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ALOE'S THERAPEUTIC INSIGHTS: IN VIVO EXPLORATION OF ANTIOXIDATIVE POTENTIAL IN RAT MODELS

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Abstract

Introduction: *Aloe vera* (*A. vera*) is commonly known as "True Aloe" has been reported to have anti-oxidative potentials, scavenging free radicals and reducing oxidative stress, which may contribute to its therapeutic benefits in promoting health and well-being. It helps protect cells from damage, supporting various physiological processes and potentially mitigating the risk of chronic disease.

Objective: This project is designed to probe anti-oxidative potential of *A. Vera* in edema induced model of rats.

Materials and Methodology: Healthy male Sprague-Dawley rats ($n=24$) 7 weeks old were used in experiments. All rats were divided into 4 groups containing 6 rats per group. Group I (Control Group, Non-edema physiological rats), Group II (edema induced rats) III and IV (edema induced rats with *A. Vera* extract treatment). Except control of non-induced rats (Group I), edema was induced in rats paws groups II, III, and IV by carrageenan and formaldehyde. Following edema induction group III and IV were administered with the crude powder extract of *A. Vera* 1.7 g and 3.4 g respectively by mixing in diet, rats were given treatment of *A. Vera* for the period of 24 days. Whole leaf aqueous extracts at various concentrations (100, 200, 400, and 600 mg/kg of bw body weight) was given to check anti-inflammatory potential of *Aloe Vera*. After treatment animals were sacrificed and samples were collected to analyze immunity and oxidative stress. Total Anti-oxidant capacity with stress biomarkers was analyzed. Oxidative stress parameters were measured spectrophotometrically by prescribed colorimetric assay. Data was subjected to ANOVA (two way) using the statistical package SPSS-22.0.

Results: Results showed that treatment with herbal plant have highly significant effect on stress biomarkers and Total anti-oxidant capacity. Leaf extracts significantly reduced formation of edema induced by carrageenan and formaldehyde and granuloma formation in a dose dependent manner.

Conclusion: *Aloe vera* showed strong anti-oxidative potential with best pharmaceutical significance.

Keywords: *Aloe vera*, Anti-oxidative potential, Herbal plant, Phytochemicals, Rats model, Therapeutic Insights

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. In this context, *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 fungi, bacteria and viruses along with major component in human and animals' medicine (3). The accumulation of oxidative damage disrupts cellular signaling pathways, compromises cellular function, and accelerates the aging process. Additionally, oxidative stress-induced inflammation further exacerbates tissue damage, creating a vicious cycle that undermines overall health and well-being at the molecular level. 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 applications 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). The anti-pyretic, anti-fungal and anti-inflammatory potential of Aloe Vera have been studied and reported (8). Various inspections were done and the sound effects of A.Vera leaf extract in rats having cisplatin induced nephrotoxicity and oxidative stress. After the administration of methanolic leaf extract of A. Vera the levels of malondialdehyde and nitric oxide were decreased, increased the levels of glutathione and activity of superoxide dismutase, catalase, glutathione reductase, glutathione-S-transferase, and glutathione peroxidase (9). A. Vera have antidiabetic and anti oxidative potential. It normalizes the antioxidant and lipid peroxidation levels. It also inhibited the formation of superoxide aim and hydroxyl free radical in enzymatic and non enzymatic systems (10). Neuroprotective effect of A. Vera extract was carried out on wistar rats with induced neuropathy by partial damage of sciatic nerve injury by surgery. After treatment A. Vera leaf extracted was revealed that A. Vera has lessened the neural apoptosis and oxidative stress by releasing pro-inflammatory cytokines and inhibiting oxidative nitrosdinne stress (11). A. Vera leaf extract has the ability to form reaction oxygen species (ROS) and treatment induced apoptosis by increased concentration of nitric oxide, hydrogen peroxides and Cytochrome-C and increased activity of Caspase 3-9 and DNA fragmentation (12). Oxidative stress is controlled by internal antioxidant system mainly including Glutathione peroxidase, SOD and Catalase.



Fig. 1. Pathology via oxidative stress

MATERIALS AND METHODS

The experimental scheme outlined in the flow diagram (Fig. 2) involves several key steps: Fresh leaves of *A.Vera* plant about 0.5 Kg were 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, were selected for the experiment. Animal's weight was 190-250grams. All rats were kept for 7 days at standard conditions (Temperature was $26^{\circ}\text{C} \pm 3.5^{\circ}\text{C}$) 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 respective head of departments from various labs at different institutes. Experimental scheme (n=25) was group I: (Non-edema/Normal Rats) Negative Control group on regular diet only, group II: (edema Rats) Positive Control group (No treatment) on CMD only, group III: (edema Rats)Treatment group1(1.5 g/kg *A. vera* in CMD), group IV: (edema Rats) Treatment group2 (3.0 g/kg *A. Vera* in CMD) and group V: (edema Rats) Treatment group3 (4.5 g/kg *A. Vera* in CMD). Hyperglycemia via intraperitoneally 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. The diabetes was confirmed and rats were given treatment of 2 different doses of *A. Vera* leaves crude extract powder for 24 days. The total anti-oxidant capacity in the serum sample was measured by using the novel automated colorimetric method along with comparative analysis of various oxidants and ROS (samrtchem@wet chemistry analyser M-6589). Total oxidant status was assessed via automated colorimetric assay along with superoxide dismutase SOD and paraoxonase activity assay. Data was subjected to one way ANOVA and SPSS-28 was used.

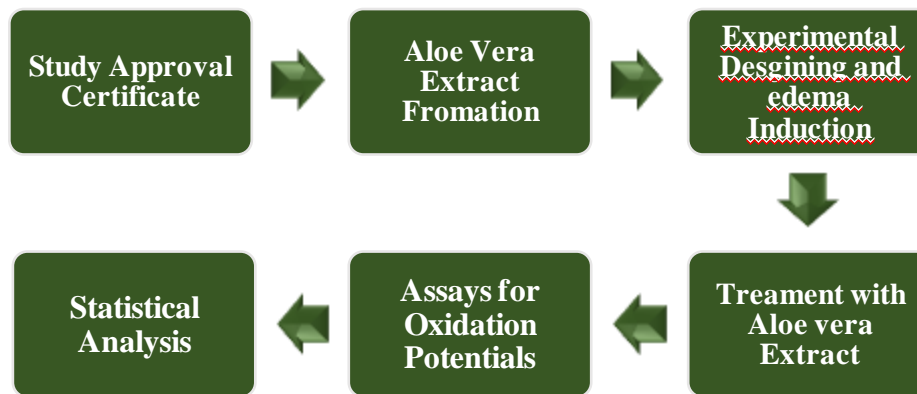


Fig. 2. Scheme of experiments

RESULTS

The overall mean of Total oxidant status is presented in Fig. 3 and Table I. One-way analysis of variance was applied to compare mean concentration of serum TOS from different groups. Positive control (diabetic rats) group showed highly significant difference ($p \leq 0.01$) with all other groups. The TOS level significantly decreased in both treatment groups as compared to positive control (diabetic rats).

Table I. Accumulative mean \pm standard error of Oxidative Parameters 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
TOS($\mu\text{mol/l}$)	20.52 \pm 0.65 ¹	72.55 \pm 10.25 ¹	23.21 \pm 2.65 ²	19.25 \pm 2.85 ²	16.97 \pm 3.12 ²	0.00
TAC(mmol/l)	2.15 \pm 0.01 ³	0.766 \pm 0.02 ³	0.79 \pm 0.06 ⁴	1.78 \pm 0.24 ⁴	1.658 \pm 0.16 ⁴	0.00
Paraoxonase (KU/min/ml)	435.25 \pm 126.51	42.55 \pm 17.56 ⁵	184.56 \pm 37.5 ⁶	235.74 \pm 64.2 ⁶	248.56 \pm 75.2 ⁶	0.01

Results are means \pm SE. NC = Negative Control, PC = Positive Control, A.V¹ = *A. Vera* 1.5g, A.V² = *A. Vera* 3.0g, A.V³ = *A. Vera* 4.5g TOS = Total oxygen status, TAC= Total Anti-oxidant capacity. Statistically Significant p-value ($p \leq 0.01$). Means values with similar numbers (superscript) were statistically found Non-significant (p -value > 0.05).

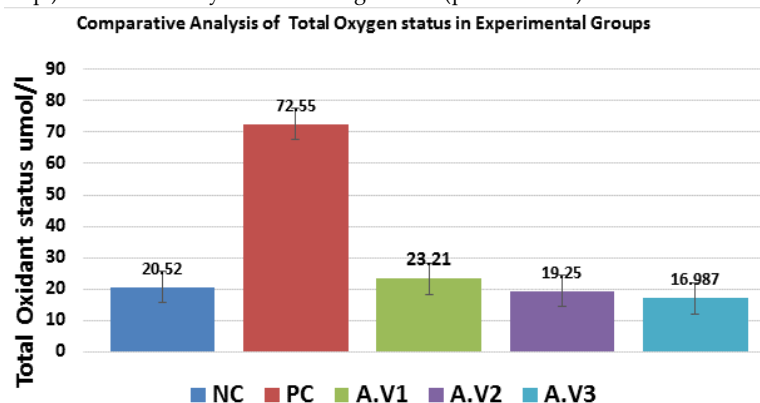


Fig. 3. Accumulative means values of TOS in experimental groups

Means \pm SE. NC=Negative Control, PC=Positive Control, A.V¹ = *A. Vera* 1.5g, A.V² = *A. Vera* 3.0g, A.V³ = *A. Vera* 4.5g, T3 = Triiodothyronine. Statistically Significant p-value ($p \leq 0.01$). Mean values with similar numbers (superscript) were statistically found Non-significant (p -value > 0.05) w.r.t Table I.

The overall mean of Total antioxidant capacity is presented in figure 4 and table 1. One-way analysis of variance was applied to compare mean concentration of serum TAC from different groups. There was no significant difference ($p \leq 0.01$) among all groups. However, the TAC level increased in high dosage group (1.55 ± 0.19) as compared to positive control (0.64 ± 0.02).

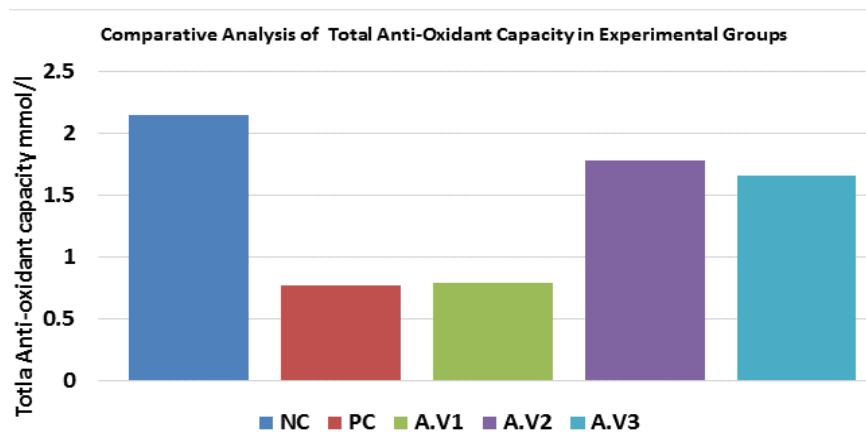


Fig. 4. Accumulative means values of TAC in experimental groups

The overall mean of Paraoxonase (PON-1) is presented in Figure 5 and table 1. One-way analysis of variance was applied to compare mean concentration of serum PON-1 from different groups. Positive control (diabetic rats) group showed highly significant difference ($p \leq 0.01$) with all groups and value of PON-1 increased in both treatment groups.

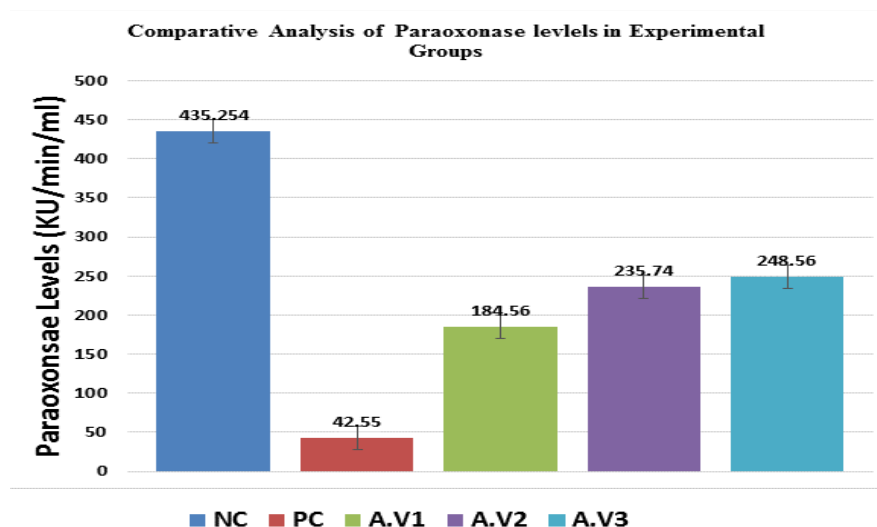


Fig. 5. Accumulative means values of PON-1 in experimental groups

Means \pm SE. NC=Negative Control, PC=Positive Control, A.V¹ = *A. Vera* 1.5g, A.V² = *A. Vera* 3.0g, A.V³ = *A. Vera* 4.5g, T3 = Triiodothyronine. Means sharing the similar numbers (superscript) were statistically found Non-significant with (p -value > 0.05).

DISCUSSION

Aloe vera exhibits potent antioxidant properties, scavenging free radicals and reducing oxidative stress, which may contribute to its therapeutic benefits in promoting overall health and well-being. Its rich antioxidant content helps protect cells from damage, supporting various physiological processes and potentially mitigating the risk of chronic diseases (13). Oxidative stress, resulting from an imbalance between free radicals and antioxidants defenses, can adversely impact at the molecular level. Free radicals, highly reactive molecules, can damage cellular components such as DNA, proteins, and lipids, leading to

functional impairment and potentially contributing to the development of various diseases mainly cancer, cardiovascular disorders, neurodegenerative diseases and aging related conditions (14).

A group of researchers supported that Acemannan, the chemical constituent of *A. vera* has anti-oxidative and anti-inflammatory properties along with medicinal efficacy as osteogenic, antibacterial and wound healing properties. Anti-tumor effects of Aloe Vera has also been reported in vivo studies (15). Elevated levels of glucose in body can cause the production of free radicals. This can play a major role in the cellular injury. Weak defensive system of body due to hyperglycemia becomes unable to react against the elevated reactive oxidative species (ROS) generation which drags body to higher oxidative stress (16). In our study when we compared the mean concentrations of total oxidative status (TOS) of different groups. Results showed that the TOS level was much elevated in diabetic group while after giving treatment the levels of TOS decreased significantly in both treatment groups. So we can say that both doses of *Azadirachta indica* were effective against oxidative stress (17). Total antioxidant capacity TAC of blood plasma is the key to assess the oxidative stress of body. Diabetic condition cause complications by increasing oxidative damage and decreased antioxidants (18). The results of study conducted also showed the antioxidative ability of neem powder. When we compared the mean concentrations of total antioxidant capacity of different groups in our study results showed that there was significant change between the groups. The value of TAC was decreased somehow in diabetic group and it was restored to normal level after treatment (19).

Paraoxonase-1 is an enzyme having antioxidative potential. It is found in plasma and have the ability to degrade the lipid peroxides. In diabetes and some other diseases its activity becomes low (20). In our study the comparison of mean concentration of PON-1 in serum of different groups revealed that level of PON-1 raised in treatment group than PC group. Another invivo study conducted experimental research on Paraoxinase which is high in normal rats but with diabetes its level decreased. After giving treatment of Aloe Vera for 21 days its level gradually increased in rats. These results showed that A.I had antioxidative effect.

Results from other studies proved that *A. Vera* have wound healing effects by increased inflammatory response neovascularization. Immunomodulatory and growth enhancing effects of *A. Vera* leaf infusion on chicks. Results revealed that *A. Vera* infusion increased growth performance and antibody titer (Durrani *et al.*, 2008). In another study effectiveness of extracts of Medicinal Aloe or True Aloe and guava extracts food pathogens stated. Results revealed that antibacterial compounds present in these extracts can inhibit these food spoiling pathogens (19). Another study was done which revealed the antiangiogenic potential of ethanolic extract of *A. Vera* leaves against mammary tumor volume and it also inhibit the new tumors development. It inhibits the development of tumors by hindering the expression of vascular endothelial factor and of proangiogenic genes (20). Oxidative stress (or oxidant-derived tissue injury) occurs when production of oxidants or reactive oxygen species (ROS) exceeds local antioxidant capacity. Destruction of body cells due to the ROS is called oxidative stress. In the body there are natural molecules that act against the ROS and counter their effects to save body from destruction. When in the body level of antioxidants becomes less than ROS body confronts oxidative stress. As a result of oxidative stress the macro molecules in the cells like DNA, proteins, carbohydrates and lipids undergo destruction (21). Results showed that treatment with herbal plant have highly significant effect on stress biomarkers and Total anti-oxidant capacity. Leaf extracts significantly reduced formation of edema induced by carrageenan and formaldehyde and granuloma formation in a dose dependent manner. Aloe Vera showed strong anti-oxidative potential with best pharmaceutical significance.

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. Aloe Vera (*A. Vera*) is commonly known as Medicinal Aloe has been reported to have anti-oxidative potentials. Hence it was concluded that Aloe Vera has anti-oxidative capacity. 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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