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ORIGINAL ARTICLE

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Multiherbal Formulations of Five Plants Ameliorate the Effects on Liver Glycogen, Serum Protein, and Lipid Profile in Alloxan-Induced Diabetic Albino Rats

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Background: *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. As per the Indian traditional system of medicine, many medicinal plants have been used to manage various health disorders, including *diabetes mellitus*. Therapeutic plants are an important source of medicine, and various active compounds were isolated from the plants. 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 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), and biochemical parameters, viz., glycogen, protein, and lipid profile (serum cholesterol, triglyceride, HDL, LDL, and VLDL) of rats, were measured.

Results: There was a significant ($p<0.01$) decrease in the glycogen and protein levels and a significant ($p<0.01$) increase in the lipid profile (except HDL) in diabetic rats when compared with the normal control. However, treatment of alloxan-injected rats with three different formulations significantly ($p<0.01$) increased the glycogen, and protein levels and significantly ($p<0.01$) decreased the lipid profile (except HDL) 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: antidiabetic properties, medicinal plants, formulations, diabetic rats

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. India is a developing country, with a large portion of the population suffering from *diabetes mellitus* and its complications. The high costs of treatment have compelled us to look for alternative and cost-effective methods to minimize complications associated with diabetes. India has a record of very long, safe, and continuous usage of many herbal drugs in the officially recognized alternative systems of health, *viz.,* Ayurveda, Yoga, Unani, Siddha, Homeopathy, and Naturopathy, and these herbal drugs and their products are more convenient for the treatment of *diabetes mellitus* due to their easy availability, low cost, minimum side effects, and greater acceptance. A large number of Indian medicinal plants have been reported for their antidiabetic effects.

Garlic (*Allium sativum)* is a member of the Alliaceae family, and all parts (inflorescence, leaves, and cloves) have been used since ancient times. Chemical constituents of garlic have been used for the treatment of hyperlipidemia, hypertension, and platelet aggregation **(Singh and Singh 2008)**. The antidiabetic effects of garlic are especially due to the volatile sulfur compounds such as allicin, diallyl disulfide, S-allyl cysteine, and diallyl trisulfide. Garlic is effective in decreasing insulin resistance as well **(Padiya and Banerjee, 2013**). Neem belongs to the family Meliaceae and exhibits many natural active compounds in almost every part of the plant (bark, branches, fruit, leaves, oil, roots, seeds, and trunk) with many curative activities. A mixture of seven isomeric compounds, azadirachtin (azadirachtin A-G), is present in it, which is the biologically most active compound, and azadirachtin E is more effective. Other active substances are azadiractol, azadirone, nimbidin, nimbinin, nimbin, nimboline, and vilosinin, with over 300 isolated and characterized constituents **(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., leaves, roots, and stems, and mostly it is known for the immense activities of fruit. It is an effective antioxidant with the richest natural source of vitamin C (200-900 mg per 100 g of edible portion). The fruit is used either alone or in combination with other plants. It is regularly used in antioxidants, chronic diseases, diabetes, grayness of hair, ophthalmic disorders, ulcers, and increased defense against many other diseases **(Jain *et al.*, 2015).** Tamarind belongs to the family Caesalpiniaceae, which is a sub-family in Leguminosae. It has been stated in *Ashtanga Sangraha, Caraka Samhita,* and *Susruta Samhita* with the name '*Amleeka*' under *Amla varga* (group of sour drugs**)**. It is cultivated mainly for the pulp in the fruit, which is used to prepare a beverage and to flavor sweets, curries, and sauces. The main phenolic compounds of tamarind are tartaric acid, pectin, triterpenes, catechin, epicatechin, procyanidin B2, arabinose, uronic acid, xylose, and many more **(Resny *et al.*, 2018).** Bioactive compounds of *Tamarindus indica* extract have a hypoglycemic effect, which may help suppress free radicals in diabetes. This will decrease blood glucose levels, have a protective effect on pancreatic β-cells, and re-establish plasma insulin levels (**Yerima *et al.,* 2014**). Ginger belongs to the family Zingiberaceae and is used worldwide as a spice, flavoring agent, and herbal remedy. It is used in Ayurveda, Siddha, and many other remedial systems to cure a variety of diseases, viz., asthma, constipation, cough, dyspepsia, indigestion, inflammation, loss of appetite, nausea, pain, palpitation, and vomiting. The plant has characteristic flavor and odor because it contains a mixture of shogaols, gingerols, zingerone, and their derivatives, and many more bioactive compounds. Because of these phytochemical compounds, it has antioxidant, anti-hyperglycemic, anti-cancer, anti-inflammatory, anti-hyperlipidemic, and many other activities **(Grzanna *et al.*, 2005; Momoh *et al.*, 2022).** 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**). 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 specific or synergistic activity of constituents like alkaloids, flavonoids, tannins, phenols, saponins, etc. 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 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.

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±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.

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 at 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). Biochemical parameters, viz., glycogen, protein, and lipid profiles of rats, were measured.

GLYCOGEN ANALYSIS (Seifter *et al.*, 1950)

Principle: The anthrone reaction is the basis of a rapid and convenient method for the determination of glycogen. Digestion of the tissue with a hot concentrated potassium hydroxide (KOH) solution, precipitation of glycogen with ethanol, and hydrolysis of the precipitate with mineral acid, i.e., sulfuric acid, cause the hydrolysis of the glycosidic bonds to give monosaccharide and the determination of the hydrolysate as reducing sugar, which is dehydrated to furfurals and its derivatives. The furfural derivative presumably condenses with anthrone to form a blue-colored complex. The blue-green color formed shows absorption maxima at 620 µm.

Reagents required

(i) 30% Potassium Hydroxide (W/V): Anhydrous potassium hydroxide (30 gm) was dissolved in 100 ml of distilled water.

(ii) 95% Ethanol (V/V): 95 ml of absolute alcohol was taken, and the final volume was made up to 100 ml by adding distilled water.

(iii) 95% Sulphuric Acid (H_2SO_4) (V/V): 5 ml of distilled water was added to 95 ml of sulphuric acid slowly with constant stirring.

(iv) 0.2% Anthrone Reagent (freshly prepared): 200 mg of anthrone was dissolved in 100 ml of 95% sulphuric acid (H_2SO_4).

(v) Standard glucose solution (20 µg/ml): 20 µg glucose solution was dissolved in 1 ml of distilled water.

Procedure: 1 ml of 30% KOH was taken in an injection vial and weighed. To it, fresh tissue was added immediately after dissection, and the vial was reweighed. The difference between the two weightings gave the weight of tissue taken. The contents from the injection vial were transferred to another tube and digested in a boiling water bath for 10 minutes with frequent stirring. The tubes were cooled, and 1.25 ml of 95% ethanol was added. The solution in the tubes was brought to a boil and then cooled. The tubes were centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded, and the precipitate was dissolved in 1 ml of distilled water. To this, 1.25 ml of 95% ethanol was again added, and the tube was brought to a boil. The tubes were cooled and centrifuged at 2000 rpm for 15 minutes, and the supernatant was discarded. Finally, the precipitates were dissolved in 5 ml of distilled water. For preparing the blank, 5 ml of distilled water in a tube was taken, and for the standard, 5 ml of 20 µg/ml glucose solution was taken in another tube. Tubes were kept in

ice-cold water, and to them, 10 ml of 0.2% freshly prepared anthrone was added. Tubes were covered with marbles and placed in a boiling water bath for 10 minutes. The tubes were cooled, and the optical density was read at 620 nm after setting the spectrophotometer to 100% absorbance with the blank.

The glycogen amount was calculated in mg/100 ml as follows:

$$GA = \frac{\frac{100}{1.11} \times \frac{\text{O.D. of Unknown}}{\text{O.D. of Known}}}{\text{Weight of tissue in mg}} \times 100$$

Here, 1.11 is the factor determined by Moris for the conversion of glucose to glycogen.

PROTEIN ANALYSIS (Lowry *et al.*, 1951)

Principle: Protein reacts with the Folin-Coicalteau reagent to give a colored complex. As in the biuret test, the color is formed due to the reaction of the protein with alkaline copper and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of color depends on the amount of these aromatic amino acids present and various proteins.

Reagents required

(i) 10% trichloroacetic acid (V/V): 10 gm was dissolved in 100 ml of distilled water.

(ii) 0.2 N NaOH: 0.8 gm anhydrous salt was dissolved in 100 ml of distilled water.

(iii) Solution A: Anhydrous copper sulfate ($CuSO_4$ 500 mg) and sodium potassium tartrate (300 mg) were dissolved in a small quantity, and then the final volume was made up to 500 ml with distilled water.

(iv) Solution B: Dissolving 8.0 gm of sodium carbonate in a 0.1 N NaOH solution, and the solution is prepared in 100 ml of distilled water.

(v) Folin's Reagent: Diluted with distilled water to make a final strength of 1 N.

Procedure: 0.2 ml of the tissue homogenate was taken in a test tube, to which 0.2 ml of 10% TCA was added. It was left for 15 minutes for precipitation. Each tube was centrifuged for 15 minutes at 2000 rpm. After centrifugation, the supernatant was discarded, and the precipitate was dissolved exactly in 1 ml of 0.2 N NaOH. In a separate tube, 0.1 ml of the test solution was taken, and for the blank and the standard, 0.5 ml of distilled water and 0.1 ml of bovine serum albumin (BSA) were taken, respectively. To each tube, 0.4 ml of distilled water was added to make the final volume up to 0.5 ml. Now in each tube, 5 ml of the mixture of solution A and solution B in the ratio of 1:1 was added, and the tubes were left as such for 10 minutes. Finally, 0.5 ml of 1 N Folin's reagent was added to each tube. After half an hour, the reading was taken at 625 nm in a spectrophotometer after setting it at 100% absorbance with the blank.

The total protein amount was calculated in mg/100mg as follows:

$$PA = \frac{\text{O.D. of Unknown}}{\text{O.D. of Known}} \times 20$$

LIPID PROFILE

(I) SERUM CHOLESTEROL (Zlatkis *et al.*, 1953)

Principle: In chloroform solution, acetic anhydride reacts with cholesterol to produce a characteristic blue-green color. The exact nature of the chromophore is not known, but the reaction probably includes esterification of the hydroxyl group in the third position as well as other rearrangements in the molecule.

Chemical required

(i) Ethanol-Ether Mixture: 1:1 (v/v)

(ii) Chloroform-Methanol Mixture: 2:1 (v/v)

(iii) Glacial Acetic Acid: Prepared Solution.

(iv) Ferric chloride solution (freshly prepared): 0.1 gm $FeCl_3$ was dissolved in 1 ml of glacial acetic acid, raising the volume to 100 ml by adding concentrated sulfuric acid.

Procedure: Freshly prepared tissue homogenate (2 ml) was centrifuged at 2000 rpm for 10 minutes. The sediments were extracted with 1 ml of an ethanol-ether mixture, and the supernatants were decanted into a separate tube. Complete extraction was ensured by repeating the whole procedure, and the filtrate collected was marked No. 1. Residue was re-extracted twice with 1 ml of a chloroform-methanol mixture, and the filtrate was marked No. 2. Suitable aliquots from filtrate 1 and 2 were taken separately in tubes, respectively, to estimate total and esterified cholesterol levels. 0.5 ml of filtrate was taken, and to it, 2.5 ml of glacial acetic acid and 0.1 ml of distilled water were mixed, and later, 2 ml of 1%

$FeCl_3$ was added to develop the color. The contents were mixed, and after an hour, the optical density was determined by a spectrophotometer at 560 nm.

The level of cholesterol was finally computed by using the following formula and expressed as mg/100 mg.

$$SC = \frac{\text{O.D. of Unknown}}{\text{O.D. of known}} \times \frac{0.2 \times 100 \times \text{m.f.}}{\text{Wt. of tissue taken}}$$

Where m.f. is the multiplying factor that depends upon dilution.

(II) TRIGLYCERIDE (McGowan *et al.*, 1983; Herbert, 1984; Roberts *et al.*, 2008)

Method: GPO-PAP, endpoint assay: (Arkray Healthcare Pvt. Ltd.)

Principle: Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to produce glycerol and free fatty acids (FFA). In the presence of glycerol kinase (GK), adenosine triphosphate (ATP) phosphorylates glycerol to produce glycerol-3-phosphate and adenosine diphosphate (ADP). Further, glycerol-3-phosphate oxidase (GPO) oxidized glycerol-3-phosphate to produce dihydroxyacetone phosphate (DAP) and H_2O_2. In the presence of peroxidase (POD), hydrogen peroxide couples with 4-aminoantipyrine (4-AAP) and 4-chlorophenol to produce red quinoneimine dye. The absorbance of colored dye is measured at 505 nm (490-550 nm) and is proportional to the triglyceride concentration in the sample.

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{4-Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Red quinoneimine Dye} + 4H_2O$$

(Table 2 and Table 3) Mix well, and incubate at 37 °C for 10 minutes. Program the analyzer as per the assay parameters.

1. Blank the analyzer with the reagent blank.

2. The absorbance of the standard is measured, followed by the test.

3. Calculate the results as per the given calculation formula.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 200$$

(III) HIGH DENSITY LIPOPROTEIN (HDL-C) (Burstein *et al.*, 1970)

Method: Phosphotungstic acid method, endpoint (Transasia Bio-medicals Ltd.)

Principle: Chylomicrons, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using the Erba cholesterol reagent.

$$\text{Serum / Plasma} \xrightarrow{\text{Phosphotungstate}} \underset{\text{(Supernatant)}}{\text{HDL}} + \underset{\text{(Precipitate)}}{(\text{LDL} + \text{VLDL} + \text{Chylomicrons})}$$

(Table 4 and Table 5) Mix well; incubate for 10 min. at 37°C or 12 min. at 30°C. Read the absorbance of the standard and each test at 505 nm or 505/670 nm for bichromatic analyzers against the reagent blank.

Calculation:

$$\underset{\text{(mg/dl)}}{\text{HDL cholesterol}} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times \underset{\text{(mg/dl)}}{\text{Concentration of Standard}} \times \text{Dilution factor}$$

(IV) AND (V) LOW-DENSITY LIPOPROTEIN (LDL-C) AND VERY LOW-DENSITY LIPOPROTEIN (VLDL-C) (Friedewald *et al.*, 1972)

For LDL cholesterol, using the Friedewald equation

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol}$$

$$\text{VLDL Cholesterol} = \frac{\text{Triglycerides}}{5}$$

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

Glycogen and Protein levels

In the present study, glycogen and protein levels 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 glycogen and protein levels increased significantly ($p<0.01$) as compared to the normal control group and standard group and recouped towards the normal group **(Table 6)**. The most significant increase in glycogen levels was obtained after 45 days, and in protein levels, it was obtained after 30 and 45 days of three different formulations of dose administration, especially in formulation 2, which was more effective in comparison to the other two formulations and exhibited an antihyperglycemic effect.

Lipid Profile (serum cholesterol, triglyceride, HDL, LDL, and VLDL)

In the present study, the lipid profiles increased (except HDL) 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 lipid profile (except HDL) decreased significantly ($p<0.01$) as compared to the normal control group and standard group and recouped towards the normal group **(Table 7, 8)**. HDL increased significantly ($p<0.01$) as compared to the normal control group and standard group in formulation 1 and formulation 3, but in formulation 2, HDL increased significantly ($p<0.05$) as compared to the normal control group and standard group. The most significant increase in lipid profiles was obtained after 30 and 45 days of three different formulations of 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.

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: Reagents composition

Reagent No.	Reagent	Composition	Concentration
1.	Triglyceride mono reagent	Pipes buffer	50.0 mmol/l
		4-Chlorophenol	5 mmol/l
		Magnesium ion	5 mmol/l
		ATP	1.0 mmol/l
		Lipase	≥ 5000 IU/l
		Peroxidase	≥ 1000 IU/l
		Glycerol kinase	≥ 400 IU/l
		4-Aminoantipyrine	0.4 mmol/l
		Glycerol 3-phosphate oxidase	≥ 4000 IU/l
		Detergents	Quantity sufficient
		Preservative	Quantity sufficient
		Activators & stabilizers	Quantity sufficient
2.	Triglyceride Standard	Triglyceride	200 mg/dl
		Stabilizers	Quantity sufficient
		Preservative	Quantity sufficient

Table 3: Procedure

Pipette into a tube marked	Reagent Blank	Standard	Test
Serum / Plasma	--	--	10 µl
Regent 2	--	10 µl	--
Regent 1	1000 µl	1000 µl	1000 µl

Table 4: Reagents composition

Reagent No.	Reagent	Composition	Concentration
1.	Precipitating reagent	Phosphotungstatic Acid	2.4 mmol/l
		Magnesium Chloride	40 mmol/l
2.	HDL cholesterol Standard	HDL cholesterol Standard	25 mg/dl

Table 5: Procedure

Pipette into a tube marked	Reagent Blank	Standard	Test
Cholesterol Working reagent	1000 µl	1000 µl	1000 µl
Distilled Water	50 µl	--	--
HDL cholesterol Standard	--	50 µl	--
Supernatant	--	--	50 µl

Table 6: Showing variations in Glycogen (mg/100mg) and Protein (mg/dl) among different experimental groups due to daily administration of different formulations (Values are expressed as Mean ± SEM, where N = 6)

Groups	Duration of Treatment	Glycogen (mg/100mg)	Protein (mg/dl)
Group I (Normal Control)	15 days	36.40±0.186	7.47±0.036
	30 days	35.95±0.201	7.34±0.043
	45 days	35.85±0.111	7.31±0.032
Group II (Diabetic Control)	15 days	23.00±0.141^{ab}	5.52±0.023^{ab}
	30 days	21.00±0.157^{ab}	5.31±0.021^{ab}
	45 days	18.70±0.201^{ab}	5.08±0.025^{ab}
Group III (Diabetic + Glibenclamide)	15 days	31.00±0.157^{a}	6.45±0.015^{a}
	30 days	33.00±0.129^{a}	6.70±0.013^{a}
	45 days	34.00±0.273^{a}	6.93±0.022^{a}
Group IV (Diabetic + formulation 1)	15 days	25.70±0.141^{ab}	6.00±0.028^{ab}
	30 days	27.80±0.163^{ab}	6.09±0.033^{ab}
	45 days	29.50±0.182^{ab}	6.20±0.031^{ab}
Group V (Diabetic + formulation 2)	15 days	29.60±0.131^{ab}	6.27±0.012^{ab}
	30 days	32.00±0.186^{ab}	6.62±0.028$^{a•}$
	45 days	34.65±0.143$^{a•}$	6.94±0.047$^{a•}$
Group VI (Diabetic + formulation 3)	15 days	27.40±0.118^{ab}	6.10±0.026^{ab}
	30 days	29.70±0.163^{ab}	6.20±0.034^{ab}
	45 days	31.50±0.173^{ab}	6.33±0.033^{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 mean ± SEM for six rats each.

Table 7: Showing variations in Lipid profile: Serum cholesterol, Triglyceride, and HDL, among different experimental groups due to daily administration of different formulations (Values are expressed as Mean ± SEM, where N = 6)

Groups	Duration of Treatment	Lipid profile (mg/dl)		
		Cholesterol	Triglyceride	HDL
Group I (Normal Control)	15 days	93.55±0.32	51.40±1.85	65.65±0.53
	30 days	94.06±0.72	53.50±1.92	64.58±0.78
	45 days	94.58±0.60	54.88±1.59	64.91±0.57
Group II (Diabetic Control)	15 days	160.51±0.80^{ab}	152.93±0.83^{ab}	38.25±0.29^{ab}
	30 days	169.82±1.24^{ab}	165.18±0.99^{ab}	35.03±0.32^{ab}
	45 days	180.36±1.25^{ab}	179.38±1.12^{ab}	31.48±0.34^{ab}
Group III (Diabetic + Glibenclamide)	15 days	115.55±0.79^{a}	93.25±1.60^{a}	54.66±0.62^{a}
	30 days	108.70±0.26^{a}	78.75±1.11^{a}	58.45±0.40^{a}
	45 days	103.40±0.81^{a}	67.95±1.51^{a}	61.05±0.57^{a}
Group IV (Diabetic + formulation 1)	15 days	143.08±1.07^{ab}	124.43±0.94^{ab}	43.23±0.46^{ab}
	30 days	134.30±1.09^{ab}	112.75±1.75^{ab}	47.34±0.59^{ab}
	45 days	123.65±1.05^{ab}	98.83±1.33^{ab}	52.53±0.64^{ab}
Group V (Diabetic + formulation 2)	15 days	121.50±0.68^{ab}	97.25±1.77$^{a•}$	52.08±0.81^{ad}
	30 days	110.80±0.48$^{a•}$	80.70±1.58$^{a•}$	57.33±0.64$^{a•}$
	45 days	101.80±0.90$^{a•}$	66.98±1.43$^{a•}$	62.43±0.63$^{c•}$
Group VI (Diabetic + formulation 3)	15 days	133.61±0.96^{ab}	113.36±1.63^{ab}	47.45±0.68^{ab}
	30 days	122.25±1.09^{ab}	98.41±1.78^{ab}	52.75±0.62^{ab}
	45 days	112.50±0.67^{ab}	82.61±1.45^{ab}	57.83±0.57^{ab}

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

Table 8: Showing variations in Lipid profile: LDL and VLDL among different experimental groups due to daily administration of different formulations (Values are expressed as Mean ± SEM, where N = 6)

Groups	Duration of Treatment	Lipid profile (mg/dl)	
		LDL	VLDL
Group I (Normal Control)	15 days	17.62±0.50	10.28±0.37
	30 days	18.78±1.12	10.70±0.38
	45 days	18.69±0.85	10.97±0.31
Group II (Diabetic Control)	15 days	91.60±0.99^{ab}	30.65±0.13^{ab}
	30 days	101.75±1.36^{ab}	33.03±0.19^{ab}
	45 days	113.00±1.37^{ab}	35.87±0.22^{ab}
Group III (Diabetic + Glibenclamide)	15 days	42.23±1.09^{a}	18.65±0.32^{a}
	30 days	34.50±0.44^{a}	15.75±0.22^{a}
	45 days	28.76±1.08^{a}	13.59±0.30^{a}
Group IV (Diabetic + formulation 1)	15 days	74.96±1.34^{ab}	24.88±0.18^{ab}
	30 days	64.40±1.33^{ab}	22.55±0.35^{ab}
	45 days	51.35±1.43^{ab}	19.76±0.26^{ab}
Group V (Diabetic + formulation 2)	15 days	49.96±1.13^{ab}	19.45±0.35$^{a•}$
	30 days	37.32±0.80$^{a•}$	16.14±0.31$^{a•}$
	45 days	25.97±1.07$^{a•}$	13.39±0.28$^{a•}$
Group VI (Diabetic + formulation 3)	15 days	63.49±1.32^{ab}	22.67±0.32^{ab}
	30 days	49.81±1.36^{ab}	19.68±0.35^{ab}
	45 days	38.14±0.95^{ab}	16.52±0.29^{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 mean ± SEM for six rats each.

DISCUSSION

Glycogen is the main biochemical component of a cell. It is vital to complete many physiological activities as a main source of energy. The liver is considered a metabolic center for the synthesis of glucose from stored glycogen and is used whenever it is required. Although the breakdown of glycogen into glucose and vice versa is a normal phenomenon in the liver, when any toxicant or xenobiotic enters the liver, it disturbs glycogen synthesis. Many xenobiotics and chemicals are known to alter the glycogen level in the liver and kidney **(Chandra and Sadiqui 1987).**

In the present study, the content of glycogen was significantly increased due to the daily administration of plant formulations 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 in comparison to the other two formulations.

S-allyl cysteine (SAC) is a sulfur-containing amino acid derived from garlic and has been reported to have antidiabetic and antioxidant activities. The SAC was administered orally (150 mg/kg b.wt.) to normal and STZ-diabetic rats for 45 days. Treatment of SAC in diabetic rats significantly stimulates the secretion of insulin, thereby reestablishing the glycogen content of the liver by reducing glycogen phosphorylase activity and enhancing glycogen synthase activity **(Saravanan *et al.*, 2009).** When an oral dose (250 mg/kg b.wt.) of aqueous homogenate of fresh raw garlic was administered simultaneously in Wistar rats for 7 days, it could inhibit nickel II- or chromium VI-induced changes, exerting a hepatoprotective effect on glycogen levels and antioxidant status in male albino rats. Study results show that liver glycogen concentration significantly ($p<0.05$) increased in rats **(Gupta *et al.*, 2009).** The supplementation of a chloroform extract (100 μg/200 μl) of *Azadirachta indica* was injected intraperitoneally in diabetic mice and showed significant enhancement in hepatic and skeletal muscle glycogen content after 21 days of treatment. This shows that plant extract has a direct effect on muscle and liver glucose metabolism, which helps in the inhibition of muscle mass loss in diabetes **(Bhat *et al.*, 2011).** The aqueous extracts of *Azadirachta indica* leaf, bark, and seed oil have been assessed for their long-term effects on alloxan-induced diabetic rats. The oral effective dose of *Azadirachta indica* leaf extract (500 mg/kg b.wt.), *Azadirachta indica* bark extract (100 mg/kg b.wt.), and *Azadirachta indica* seed oil (5 ml/kg b.wt.) was given once daily for two months. The decrease in glycogen content in hepatic tissue and skeletal muscle of the *Azadirachta indica* leaf extract-treated (17.9% and 29.3%), *Azadirachta indica* bark extract-treated (22.4% and 36.2%), and *Azadirachta indica* seed oil treated diabetic rats were much less ($p<0.05$) than in the untreated diabetic controls, showing that the flawed glycogen storage of the diabetic state was moderately corrected by the treatment. Two-month treatment of diabetic rats with *Azadirachta indica* leaf extract, *Azadirachta indica* bark extract, and *Azadirachta indica* seed oil enhanced the glycogen level, which may be due to the revival of the glycogen synthase system involved in glycogenesis in muscle and liver tissue **(Shailey and Basir 2011).** Oral administration of hyponidd (a herbo-mineral formulation composed of ten medicinal plant extracts, containing *Emblica officinalis*) at doses of 100 mg/kg and 200 mg/kg for 45 days. Hyponidd-treated diabetic groups showed significant ($P<0.05$) increases in liver glycogen, possibly because of the reactivation of the glycogen synthase system as a result of enhanced insulin secretion. The effect of hyponidd at a dose of 200 mg/kg was more effective than that of glibenclamide (600 µg/kg) in restoring the values to near normal **(Babu and Prince 2004).** *Emblica officinalis* fruit powder was assimilated into the diet (2.5, 5, and 10 gm %) of fluoride-exposed animals for 30 days. An inclusion of *Emblica officinalis* fruit powder significantly improved hepatic glycogen content activity. The enhancement of hepatic glycogen content activity was more detectable with an increase in the dose of *Emblica officinalis* fruit powder. *Emblica officinalis* fruit powder supplementation reversed this trend in a dose-dependent manner, indicating the hepatic restoratory effect of *Emblica officinalis* fruit **(Narsimhacharya and Vasant 2012).**

The mild and severe diabetic rats were fed an aqueous extract of the seed of *Tamarindus indica* at doses of 80 mg and 120 mg/0.5 ml distilled water/100g b.wt./day/rat, respectively, for 14 days. After 14 days of an aqueous extract of the seed of *Tamarindus indica* supplementation in mild and severe diabetic rats, there was a significant increase in liver and skeletal muscle glycogen levels, and this was shifted towards the control level **(Maiti *et al.*, 2005).** The effects of constant oral administration of a hydroalcoholic extract of *Tamarindus indica* leaves and an aqueous extract of *Tamarindus indica* leaves in STZ-diabetic rats at doses of 100 and 200 mg/kg were determined at 0, 7, and 14 days of treatment. Both extracts of *Tamarindus indica* leaf doses caused a significant ($P<0.05$) increase in hepatic glycogen levels **(Meher and Dash 2013).** Daily oral administration of 500 mg/kg body weight of free and bound polyphenols from *Zingiber officinale* to STZ-induced diabetic rats significantly increased ($P<0.05$) liver glycogen in 28 days. The results from this study have shown that both free and bound polyphenols from *Zingiber officinale,* mainly the free polyphenol, could improve liver disorders caused by diabetes mellitus in rats **(Kazeem *et al.*, 2013).** The hepatic glycogen content of Type 2 diabetic rats was studied after 42 days of chronic oral administration (on the 42nd day) of 50% ethanol extract (1.25 g/10 ml/kg b.wt.) of *Zingiber officinale* for six weeks. It was observed from this study that hepatic glycogen content was significantly increased ($p = 0.002$) after 42 days, and it may be as certain that the hypoglycemic activity of *Zingiber officinale* in type 2 rats is due to increased uptake of glucose for the formation of glycogen by enhanced glycogenesis **(Shadli *et al.*, 2014).** Diabetic rats treated with a 400 mg/kg dose of the polyherbal antidiabetic tablet (*Gymnema sylvestre, Momordica charantia, Phyllanthus amarus, Ocimum sanctum, Trigonella foenum-graecum,* and *Allium sativum*) showed a significant decrease in liver glycogen levels, but muscle glycogen levels increased significantly **(Suman *et al.*, 2016).** Decreased liver glycogen content was significantly increased by treatment with Ojamin (aqueous extracts of fourteen herbs), Metformin, and Ojamin-Metformin. Ojamin, Metformin, and Ojamin-Metformin were equipotent in this regard **(Choudhari *et al.*, 2017).** A reduction in glycolytic enzyme activities might be responsible for the depletion of glycogen content in diabetic rats. Oral administration of polyherbal formulations (aqueous extracts of *Momordica charantia, Syzygium cumini, Acacia nilotica, Elettaria cardamomum, Cicer arietinum, Foeniculum vulgare,* and *Gymnema sylvestre*) at doses (200, 400, and 600 mg/kg) in treated groups significantly ($P\leq0.01$) increased the liver glycogen concentration after 8 weeks **(Majeed *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 sylvestra, Sphaeranthus indicus,* and *Swertia chirata*) in diabetic treated rats significantly ($P\leq0.01$) recovered liver glycogen **(Iftikhar *et al.*, 2019).** 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 two doses (100 mg/kg and 200 mg/kg) in the diabetic rats demonstrated a significantly elevated glycogen content, which was on par with that of standard glibenclamide. This elevated level of glycogen was mainly attributed to an increase in peripheral uptake of glucose by the stimulated insulin released from pancreatic β-cells. The phytoconstituents present in the PHP play a major role in mimicking insulin action, thereby promoting the entry of glucose into the tissues. **(Manorama *et al.,* 2022).** In the present study, a combined herbal aqueous extract of *Tribulus terrestris* and *Curcuma amada* was administered to diabetic-induced rats for 37 days. The liver glycogen level is found to be lower in diabetic rats (23.14 ± 2.14 mg/g). At the same time, combined TT and CA administration significantly recovered the glycogen level (34.94 ± 4.14 mg/g), which is equally significant to the glibenclamide-treated group (35.54 ± 4.12 mg/g), and the combined formulation of TT and CA showed a better antidiabetic effect **(Kaliaperumal *et al.*, 2024).** All of these earlier findings support our results that selected formulations improved hepatic glycogen levels in alloxan-induced diabetic rats. Our results are in accordance with these previous studies**.**

Proteins are considered the building blocks of tissues. This parameter shows a great degree of alteration in both the vital and reproductive organs. Several toxicants of chemical or synthetic origin are known to reduce protein contents through the process of degradation. As these compounds stimulate significant changes in physiological functions, it is expected that these changes are maintained through the conversion or degradation of new proteins.

In the present study, during the daily administration of formulations, protein content was significantly increased as compared to the normal control group at various durations. Out of three formulations, formulation 2, which contains *Allium sativum, Azadirachta indica,* and *Zingiber officinale*, was more effective in increasing protein contents compared to the other two formulations.

Similar results were reported where STZ-induced diabetic rats were treated with S-allyl cysteine (SAC), a garlic constituent, at two different doses (100 and 150 mg/kg b.wt.) for 45 days. Treatment with SAC significantly increased the levels of protein and maintained the protein level near normal. A 150 mg/kg b.wt. dose produced a better effect than a 100 mg dose **(Saravanan *et al.*, 2009).** Azadirachtin, a tetra or triterpenoid class of limonoids, is found principally in the seeds of the neem tree (*Azadirachta indica*). Pre-treatment with azadirachtin-A at 100 and 200 µg (kg body mass) doses showed significant dose-dependent increases ($P \leq 0.05$) in total protein levels compared with the CCl_4-treated group and were observed to restore the levels of total protein to normal. The fact that pre-treatment with azadirachtin-A inhibited a decrease in total protein levels caused by CCl_4-induced hepatotoxicity shows the hepatoprotective nature of this active component **(Baligar *et al.*, 2014).** In a study, *Emblica officinalis* fruit extract orally administered (250 mg/kg b.wt./day) to alcohol-administered rats for 60 days significantly ($p<0.05$) increased plasma total protein levels. Results showed that remedies for alcoholism, including phytochemicals and the use of natural extracts from plant foods, are capable of decreasing the risk of oxidative stress. This might be due to the free radical and hydroxyl radical scavenging activity of tannoid compounds present in *Emblica officinalis* fruit extract **(Reddy *et al.*, 2010).** When adult female Wistar rats were treated daily for 45 days with sodium fluoride in drinking water, alone or in combination with tamarind pulp (20 mg/kg b.wt. by oral intubation), tamarind stabilized the toxic effects of sodium fluoride (NaF) by enhancing the protein content in the serum. The concentration of F was decreased markedly ($p<0.01$) in the serum of NaF+ and tamarind-treated animals, showing that tamarind pulp can inhibit free radical-induced oxidative stress by F, attributable to its antioxidant activity **(Ekambaram *et al.*, 2010).** Similarly, administration of paracetamol significantly reduced serum total protein. When rats were fed a 1% ginger powder-containing diet one week earlier, along with oral administration of paracetamol for three weeks, they significantly increased serum total protein **(Lebda *et al.*, 2013).** In a study, ginger powder was added as 7% of the standard diet. Ginger oil (4 ml/kg b.wt.), aqueous ginger extract (500 mg/kg), and methanolic ginger extract (200 mg/kg) were given to diabetic rats orally for 60 days and showed a significant increase in total protein. Diabetic rats treated with ginger oil showed normal values of serum total protein **(Anfenan, 2014).** The level of total protein (30.99%) was increased significantly ($p \leq 0.05$) after oral administration of swertiamarin (the active compound of *Enicostemma littorale*) at a dose of 50 mg/kg in diabetic-treated rats for 28 days **(Dhanavathy *et al.*, 2015)**. The mean value of protein (gm/dl) in the diabetic control group was non-significantly ($p>0.05$) decreased as compared to the control group. Whereas, the mean values of protein (gm/dl) were non-significantly ($p>0.05$) increased in polyherbal extract mixtures (*Allium cepa, Trigonella foenum-graecum, Tinospora cordifolia, Gymnema sylvestre, Syzygium cumini,* and *Momordica charantia*) at doses (100 and 200 mg/kg) for 28 days **(Ahmed *et al.*, 2018).** Oral administration of Poorna Chandrodayam Chendooram (gold, sulfur, and mercurial preparation with *Alovevera brobadensis* and *Hibiscus* extracts) significantly ($p<0.01$) enhanced the total protein level of plasma, and the increased level was 35.24% **(Muthukumaran and Begum 2020).** In an in-vivo study, on the 21st day, diabetic control rats showed significantly decreased protein levels. Dried raw

powders of *Cassia auriculata* leaf, *Centella asiatica* leaf, and *Zingiber officinale* rhizome were combined in three different ratios (a dose of 200 and 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.) were administered to diabetic rats for 21 consecutive days. PHF and APHF showed a significant ($p<0.05$) improvement in protein levels. PHF (A, B, and C) at 400 mg/kg b.wt. and APHF (A, B, and C) exhibit a substantial rise in total protein (**Alhamhoom *et al.,* 2023).** In a study, it was determined that the total protein values decreased in diabetic rats compared to the control group. Oral gavage of 200 mg/kg ginger extract was administered to streptozotocin-induced diabetic rats for 30 days, which increased protein values and meant improvement of liver functions. This result may be due to the antidiabetic and hepatoprotective effects of ginger and its ability to mitigate liver damage in diabetes **(Seyidoglu *et al.*, 2023).** Black pepper (BP), turmeric (T), ajwa pulp (AP), and ajwa seeds (AS) aqueous extracts and their various combinations were administered orally once daily for eight weeks to diabetic rats. The dose of BP aqueous extract was 50 mg/kg b.wt., while the dose of the other materials was 500 mg/kg b.wt. Phytochemical screening detected the presence of flavonoids, tannins, saponins, steroids, and alkaloids in these extracts. Treatments resulted in a significant ($P\leq0.05$) reduction in serum protein levels. In this study, the serum protein level was highest in the control group and the group treated with a mixture of BP+T+AP+AS at the end of the experiment **(Sarfraz *et al.*, 2024).** 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. Our results are in agreement with these previous studies.

Lipid is a waxy compound and a type of sterol occurring naturally in the tissues of plants and animals, but it is shown only in animals. High cholesterol levels can result in severe concerns, such as heart disease or a stroke. Cholesterol in blood and tissue is considered the main biochemical component, as the cell membrane has a considerable amount of cholesterol and cholesterol esters. The cell membrane, which is a bilipid layer, performs many complex dynamic functions and has significant biological properties. Moreover, cholesterol is not only a main component of the cell membrane of plasma lipoprotein but also the precursor of many biologically essential steroids, such as bile acids and many steroidal hormones. It is synthesized in the tissues and has a specific concentration that is involved in executing several physiological functions. The liver, being the principal organ, is concerned with the synthesis of lipoprotein, phospholipids, and cholesterol, as well as the metabolism of these components.

The results of the present study represent a preliminary assessment of the biochemical status of albino rats due to the effects of three different herbal formulations at a dose of 300 mg/kg. Daily administration of different plant formulations significantly reduced the content of total cholesterol, triglycerides, LDL, and VLDL and increased the value of HDL in body tissues as compared to their normal control group at various durations. Out of three formulations, formulation 2, which contains *Allium sativum, Azadirachta indica,* and *Zingiber officinale*, was more effective in increasing protein contents compared to the other two formulations. It may be possible just because the plants contain some phytochemicals such as alkaloids, saponins, and tannins.

Similarly, in a small Indian study, 16 hypercholesterolemic subjects (age range: 30-60 years) with total cholesterol above 220 mg/dl, 600 mg of fish oil, and 500 mg of garlic oil were administered per day for 60 days. Significant decreases were seen in all the lipid parameters (except HDL, which was increased). After 60 days of supplementation, total cholesterol, serum triglyceride, LDL, and VLDL decreased by 20%, 21%, 37%, and 36.7%, and HDL increased by 5.1% **(Jeyaraj *et al.*, 2006).** When hyperlipidemia-induced rats were supplemented with garlic extract (2 ml/kg b.wt./day) for four weeks at the end, a significant decrease ($P<0.001$) in serum cholesterol, serum triglycerides, LDL, and VLDL was noted, whereas HDL

levels were significantly increased. This occurs because garlic has a high content of organosulfur substances, which lower serum cholesterol levels, or bad cholesterol, and increase HDL, or good cholesterol **(Gupta, 2010)**. Ethanolic extract of *Azadirachta indica* leaves (200 mg/kg) after induction of diabetes normalized glucose levels and lipid profiles such as total cholesterol, triglyceride, LDL, and VLDL were reduced, whereas the level of HDL was increased significantly (P<0.05) in extract-treated diabetic rats. The ethanolic extract of *Azadirachta indica* leaves is beneficial for the treatment of diabetes related to hyperlipidemia **(Bisht and Sisodia *et al.*, 2010).** Supplementation of one medium-sized Amla (35 gm) for 6 months led to a significant decrease in the fasting blood sugar and lipid profile in the type 2 *diabetes mellitus* experimental patients' group. The total cholesterol, LDL, VLDL, HDL, and triglycerides were determined at the start and 6 months of treatment. There was a significant decrease in total cholesterol, LDL, VLDL, and triglycerides, whereas there was a significant increase in HDL levels **(Sri *et al.*, 2013).** Free radical generation by the alloxan causes damage to β-cells of the pancreas, which is prominent in insulin deficiency, which results in hyperglycemia and is also associated with hyperlipidemia. However diabetic rats, when treated with an ethanolic extract of *Tamarindus indica* fruit pulp (300 and 500 mg/kg), significantly (p<0.01) decreased the raised cholesterol, triglycerides, LDL, and VLDL levels and significantly increased HDL levels **(Koyaguru *et al.*, 2013).** 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) decreased cholesterol, triglycerides, LDL, and VLDL levels, and increased HDL levels. This may be an indication of the positive metabolic control of *Tamarindus indica* extract on mechanisms involved in the exclusion of lipids from the body. This effect may be due to the antioxidant effect of *Tamarindus indica* extract **(Al Ahdab, 2015).** Aqueous extract of ginger at concentrations of 0.4 and 0.6% with water was offered to broiler chicks (ROSS) treated groups, respectively. The ginger extract caused a statistical decrease in plasma cholesterol, triglycerides, LDL, and VLDL and increased HDL **(Saeid *et al.*, 2010).** Diabetic group animals fed on a diet having curcumin powder (0.5 gm/100gm diet), ginger powder (3 gm/100gm diet), and a curcumin and ginger powder mixture (0.5% and 3% diet) showed remarkably improved effects in lipid profile levels, and their mixture was more effective. Curcumin and ginger significantly lower total cholesterol, triglycerides, LDL, and VLDL and enhance HDL levels **(Khattab *et al.*, 2013).** Treatment of diabetic rats with zingerone (an active constituent of ginger) doses (50 and 100 mg/kg) has significantly reduced all elevated levels of lipids like total cholesterol, triglycerides, LDL, and VLDL, and significant augmentation in HDL was also noted **(Anwer *et al.*, 2019).** Oral administration of swertiamarin, an active compound of *Enicostemma littorale* (50 mg/kg), for 28 days significantly reversed the levels of serum lipids to near normal **(Dhanavathy *et al.*, 2015)**. The increased lipid profile was brought back to near normal by the treatment with a 400 mg/kg dose of the polyherbal antidiabetic tablet (*Gymnema sylvestre, Momordica charantia, Phyllanthus amarus, Ocimum sanctum, Trigonella foenum-graecum,* and *Allium sativum*), and the decreased HDL was significantly increased. Restoration of these evoked changes in the serum lipid profile shows the protective nature of polyherbal antidiabetic tablets **(Suman *et al.*, 2016).** Increased serum total cholesterol, triglyceride, LDL, and VLDL levels were significantly decreased, and HDL levels were significantly increased by treatment with Ojamin (aqueous extracts of fourteen herbs), Metformin, and Ojamin-Metformin. This effect might be due to the increased secretion of insulin from β cells of the pancreas that further stimulates fatty acid synthesis and also the incorporation of fatty acids into triglycerides in the liver and adipose tissue **(Choudhari *et al.*, 2017).** Total cholesterol, triglycerides, and LDL levels were significantly (p<0.05) decreased in polyherbal extract mixtures (*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 in treated rats, while HDL levels were significantly (p<0.05) increased in polyherbal extract mixtures treated groups, showed their potential to have hypolipidemic action **(Ahmed *et al.*, 2018).** The

administration of xanthine derivatives (6 and 7) and chitosan microparticles loaded with xanthine derivatives (CS-6 and CS-7) were associated with the improvement of values of the lipid parameters (total cholesterol and triglycerides, LDL, and HDL). Total cholesterol, triglycerides, and LDL were significantly decreased, and HDL was significantly increased in xanthine derivatives (6, 7) and chitosan formulations (CS-6, CS-7)-treated rats **(Lupascu *et al.*, 2019).** The values of cholesterol, triglyceride, and LDL were significantly ($p<0.01$) diminished when treated with Poorna Chandrodayam Chendooram (gold, sulfur, and mercurial preparation with *Alovevera brobadensis* and *Hibiscus* extracts). The decreased levels were 27.58% for cholesterol, 32.23% for triglycerides, and 19.29% for LDL, but the HDL level was significantly improved. The increased level was 44% in PCM treatment compared to the normal control group **(Muthukumaran and Begum, 2020).** In a study, there was a significant elevation in the level of total cholesterol, triglycerides, LDL, and VLDL (except HDL) in diabetic rats as compared to the control group. Daily treatment with diaronil for 3 weeks at a dose of 200 and 400 mg/kg was effective in decreasing serum lipid profiles (except HDL). Diaronil at a dose of 400 mg/kg showed more significant antihyperlipidemic effects than the 200 mg/kg dose. **(Amrutha Raj *et al.,* 2022).** In an in-vivo study, on the 21st day, diabetic control rats showed significantly higher levels of total cholesterol, triglycerides, LDL, VLDL, and lower levels of HDL. Dried raw powders of *Cassia auriculata* leaf, *Centella asiatica* leaf, and *Zingiber officinale* rhizome were combined in three different ratios (a dose of 200 and 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.) were administered to diabetic rats for 21 consecutive days. PHF and APHF showed a significant ($p<0.05$) reduction in total cholesterol, triglycerides, LDL, and VLDL and increased HDL levels in a dose- and time-dependent manner. PHF B produced the most notable outcomes among all PHFs, while among APHFs, APHF C had the most prominent activity. The improvements in lipid profile are greater in APHF C than in metformin. (**Alhamhoom *et al.,* 2023).** All of these previous findings support our results that selected formulations have antihyperglycemic, antihyperlipidemic, and antioxidant effects in alloxan-induced diabetic rats. Our results are in accordance with these earlier studies.

Based on the present study on multiherbal formulations consisting of *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Phyllanthus emblica* (Amla), *Tamarandus indica* (Imli), and *Zingiber officinale* (Ginger) in a ratio of 1:1:1:1:1 and studies done by various scientists they were revealed that medicinal plants are effective against various ailments. The medicinal plants possess fewer side effects as compared to allopathic medicines. In the present study, the level of blood sugar declined significantly due to the combined administration of formulation 2, which contains *Allium sativum* (Garlic), *Azadirachta indica* (Neem), and *Zingiber officinale* (Ginger). These plants collectively contain antidiabetic activity and are useful in lowering blood sugar levels due to the presence of certain active phytochemical constituents like alkaloids, flavonoids, etc. Moreover, herbal medicines have the least side effects, and they are also easy to procure. Hence, further study on active phytochemical constituents is needed in this area.

CONCLUSION

Our results are in agreement with all of the above earlier findings of a single plant, formulations, or extracted phytochemical constituents. The medicinal properties of these different formulations (F1, F2, and F3) might be due to the specific or synergistic activity of alkaloids, flavonoids, glycosides, steroids, and other active phytochemical compounds of the plants, which were alternative therapeutic agents for the management of *diabetes mellitus*. Treatment with the polyherbal formulation extracts was more effective than the standard drugs (Glibenclamide) in the amelioration of hyperglycemia and hyperlipidemia and also prevented the alteration in protein levels and glycogen content of liver tissue. This combined mixture is synergistically viable and may be an ideal alternative medicine because of its antihyperglycemic and antihyperlipidemic actions. The need of this study is to develop a potent herbal preparation that could be easily afforded by people and has the fewest side effects or no side

effects.

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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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