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IN VIVO EXPLORATION OF ALOE VERA: ASSESSING ANTI-HYPERGLYCEMIC EFFECTS IN RATS MODELS FOR THERAPEUTIC INSIGHTS

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Abstract

Introduction: *Aloe Vera (A. Vera) commonly known as "Medicinal Aloe" has been reported to have antihyperglycemic potentials addressing the multi-directional role of phytochemicals in therapeutic insights.*

Objective: *The objective of this study is to explore the antihyperglycemic potential of A. Vera in diabetes-induced models of rats.*

Methodology: *Healthy male Sprague-Dawley rats (n=25) 7 weeks old were used in experiments. Our experimental rats were 25 by numbers being divided into the five designated groups. Group I (Control Group, Non-induced physiological rats), Group II (diabetes-induced rats) III and IV (diabetes-induced rats with A. Vera extract treatment). Except for control of non-induced rats (Group I), Diabetes was induced in groups II, III, IV and V by Streptozotocin (STZ) intraperitoneal injections (200mg/kg). Following diabetes induction groups III, IV and V were administered with the crude powder extract of A. Vera 1.5 g, 3.0 g and 4.5g respectively by mixing in diet, rats were given treatment of A. Vera for 24 days. Metabolic hormones (thyroid hormones) and cortisol serum levels were measured by commercially available ELISA Kits in an automated chemiluminescence Immunoassay CLIA analyzer. Data was analyzed by one-way ANOVA using SPSS 28.*

Results: *Results showed that treatment with Medicinal Aloe has highly significant effects. Among T3 and T4 and cortisol, T3 and cortisol levels were highly significant.*

Conclusion: *Aloe Vera has anti-hyperglycemic capacity and has variable effects on hormonal dynamics.*

Keywords: *Aloe Vera, Antihyperglycemic effects, Herbal plant, Hormonal dynamics, Rats model*

INTRODUCTION

Since decades the research to diagnostics technologies and medications has been drifted towards the usage of natural compounds and the compounds based on combinations of these compounds with various other natural compounds of therapeutic significance majorly consisting of alkaloids, flavonoids and phenolic compounds (1).

The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (1). In this context, our selected phytobiochemical and botanical target is Aloe Vera popularly known as Medicinal Aloe or True Aloe, has prime importance in natural treatments efficacies (2). In ancient immemorial period Medicinal Aloe has been used as a disincentive agent against highly contagious smallpox and other infectious diseases. A. Vera possesses the history of medicinal usage in various purposes against fungai, becteria and viruses along with major component in human and animals' medicine (3). Phytochemical screening of Medicinal Aloe leaves depicts the composition of polysaccharides, anthraquinone, sterols, saponins including alkaloids (4). The main organic chemicals are the alkaloids exhibiting a series of diagnostic and pharmaceutical importance working as cardiac cancer and anti-hyperglycemic agents (5). Other compounds with hydrocarbons like compositions are Glycosides. Greater

than 30 compounds have been isolated from past of Medicinal Aloe for its various application in diagnostics. The crude extract of Medicinal Aloe has been primarily used for anti-inflammatory effects (6).

As per its historical importance, Medical Aloe has been widely used in human ailments. Traditionally for bitter juice extract, Medical Aloe leave have been used in cleaning of blood from infection and anti-bacterial effects. Aloe Vera Juice is considered to be a good tonic to increase appetite and cure fever in order to kill the intestinal worms as well (7,8). The anti-pyretic, anti-fungal and anti-inflammatory potential of Aloe Vera have been studied and reported (9).

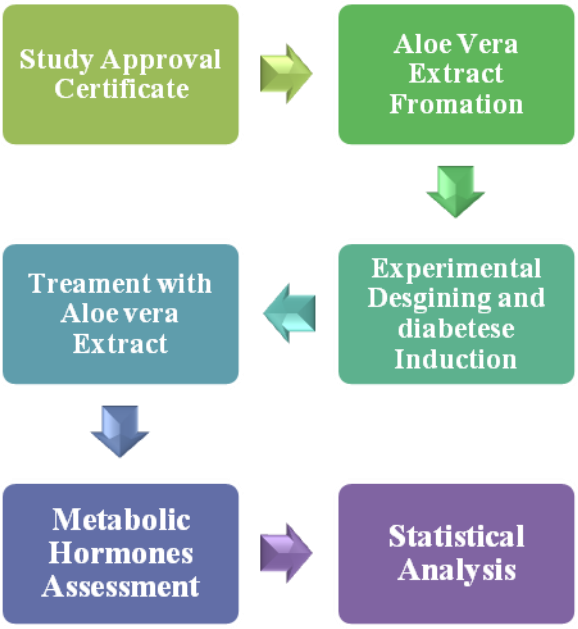
Reduced glucose levels were seen in the treatments with Aloe Vera extract when streptozotocin-induced diabetic rabbits were used in a series of experiments (10). Diabetes exhibits a complex pathology of microvasculature and microvasculature complexities from blood vessels, skin, eye, kidney, and the nervous system during the course of the disease process (11). During prolonged diabetic condition, insulin resistance results in the depletion of glycogen stores in liver and tissue muscles which leads to hyperglycemia and weight loss. One of the common complications seen in diabetic patients is postprandial hyperglycemia (PPHG), and therapeutic approaches for decreasing PPHG is to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes, namely, glucosidases (12).

The Aloe vera leaves extract was found to be an effect medicine in lowering blood glucose levels with minimal side-effects. Variety of plants has been used in medicine and human's cancer cells have been treated by assessing the cytotoxic nature of chemical constituents of these compunds. This study focusses on examination of antihyperglycemic potential of A. Vera in diabetes induced models of rats.

MATERIALS AND METHODS

EXPERIMENTAL SCHEME GLASS WARE PREPARATION

The experimental scheme outlined in the flow diagram (Fig. 1) involves several key steps:
The initial step involves obtaining approval for the study from relevant regulatory bodies or ethics committees. This certificate ensures that the study meets ethical standards and guidelines. In next step, Aloe Vera extract was prepared. This may involve the extraction of active compounds or ingredients from the Aloe Vera plant using appropriate methods and solvents.



Fig, 1. Scheme of experiments

The experimental design was planned, outlining the parameters, groups, and methodologies to be used in the study. Diabetes was then induced in the experimental subjects, likely through the administration of chemicals or genetic manipulation to simulate diabetic conditions. The experimental subjects were treated with the Aloe Vera extract. This treatment may involve oral administration, injection, or other methods depending on the experimental design. After treatment, the levels of metabolic hormones such as thyroid

hormones (T3, T4) and cortisol were assessed. This assessment may involve blood sampling and laboratory analysis to measure hormone concentrations. Finally, statistical analysis was performed on the data collected from hormone assessments and other relevant measurements. This analysis helped to determine the significance of any observed effects of Aloe Vera extract treatment on metabolic hormone levels.

ALOE VERA EXTRACT FORMATION

Fresh leaves of *A. Vera* plant about 0.5 Kg was obtained from the local areas of Lahore which were identified and authenticated from botanist of Kinnaird College Lahore and University of Gujrat Pakistan. Leaves were shade dried and were grinded to make powder. A total of 24 male Sprague-Dawley rats of 7-week-old, reared in animal nursery of Department of Zoology KCWU Lahore, were selected for the experiment. Animal's weight was 190-250grams. All rats were kepp for 7 days at standard conditions (Temperature was 26°C ± 3.5 °C) and humidity (30-50%) with the light maintained at 10h (light-dark cycle). Chow maintained diet (CMD) was given to animals and water ad libitum. Their daily consumption of diet was measured at fixed intervals. All procedures and protocols used during animal handling were approved from Forman Christian College Lahore and Government College University Faisalabad.

Experimental Scheme: (n=25)

GROUP I: (Non-Diabetic/Normal Rats) Negative Control group on regular diet only.

GROUP II: (Diabetic Rats) Positive Control group (No treatment) on CMD only.

GROUP III: (Diabetic Rats)Treatment group1(1.5 g/kg*A. Vera* in CMD).

GROUP IV: (Diabetic Rats) Treatment group2 (3.0 g/kg *A. Vera*in CMD).

GROUP V: (Diabetic Rats) Treatment group3 (4.5 g/kg *A. Vera*in CMD).

DIABETIC INDUCTION

Hyperglycemia via intraperitonialy Streptozotocin (STZ) (200mg/kg) monohydrate in normal saline. Rats were kept under observation for few hours after giving injection. Blood glucose level was measured by glucometer through tail tipping method. When diabetes was assured, after 4 days of injection then hyperglycemic rats (glucose level > 190mg/dl) were separated and used for the experimental study. The diabetes was confirmed by measuring fasting blood glucose level of rats. So, rats were given treatment of 2 different doses of *A. Vera*leaves crude extract powder for 24 days.

METABOLIC HORMONES ASSESSMENT

Triiodothyronine (T3), Thyroxine (T4) including Cortisol for the quantitative determination of serum levels of these hormones via CLIA based kits were analyzed on Accre-6 CLIA Automated (Wondfo TISENC, China) system.

STATISTICAL ANALYSIS

Data was analyzed by using two way ANOVA to compare the selected variables among different experimental groups using the statistical package SPSS-28. Graph Pad Prism 9.0 was used to illustrate the concepts in graphical form of significant and non-significant values.

RESULTS

METABOLIC HORMONAL ASSESSMENTS

The metabolic hormonal assessments were conducted across five different groups: Group I (Negative Control), Group II (Positive Control), Group III (Aloe Vera 1.5g), Group IV (Aloe Vera 3.0g), and Group V (Aloe Vera 4.5g). Serum levels of T3 (Triiodothyronine), T4 (Thyroxine), and Cortisol (Cort) were measured to evaluate hormonal variations among these groups. Table I presents the accumulative mean values ± standard error (SE) of serum T3 and T4 levels in each group. Overall, significant differences were observed in the T3 and T4 levels among the groups, as determined by one-way ANOVA analysis. The results indicated a notable decrease in T3 levels across all treatment groups compared to the Positive Control group (PC), with statistically significant differences ($p \leq 0.01$). Similarly, T4 levels showed a decreasing trend

in the treatment groups compared to the PC group, albeit with less prominent results. Fig. 2 illustrates the accumulative mean values of metabolic hormones (T4 and T3) in the experimental groups.

Table I. Accumulative mean ± standard error of serum T3 and T4 levels in Five Groups

Groups	Group I	Group II	Group III	Group IV	Group V	Statistics
Parameters	NC	PC	A.V[1]	A.V[2]	A.V[3]	*P*-Value
T4 (ng/ml)	8.96±0.07[1]	10.56±0.17[1]	8.25±0.02[2]	6.35±0.06[2]	5.65±0.09[2]	0.12
T3 (µg/ml)	14.25±0.07[3]	13.62±0.17[3]	9.85±0.19[4]	8.125±0.17[4]	5.98±0.21[4]	0.01
Cort (µg/dl)	53.25±6.01[5]	165.25±26.57[5]	62.5±8.07[6]	46.9±8.15[6]	19.65±0.07[6]	0.00

Results are means ± SE. **NC** = Negative Control, **PC** = Positive Control, A.V[1] = *A. Vera* 1.5g, A.V[2] = *A. Vera* 3.0g, A.V[3] = *A. Vera* 4.5g **T3** = Triiodothyronine, **T4** = Thyroxine. **Cort** = Cortisol. Means values with similar numbers (superscript) were statistically found Non-significant with (p-value >0.05).

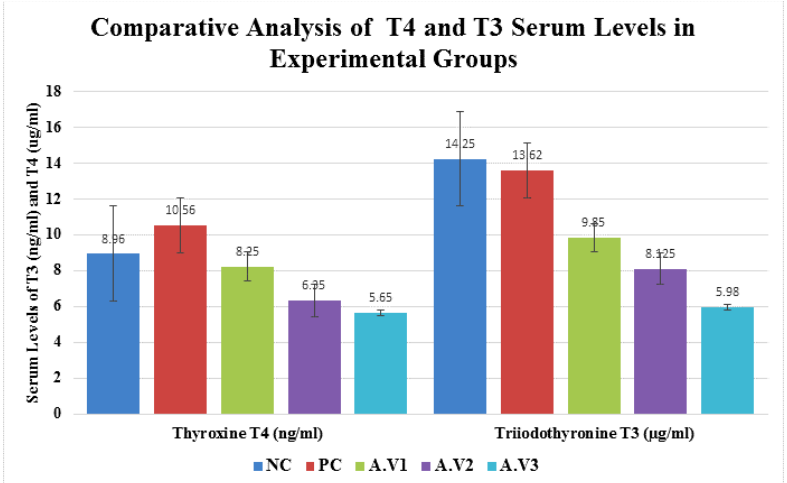


Fig. 2. Accumulative means values of Metabolic Hormones (T4 and T3) in experimental groups. (Means±SE. **NC**=Negative Control, **PC**=Positive Control, A.V[1] = *A. Vera* 1.5g, A.V[2] = *A. Vera* 3.0g, A.V[3] = *A. Vera* 4.5g, T3 = Triiodothyronine. Means sharing the similar numbers (superscript) were statistically found Non-significant with (p-value >0.05).

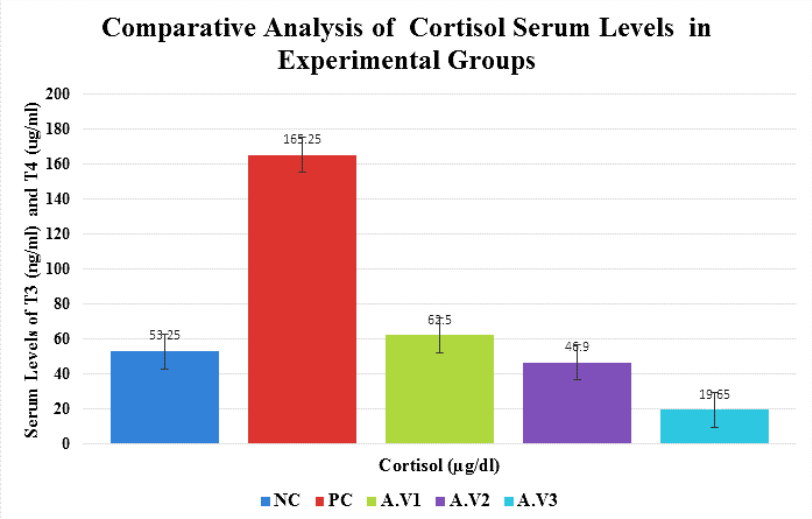


Fig. 3. Accumulative means values of Cortisol in experimental groups (Means±SE. **NC**=Negative Control, **PC**=Positive Control, A. VERA 1 = *A. Vera* 1.25g, A.V[2] = *A. Vera* 3.0g, A.V[3] = *A. Vera* 4.5g. Means sharing the similar letters were statistically found Non-significant with (p-value >0.05).

The accumulative means of cortisol levels are presented in Fig. 3 and Table I. Similar to T3 and T4, one-way ANOVA was employed for comparative analysis of cortisol levels among the experimental groups. The Positive Control group (diabetic rats) exhibited a significantly higher cortisol level compared to all other

groups, with p-values ≤ 0.01. Furthermore, cortisol levels significantly decreased in all five treatment groups compared to the Positive Control group.

These findings suggest that the administration of Aloe Vera in varying doses has a discernible impact on the metabolic hormonal profile, particularly in terms of T3, T4, and cortisol levels. Further analysis and interpretation of these results are discussed in subsequent sections.

DISCUSSION

Metabolic hormones have prime importance in regulation of varieties of mechanisms of our body. Thyroid hormones affect every cells and all organs of the body. Majorly patients with weight loss and weight gain phenomena confer thyroid disorders with altered heart rates predominantly. A.vera and its phytocomponents have been seen to decrease the pro-inflammatory cytokines levels and reduced blood glucose levels by improved insulin sensitivity mechanisms resulting in the reduction of blood glucose levels (13). In the present study, we evaluated the mean concentrations of serum T4, T3 and cortisol levels to assess the effect of the extract of A.vera in diabetic control along with the experimental assessment of anti-stressor efficacy. The thyroid controls and manages the essential metabolic processes of the body for growth and metabolism. It also regulates the adult metabolism. The thyroid hormone relates to the expenditure of energy and weight of the body, temperature metabolism and heart rate (14).

A variety of studies have been conducted on medicinal properties of A.vera with special reference to the anti-diabetic efficacy. A group of researcher conducted a study on major risk in cardiovascular complications in associations with hyperglycaemia with an alteration of lipid parameters presenting a major risk. Oral administration of Aloe vera gel extract at a dose of 300 mg/kg bodyweight per day to STZ-induced diabetic rats for a period of 21 days resulted in a significant reduction in fasting blood glucose, hepatic profile, triglycerides, free fatty acids and phospholipids and a significant improvement in plasma insulin. It was also observed that the altered fatty acid composition in the liver and kidney of diabetic rats was restored following treatment with the A.vera extract (15, 16).

Researchers conducted a study to evaluate T3 and T4 levels in male mice and found that the T3 levels decreased while T4 levels increased showing inhibitory effects of A. Vera on thyroid function (17). Our study showed somewhat similar results in which we evaluated the effect of A. Vera on the levels of T3 and T4 hormones we compared the concentration of means of serum T4 in different groups. Our results showed that there was no significant effect of A. Vera on T4 levels but there was a slight decrease in the serum T4 levels. Although, there was less significant change observed in the T4 level but we can say that high dose of A. Vera affects the levels of T4 and the higher dose may have an inhibitory effect on T4 level (18,19). While comparing the means of serum T3 concentrations of different groups our results showed that T3 levels decreased in treatment groups. The results were highly significant with positive control group suggesting the inhibitory effect of both doses of A. Vera on T3 levels in serum (20). The most investigated active compounds are aloe-emodin, aloin, aloesin, emodin, and acemannan which have been prominently seen in anti-stressor efficacy of A.vera for medicinal purposes (21).

The relationships among hyperglycemia, oxidative stress and pro-inflammatory signals are closely interconnected in numerous tissues. In turn, all the above-mentioned pathological conditions involve a high percentage of the worldwide population, especially older adults (>60 years old), and a comprehension of the molecular biochemical mechanisms is essential for the advance of novel therapeutical approaches to ameliorate or neutralizing harmful effects (22, 23). Elevated levels of glucose in body can cause the production of free radicals. This can play a major role in the cellular injury. The weak defensive system of the body due to hyperglycemia becomes unable to react against the elevated reactive oxidative species (ROS) generation which drags the body to higher oxidative stress (24). Diabetes mellitus with Psychological stress has short-term and long-term effects. In short term stress and diabetic condition, the basal cortisol level and secretion of corticotrophin releasing hormone increases so that the body adapts itself to a hyper catabolic condition (25). Chronic elevation of serum corticosteroids may lead to the non-insulin dependent diabetes mellitus (NIDDM) or diabetes type-II. We evaluated the mean concentrations of serum cortisol levels and our results showed that that in the diabetic condition there was an elevation in the levels of

cortisol but after treatment cortisol levels significantly decreased. This is because of the anti-stressor effect of Aloe Vera. A. vera in most studies refer to anti-cancer action, skin and digestive protective activity, and antimicrobial properties. Potential clinical implications of the findings, including how the anti-hyperglycemic and anti-stressor effects of A. vera could be translated into therapeutic interventions for diabetes and stress-related conditions.

CONCLUSION AND FUTURE PROSPECTS

Herbal medicines have extreme importance in the field of medicine and have no side effects. As diabetes is one of the most spreading disease in this era and number of patients is increasing day by day. So we have planned this project for cure of diabetes with plant treatment. In this study *Aloe Vera (A. Vera)* is commonly known as Medicinal Aloe has been reported to have antihyperglycemic potentials.The pharmaceutical research for *A. vera* based on nano-formulations with various drugs is of great prospects in the field of medical Diagnostics in order to enhance the efficacy of these compounds with lower doses and less post-treatment complications.

Conflict of Interest:

Authors have no conflict of interest.

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