



ALTERATIONS IN TOTAL PROTEIN CONCENTRATION, SERUM PROTEIN FRACTIONS AND ALBUMIN/GLOBULIN RATIO IN HEALTHY RABBITS

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ABSTRACT

This study assessed the effect of oral administration of *Aloe vera* and was to evaluate total serum protein, albumin and globulin concentrations as well as albumin / globulin (A / G) ratio. Twenty rabbits weighing 1000 – 1800 g were divided into 2 groups. Each group consisted of ten animals. One served as control and other group served as experimental group. Results show that animals after 07, 15 and 30 days dosing of *Aloe vera* showed highly significant decrease in total protein and globulin and highly significant decrease in Albumin after 15 and 30 days of dosing of *Aloe vera* in comparison to control animals group. It is concluded that the long-term use of *Aloe vera* may cause hypoglobulinemia and hypoalbuminemia at 30 days of dosing and it could be due to the liver diseases, evidence of hepatotoxicity induced *Aloe vera* also reported in previous studies.

Keywords: albumin, *Aloe vera*, hepatotoxicity

INTRODUCTION

Liver has great capacity to synthesize and detoxify the drugs and other substances. Many of the toxic effects are associated with the use of herbal drugs. Excessive use of herbal drugs damages multiple organs, especially the liver and kidney. In various cases damaging of hepatocytes has ranged to mild increase in liver enzymes to the liver damage¹. Low level of protein is the indicator of inflammatory bowel disease, liver problems and mal absorption etc. When albumin : globulin (A / G) ratio is low, which is associated with autoimmune diseases and nephrotic syndrome². Traditional herbal medicines or folk medicines are widely used in almost all region of the world population. A variety of herbal drugs can cause cardiovascular abnormalities, renal and liver damage. *Aloe vera* (L) Burm. f. has transparent³ colourless gel from fresh leaves. Major chemical constituents of leaf gel are polysaccharides⁴ (hemicelluloses, pectins, mannose, glucomannan and acemannan derivatives) and tannins. It also contains water, enzymes, lipids, amino acids⁵, sterols such as β -sitosterol, lupeol, campesterol. The use of fresh *Aloe vera* gel is recommended because high temperature and humidity may alter the chemical composition of leaves of gel. *Aloe vera* gel has been used for the treatment of acute inflammation⁶ via bradykinase activity and it may be due to plant sterol especially lupeol found in *Aloe vera*, also effective in burns and is treated with *Aloe vera* gel. Synthesis of proteoglycan and collagen will increase by stimulation of macrophages and fibroblast activity. *Aloe vera* promotes the synthesis of both and plays an important role in wound healing. *Aloe vera* has been widely used in phytomedicine. The drug is widely used as a self-prescribed anti-aging drug⁷. It has anti-pruritic, anti-inflammatory, anti-diabetic, anti-proliferative, anti-aging effects, but several cases of *Aloe vera* -induced hepatotoxicity were also reported⁸. It increases the level of gamma-glutamyl transferase, alkaline phosphatase when administered for two weeks daily⁹. Furthermore, the cytotoxic effect of *Aloe vera* may be due to different other chemicals and substances added during manufacturing process.

MATERIALS AND METHODS

Animal Selection

The study was conducted on 20 healthy white rabbits of both sexes weighing from 1000 – 1800 g. Animal were housed in separate cages, under controlled conditions of temperature ($22 \pm 1^\circ\text{C}$). The standard laboratory feed given to the control and experimental groups of animals that contained the same quantity of daily calories and protein.

Experimental Design

Animals were uniformly divided into two groups. Each group consists of ten animals; one group serves as a control while the other group received the drug. Body weight of rabbits was measured weekly. Before administration of drug, observe health of rabbits during the total experimental period for a week specifically noticing weight loss, loss of hair, passivity, diarrhea, sedation, edema, ulceration and irritability.

Dosing Protocol

The daily dosing of *Aloe vera* for the assessment of protein was done for a period of 30 days, in the dose of 500 mg / 70 kg orally¹⁰ according to the body weight of animal. 0.9 % saline (NaCl) was given to control animals group by the same route as the treated group. Blood samples were collected after 07, 15 and 30 days of dosing of *Aloe vera*.

Estimation of Protein

Blood sample for the estimation of albumin was collected in gel tubes through cardiac puncture technique after 7, 15 and 30 days of dosing serum was separated by centrifugation at 3000 rpm for 15 minutes in 14 K Humax centrifuge. All analysis was done within 3 h of sample collection and biochemical assays of serum total protein, albumin and globulin were carried out using Humalyzer 3000 is a semi-automatic chemistry analyzer Model no. 16700 (Human Germany) and using the standard kits supplied by Human Gesellschaft fur Biochemical and Diagnostic mbh, Germany. Serum total protein was determined by biuret method. Dye-binding technique was used for the measurement of albumin¹¹.

Statistical Analysis

All results were expressed as Average value ± Standard Deviation (St. Dev). The significance of difference between averages was determined by Test^{12,13}. Whereas the data

obtained from present study was analyzed for P-value < 0.01 was considered significant and P-value < 0.001 was considered highly significant, following the one way ANOVA.

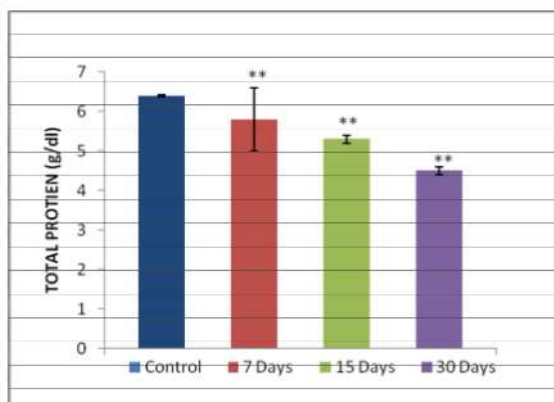


Figure 1: Effect of *Aloe vera* on Total Protein
 n = 10, Average value ± St. Dev, Significant difference by Newman keuls test
 **p < 0.001 as compared to control rabbits, following one way ANOVA

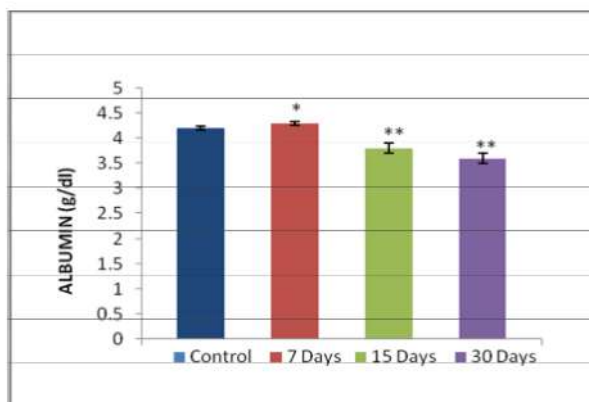


Figure 2: Effect of *Aloe vera* on Albumin
 n = 10, Average value ± St. Dev, Significant difference by Newman keuls test
 *p < 0.01 as compared to control rabbits **p < 0.001 as compared to control rabbits, following one way ANOVA

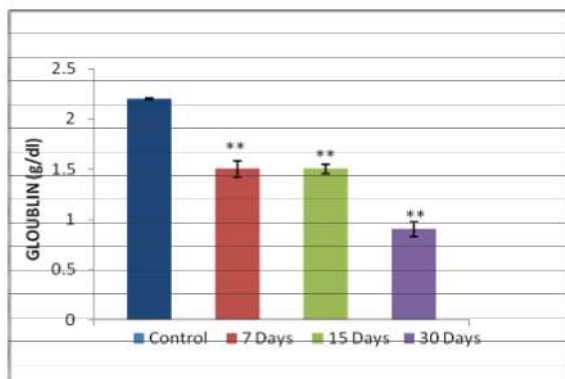


Figure 3: Effect of *Aloe vera* on Globulin
 n = 10, Average value ± St. Dev, Significant difference by Newman keuls test
 **p < 0.001 as compared to control rabbits, following one way ANOVA

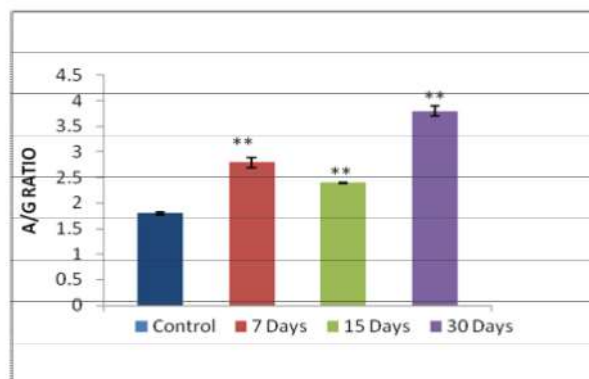


Figure 4: Effect of *Aloe vera* on A / G Ratio
 n = 10, Average value ± St. Dev, Significant difference by Newman keuls test, **p < 0.001 as compared to control rabbits, following one way ANOVA

RESULTS

Gross Examination

No any gross toxicity was observed in test group animals during the total period of experiment.

Protein Estimation

Figures 1, 2, 3 and 4 show the statistical analysis of total protein, albumin, globulin and A / G Ratio after 07, 15 and 30 days of dosing of *Aloe vera* and data analyzed by One way ANOVA show significant effect of drug *Aloe vera*.

Effect on Total Protein

Figure 1, Post-hoc analysis by Newman – Keuls test shows that animals after 07, 15 and 30 days dosing of *Aloe vera* showed highly significant decrease in total protein, i.e. 5.8 ± 0.8 , 5.3 ± 0.1 and 4.5 ± 0.1 (g/dl) respectively in comparison to control animals group, i.e. 6.4 ± 0.03 (g/dl). Results showed that the total protein level in *Aloe vera* treated group after 7, 15 and 30 days was decreased much significantly.

Effect on Albumin

Figure 2, Post-hoc analysis by Newman – Keuls test shows that animals after 15 and 30 days dosing of *Aloe vera* showed

highly significant decrease in albumin, i.e. 3.8 ± 0.1 and 3.6 ± 0.1 (g/dl) respectively and animals after 07 days dosing of *Aloe vera* showed significant rise in albumin, i.e. 4.3 ± 0.04 (g/dl) in comparison to control animals group, i.e. 4.2 ± 0.04 (g/dl). Results showed that the albumin level in *Aloe vera* treated group after 15 and 30 days was decreased much significantly than after 7 days of dosing.

Effect on Globulin

Figure 3, Post-hoc analysis by Newman – Keuls test shows that animals after 07, 15 and 30 days dosing of *Aloe vera* showed highly significant decrease in globulin, i.e. 1.5 ± 0.08 , 1.5 ± 0.05 and 0.9 ± 0.07 (g/dl) respectively in comparison to control animals group, i.e. 2.2 ± 0.01 (g/dl). Results showed that the globulin level in *Aloe vera* treated group after 7, 15 and 30 days was decreased much significantly.

Effect on A / G Ratio

Figure 4, Post-hoc analysis by Newman – Keuls test shows that animals after 07, 15 and 30 days dosing of *Aloe vera* showed highly significant increase in A / G Ratio, i.e. 2.8 ± 0.1 , 2.4 ± 0.01 and 3.8 ± 0.1 respectively in comparison to

control animals group, i.e. 1.8 ± 0.03 . Results showed that the A / G Ratio level in *Aloe vera* treated group after 7, 15 and 30 days was increased much significantly.

DISCUSSION

Folk medicines and traditional herbal drugs have been used in different cultures to treat kidney, liver and CV diseases and they obtained from natural resources such as plants. During manufacturing quality control and standardization of scientific techniques and knowledge are essential from harvesting and collection to the finished products. For the assessment of folk medicines and traditional herbal drugs specific evaluation for quality, efficacy and safety is required and should be properly documented¹⁴. Serum protein has a role in oncotic pressure and reduction in oncotic pressure resulting in excess body fluid build up in the tissues causing oedema¹⁵. If serum total protein level is not normal, further testing should be continued to identify that which type of specific protein level is decrease or increase. If the level of serum total protein is below the range that usually reflect low albumin level and it may be due to liver disease or acute infection¹⁵. Globulin is produced in liver¹⁶. Low levels could reflect impaired synthesis of proteins¹⁷. In present study *Aloe vera* showed significantly decreased levels of serum protein and protein fractions such as albumin and globulin, observed in the experimental groups was due to the low protein content of *Aloe vera* gel and it could be due to constituents found in plant gel. *Aloe vera* significantly decreased the level of albumin. Albumin is mainly produces in the liver¹⁸. The decreased level could be due to decreased production or increased loss, but this effect could be related to the effects of *Aloe vera* on liver. It contains several alkaloids that may induce or inhibit liver enzymes such as cytochrome P₄₅₀ as well as the enzymes required for ethanol metabolism¹⁹. In other studies *Aloe vera* and its constituents are associated with liver²⁰ and kidney. It is concluded that the long-term use of *Aloe vera* may cause hypoalbuminemia and hypoglobinemia at 15 and 30 days of dosing. Aloe products are one of the most selling herbal products in Pakistan but the accurate doses of intake to achieve desirable therapeutic effects are not well understood. Finally, the overdose of a variety of herbal drugs has led to acute liver failure due to increase in hepatic enzymes.

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