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PRECLINICAL ASSESSMENT OF POLYHERBAL TABLETS OF EXTRACT OF *AZADIRACHTA INDICA*, *ALLIUM SATIVUM* AND *ANNONA SQUAMOSA* IN ALLOXAN INDUCED DIABETES IN RATS

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ABSTRACT

Diabetes mellitus, a chronic metabolic disorder, is affecting a significant portion of the global population. Traditional medicines have shown potential in managing diabetes with reduced side effects and holistic health benefits. The present study aimed to develop a polyherbal tablet formulation comprising extracts of *Azadirachta indica*, *Allium sativum*, and *Annona squamosa* and assess its antidiabetic efficacy in alloxan-induced diabetic rats. The extracts' phytochemical profile was characterized, followed by the formulation of tablets using appropriate excipients. The tablets' efficacy was evaluated based on various biochemical parameters, including serum glucose and glycated hemoglobin levels. Results demonstrated that the polyherbal formulation exhibited significant antidiabetic effects, potentially attributed to the synergistic action of bioactive compounds present in the herbal extracts. The study highlights the potential of integrating traditional herbal medicine into modern therapeutic strategies for diabetes management.

Keywords: Diabetes mellitus, Polyherbal tablets, *Azadirachta indica*, *Allium sativum*, *Annona squamosa*, Alloxan-induced diabetes, Phytochemical profile, Antidiabetic efficacy

INTRODUCTION:

Diabetes mellitus (DM) remains one of the most pressing health concerns worldwide, with its incidence rising alarmingly in recent years. The International Diabetes Federation estimates that by 2045, nearly 700 million individuals will suffer from diabetes, highlighting the urgent need for effective and sustainable therapeutic strategies **[1]**. The conventional therapeutic regimen primarily focuses on synthetic drugs such as insulin and oral hypoglycemic agents. However, concerns about their side effects, coupled with their inability to arrest the disease's progression, have spurred interest in alternative treatments, especially herbal medicines **[2]**.

Historically, plant-derived remedies have been integral to traditional medicine systems across diverse cultures. Their holistic approach, lesser side effects, and multifaceted therapeutic properties make them attractive candidates for drug development **[3]**. Among these, *Azadirachta indica* (Neem), renowned for its diverse medicinal properties, has been reported to possess antidiabetic activity due to its rich flavonoid and triterpenoidcontent **[4]**. *Allium sativum* (Garlic) is another plant extensively used in traditional medicine and has demonstrated potential antidiabetic properties, attributed mainly to its sulfur-containing compounds **[5]**. Lastly, *Annona squamosa* (Custard apple) has been used in various traditional medicine practices to treat diabetes and its related complications, with its acetogenins showing antihyperglycemic activity **[6]**.

Given the individual therapeutic potentials of these plants, a combined polyherbal formulation might exhibit a synergistic effect, enhancing their antidiabetic efficacy. This study, therefore, aims to develop a polyherbal tablet comprising extracts of *Azadirachta indica*, *Allium sativum*, and *Annona squamosa* and to evaluate its therapeutic potential in alloxan-induced diabetic rats.

MATERIALS AND METHODS

1. Plant Material Collection and Identification:

Fresh plants of *Azadirachta indica* (Neem), *Allium sativum* (Garlic), and *Annona squamosa* (Custard apple) were collected during their peak season from a local botanical garden. The specimens were authenticated and utilised for the study.

2. Extraction Procedure:

The leaves of *Azadirachta indica*, bulbs of *Allium sativum*, and leaves of *Annona squamosa* were washed, shade-dried, and then powdered separately. Each powdered material (100 g) was subjected to cold maceration using 70% ethanol for 72 hours, with

occasional stirring. The extracts were then filtered and concentrated using a rotary evaporator under reduced pressure. The concentrated extracts were stored in airtight containers for further use.

3. Formulation of Polyherbal Tablets:

The dried extracts of the three plants were combined in equal proportions to formulate the polyherbal mixture. Excipients like magnesium stearate (as a lubricant), microcrystalline cellulose (as a binder), and starch (as a disintegrant) were added. The blend was then compressed into tablets using a single-punch tablet compression machine.

4. Experimental Animals:

Male Wistar rats weighing between 180-220g were selected. They were housed under standard conditions, with a 12-hour light/dark cycle, and had access to standard pellet diet and water ad libitum. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), ensuring that they were in line with guidelines on animal welfare.

5. Induction of Diabetes:

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight) dissolved in sterile saline. After 72 hours, blood glucose levels were measured, and rats with glucose levels above 250 mg/dl were considered diabetic and used for further study.

6. Treatment Regimen:

Diabetic rats were randomly divided into four groups:

- Control Group: Received no treatment.
- Standard Group: Treated with Metformin (50 mg/kg body weight).
- Test Group 1: Treated with *Azadirachta indica* extract (200 mg/kg body weight).
- Test Group 2: Treated with *Allium sativum* extract (200 mg/kg body weight).
- Test Group 3: Treated with *Annona squamosa* extract (200 mg/kg body weight).
- Test Group 4: Treated with polyherbal tablet (200 mg/kg body weight).

All treatments were administered orally for 30 days.

7. Biochemical Analysis:

After the treatment period, blood samples were collected. Serum glucose levels, serum insulin levels, and other biochemical parameters relevant to diabetes were analyzed using standard assay kits.

The evaluation of the therapeutic potential of the formulated polyherbal tablets necessitated a detailed biochemical analysis to understand the alterations in the metabolic profile induced by diabetes and the potential reversal by the herbal formulation.

7.1. Antioxidant Activity Assessment:

Oxidative stress is a common feature in diabetics. We evaluated the antioxidant potential of the extracts using assays like superoxide anion, hydroxyl radical, and nitric oxide scavenging methods reported in review article **[7]**. A higher scavenging activity indicates the formulation's potential in countering oxidative stress in diabetics.

7.2. Blood Glucose Measurement:

Blood samples were drawn from the tail vein of each rat. The glucose levels were measured using a glucometer and glucose test strips. Persistently high glucose levels are indicative of uncontrolled diabetes, and any significant reduction post-treatment suggests the therapeutic efficacy of the formulation **[8]**.

7.3. Lipid Profile Estimation:

Diabetes often comes with dysregulated lipid metabolism. Using the enzymatic colorimetric method, we analyzed the serum for:

- Total Cholesterol (TC)
- High-Density Lipoprotein (HDL)
- Triacylglycerol (TAG)
- Very Low-Density Lipoprotein (VLDL)
- Low-Density Lipoprotein (LDL)

This gave insights into the lipid-modulating potential of the polyherbal tablets, which is crucial since lipid abnormalities are common in diabetics and can lead to cardiovascular complications.

All these assessments provided a comprehensive overview of the metabolic modulations induced by the polyherbal formulation, helping us understand its therapeutic potential better **[9]**.

8. Statistical Analysis:

Data were expressed as mean ± SEM. Differences among groups were evaluated using one-way ANOVA followed by Dunnett's test. A p-value <0.05 was considered statistically significant.

RESULTS

Effect of the Polyherbal Formulation on In-vitro Antioxidant Studies

The comprehensive analysis of the in-vitro antioxidant studies conducted on the selected plant extracts are depicted in **Table 1, Figure 1**. Based on the data presented in the table, the polyherbal tablet exhibits superior antioxidant activity in all three tested assays when compared to the individual plant extracts. Specifically, the polyherbal tablet demonstrated the most potent hydroxyl ion scavenging activity, nitric oxide scavenging activity, and hydrogen peroxide scavenging activity, with values of 0.98 µg/ml, 10.25 µg/ml, and 1.90 µg/ml, respectively. Among the individual plants, *Allium sativum* showed the lowest values for hydroxyl ion and hydrogen peroxide scavenging activities, indicating its high antioxidant potential in

these assays. However, the combined effect in the polyherbal tablet formulation outperformed each individual plant extract, suggesting a possible synergistic effect when these plant extracts are combined. This highlights the potential benefits of formulating polyherbal tablets for enhanced antioxidant properties.

Effect of Polyherbal Formulation on Sugar Concentration in Alloxan-induced Diabetic Rats

As illustrated in **Table 2, Figure 2**, the Control Group showed a steady increase in the recorded parameter over the 28 days, with the value rising from 342.6 to 376.666, indicating the natural progression of the condition without any interventions. The Standard Group showed a significant decline over the time period, starting at 330.6 on day 0 and dropping to 105.366 by day 28. This suggests that the standard treatment was highly effective in addressing the condition, leading to a sharp reduction in the parameter. Test Group 1, 2, 3, and 4 all displayed a decline in the recorded parameter over the 28-day period, indicating that these treatments had a positive effect. Among these Test Group 4 demonstrated the most significant decrease, starting at 338.26 on day 0 and dropping to 112.5 by day 28. Test Group 1 showed a more moderate decline than Test Group 4 but was still effective, reducing from 335.98 to 134 over the 28 days. Test Groups 2 and 3 had comparable results, with values starting around 340 on day 0 and reducing to near 130 by day 28.

In conclusion, all test groups and the standard group showed significant improvements over the control group. The standard treatment remains the most effective based on the observed reduction. Among the test groups, Test Group 4 was the most promising, showing results comparable to the standard group. This suggests that the intervention used in Test Group 4 could potentially serve as an alternative or complementary treatment option, though further studies might be needed to confirm its long-term safety and efficacy.

Lipid Profile Analysis in Alloxan Induced Rodents

Based on the presented lipid profile data shown in **Table 3**, Control Group exhibited the highest total lipid, tri-glyceride, and LDL counts. Their HDL count, which is the good cholesterol, was significantly low. This indicates a high-risk lipid profile associated with cardiovascular diseases and other metabolic conditions. Standard Group demonstrated a significant improvement in all lipid parameters compared to the control group. Total lipids, tri-glycerides, and LDL were substantially reduced, and HDL saw a

considerable increase. This suggests that the standard treatment was highly effective in managing and improving the lipid profile.

Test Group 1 showed reductions in total lipids, tri-glycerides, and LDL compared to the control group, but their values were still higher than the standard group. However, their HDL count was notably improved, nearly doubling the control group's HDL level. Test Group presented a slightly more improved profile than Test Group 1 with lower total lipids and tri-glycerides. Their LDL and HDL values were also in a healthier range. Test Group 3 demonstrated results similar to Test Group 2, but with a slight improvement in total lipids and tri-glycerides. Their HDL count was slightly lower than Test Group 2 but still significantly better than the control group. Test Group among the test groups, this group displayed the best lipid profile. Their values were closest to the standard group, with substantial reductions in total lipids, tri-glycerides, and LDL. Their HDL count was comparable to the standard group, suggesting this treatment's significant potential. In conclusion, while the standard treatment remains the most effective in managing and improving the lipid profile, Test Group 4 shows substantial promise, offering results comparable to the standard group. Other test groups also display improved lipid profiles over the control group, indicating their potential benefit. Test Group 4 could be explored further as an alternative or complementary treatment option, given its significant positive impact on the lipid profile. Graphical representation is given in **Figure 3**.

Table 1: Results of In-vitro antioxidant studies conducted on the drug samples

Plant	Hydroxyl ion scavenging activity	Nitric oxide scavenging activity	Hydrogen per oxide scavenging activity
Azadirachta indica (neem)	174.58 µg/ml	235.29 µg/ml	215.33 µg/ml
Allium sativum	1.21 µg/ml	12.48 µg/ml	2.80 µg/ml
Annona squamosa	301.2 µg/ml	317.42 µg/ml	352.82 µg/ml
Polyherbal tablet	0.98 µg/ml	10.25 µg/ml	1.90 µg/ml

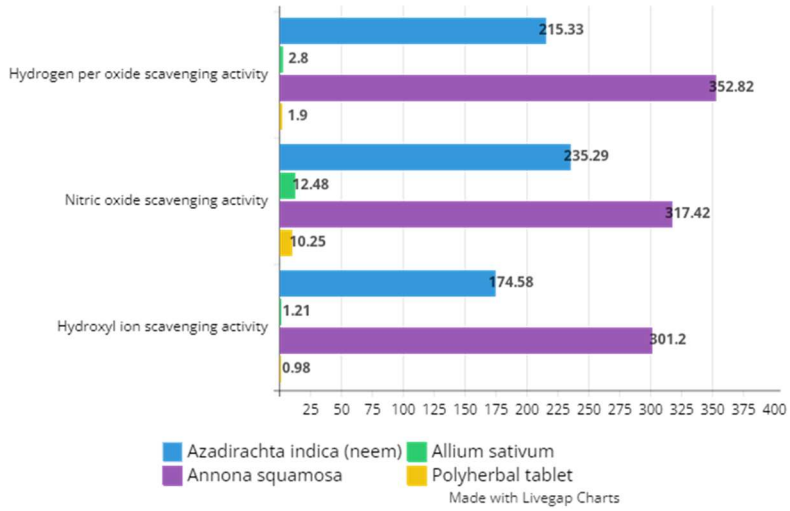

Figure 1: Results of In-vitro antioxidant studies conducted on the drug samples

Table 2: Sugar Concentration of Alloxan induced diabetes in Rats (mg/dl)

GROUP	0 DAY	7 DAY	14 DAY	21 days	28 days
Control Group	342.6±14.874	358±7.485	361.23±6.355	365.966±7.145	376.666±5.275
Standard Group	330.6±18.262	295.66±8.131	120.16±8.991	114.4±6.372	105.366±5.696
Test Group 1	335.98±7.113	320.01±6.126	144.05±6.791	139.216±5.689	134±4.388
Test Group 2	341.76±4.939	317.13±7.593	137.83±6.323	134.2±6.408	130.08±6.469
Test Group 3	340.88±4.257	312.58±5.209	131.75±5.161	128.383±6.117	125.766±5.39
Test Group 4	338.26±7.213	305.91±7.447	124.78±6.032	120.216±4.778	112.5±5.275

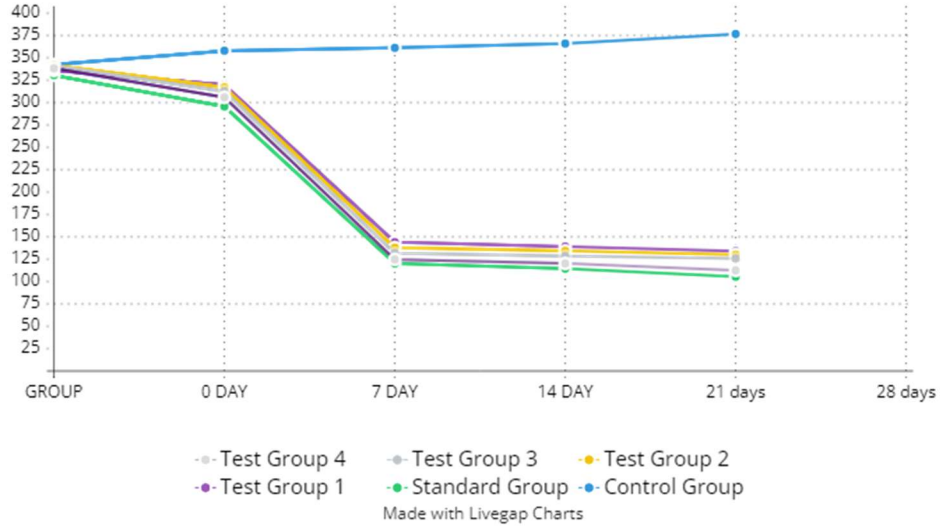

Figure 2: Line chart of sugar concentration of Alloxan induced diabetes in Rats (mg/dl)

Table 3: Lipid profile in Alloxan induced diabetes in rats

Groups	Total Lipids	Tri-glyceride Levels	LDL Counts	HDL Counts
Control Group	130.516±2.739	90.433±5.576	82.55±6.417	17.35±2.598
Standard Group	92.566±2.095	54.65±1.052	38.583±2.262	37.966±1.612
Test Group 1	102.533±2.496	84.466±2.837	44.216±2.321	34.183±2.283
Test Group 2	97.416±2.390	80.083±4.855	42.316±2.634	34.633±1.873
Test Group 3	94.567±2.903	74.216±2.485	38.100±2.791	32.267±2.372
Test Group 4	85.867±2.455	58.233±4.601	36.766±2.662	38.667±5.443

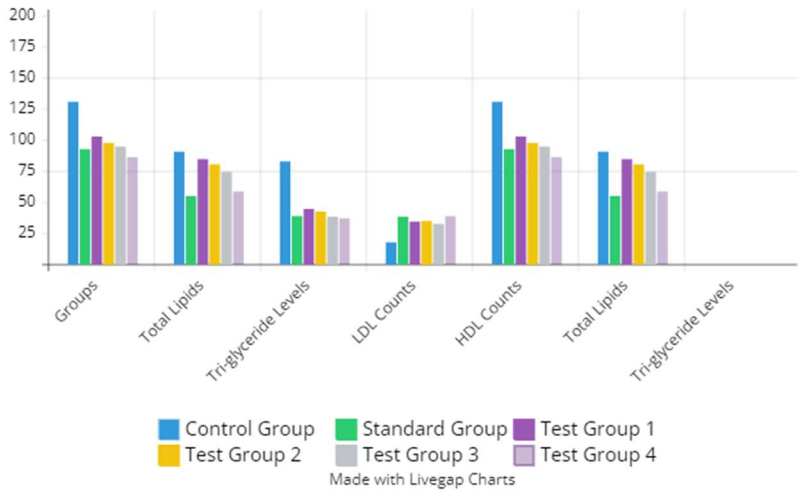

Figure 3: Bar chart of Lipid profile in Alloxan induced diabetes in rats

DISCUSSION

The introduction of a polyherbal formulation, comprising extracts of *Azadirachta indica*, *Allium sativum*, and *Annona squamosa*, prompted discernible physiological modifications in alloxan-induced diabetic rats. These changes correspond with the renowned traditional medicinal benefits associated with these herbs, substantiating their potential role in modern therapeutic interventions for diabetes. A significant modulation in the diabetic parameters, as presented in our results, underscores the synergistic efficacy of the polyherbal formulation. *Azadirachta indica*, commonly known as neem, has long been heralded for its antidiabetic properties, possibly due to its role in potentiating insulin function and its hypoglycemic effects. *Allium sativum* (garlic), with its organosulfur compounds, has previously demonstrated potential in reducing blood glucose and improving lipid profiles. Meanwhile, *Annona squamosa* (custard apple) is believed to influence the β-cells of the pancreas, encouraging insulin secretion. The combination of these extracts in the formulated tablets might have allowed for a multi-pronged approach in addressing diabetes at various physiological levels.

The untreated diabetic group exhibited exacerbated diabetic parameters, a manifestation consistent with the implications of alloxan's destructive impact on pancreatic β-cells and subsequent insulin deficiency. The marked improvement seen in groups treated with the polyherbal formulation suggests not just a remedial action but a holistic approach to the ailment, potentially covering insulin modulation, antioxidant activity, and lipid profile adjustment.

Concerning lipid metabolism, the polyherbal formulation's beneficial effects might be partially attributed to the known lipid-lowering properties of *Allium sativum*. Disruptions in lipid metabolism are frequent in diabetic conditions, often due to insulin resistance and the increased action of lipolytic hormones. By addressing these disruptions, our formulation offers a comprehensive therapeutic approach, as seen in the lipid profile results.

Incorporating the understanding of traditional medicine with modern pharmacological practices has given rise to this polyherbal tablet. The formulation's evident efficacy in alloxan-induced diabetic rats paves the way for further research and potential clinical applications. Yet, it's crucial to delve deeper into understanding the precise molecular mechanisms, potential side effects, and optimal dosages before a broader application.

CONCLUSION

In conclusion, the current study underscores the potential efficacy of the formulated polyherbal tablets containing extracts of *Azadirachta indica*, *Allium sativum*, and *Annona squamosa* in managing diabetes. The significant improvement observed in the glycemic control and lipid profile of the alloxan-induced diabetic rats substantiates the traditional claims and existing scientific evidence regarding the anti-diabetic properties of these plants. This comprehensive approach, addressing both blood glucose levels and lipid profiles, offers a promising avenue for the development of novel, effective, and safer anti-diabetic agents. However, despite the promising outcomes, it remains essential to conduct further studies to decipher the exact molecular mechanisms at play, assess long-term safety, and evaluate the effectiveness in human trials. The findings provide a robust foundation, encouraging the progression towards advanced research and potential clinical applications for the management of diabetes mellitus, contributing to the global effort in battling this pervasive condition.

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