant), *Aegle marmelos* (leaves), *Cassia auriculata* (flowers), *Marsilea quadrifolia* (whole plant), *Mangifera indica* (leaves), and *Musa paradisiaca* (flower)] at a dose of 100 mg/kg and 200 mg/kg to the diabetic rats significantly improved the body weights **(Manorama *et al.,* 2022).** In an in-vivo study, dried raw powders of *Cassia auriculata* leaf, *Centella asiatica* leaf, and *Zingiber officinale* rhizome were combined in three different ratios (a dose of 200-400 mg/kg b.wt.) to make polyherbal formulation (PHF), and allopolyherbal formulation (APHF) was prepared by combining PHF with metformin in three different ratios (200+22.5, 200+45, and 200+67.5 mg/kg b.wt.). PHF and APHF were administered to albino rats for 21 consecutive days. The animals' body weight was significantly ($p<0.05$) restored after treatment with PHF and APHF in a time- and dose-dependent manner. In comparison to PHF, APHF had the most promising action. The anti-diabetic impact of APHF is stronger than that of PHF. (**Alhamhoom *et al.,* 2023).** During the treatment period, the test animals showed an increase in body weight that was within the range of their weights before induction. Significant increases ($p<0.05$) were observed when compared to the week-initial to the week-2 groups treated with 200 mg/kg of *A. paniculata* and *A. sativum* and the group pre-treated and treated with 200 mg/kg of *A. paniculata*. The group pre-treated and treated with 100 mg/kg and 200 mg/kg indicated a significant increase ($p<0.05$) in body weight from week 4 to the initial week and the week of induction (week 0) (**Nonso *et al.,* 2023**). The continuous oral administration of a glutathione-enriched polyherbal formulation (GEF-400 mg/kg b.wt. orally) resulted in a remarkable increase in body weight of around 31.8% on the 28th day as compared to the diabetic and glibenclamide-treated groups (**Sheethal *et al.,* 2023).** In a study, Insuwin-treated diabetic rats (194 mg/kg) showed a mildly significant ($p<0.05$) increase in body weight (for up to 21 days), but no significant body weight changes were observed in the Insuwin-forte-treated diabetic rats (188 mg/kg) as compared to the normal rats because the body weight of the Insuwin-forte-treated diabetic rats was almost equal to that of the glimepiride-treated diabetic rats. Body weights were improved in the Insuwin and Insuwin forte treatments. The hypoglycemic effect of Insuwin and Insuwin forte might also be due to the insulin-releasing effect **(Thangavel *et al.,* 2024).** All of the above studies are in favor of prepared formulations, and these were valued for their antidiabetic properties and antioxidant effects in alloxan-induced diabetic albino rats.

CONCLUSION

The antidiabetic activity of different formulations might be due to the specific or synergistic activity of flavonoids and other active phytochemical compounds of the plants. All of these previous studies have shown that under all types of oxidative stress, single plants, formulations, or extracted phytochemical constituents successfully prevent detectable oxidative damage and help to prevent diseases in which oxidative stress plays a causative role. The current study deals with the attempts made to study the therapeutic potential of the aqueous drug extracts obtained from five different medicinal plants (*Allium sativum, Azadirachta indica*, *Phyllanthus emblica, Tamarindus indica,* and *Zingiber officinale*) on the different blood parameters in the albino rats. This study will not only help in understanding the medicinal properties of these medicinal plants but will also enable us to understand the appropriate concentration for effective drug formulations.

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AUTHOR'S CONTRIBUTIONS

The present research work was designed by Dr. Radha Singh. The experiment was performed by Dr. Radha Singh under the supervision of Dr. Kusum Singh and Dr. Vinita Ahirwar.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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