EFFECT OF CHLOROFORM EXTRACT OF *ALOE VERA* GEL ON SODIUM AND WATER RETENTION IN RATS

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ABSTRACT

Background and Objective: Non-steroidal anti-inflammatory drugs are popular to treat acute and chronic inflammatory conditions. These drugs cause salt and fluid retention among other side effects. Aloe vera is a well – known medicinal plant that reduces inflammation but its effect on salt and fluid retention has not been studied. The objective of the study was to evaluate the effects of chloroform extract of Aloe vera leaf – gel on sodium and water retention in rats.

Methods: A randomized controlled experimental study was conducted at Post Graduate Medical Institute, Lahore in which twelve adult healthy male Sprague Dawley rats were divided into two groups each containing six rats. First group served as a control and was given 0.5 ml distilled water while second experimental group was given chloroform extract of Aloe vera gel, 200 mg/kg dissolved in 0.5 ml distilled water orally by one ml syringe as a single morning dose for a period of 28 days. Body weight, urine sodium concentration, blood haematocrit and serum sodium and potassium concentrations were measured on Day 0, 14 and 28.

Results: It was found that Aloe vera extract did not cause significant change in body weight and urine sodium concentration, caused a slight fall in haematocrit, rise in serum sodium concentration within normal range and no change in serum potassium concentration.

Conclusion: The present study shows that Aloe vera extract does not cause sodium and water retention when given orally in 200 mg/kg dose, but needs evaluation by using gradually increasing doses.

Keywords: Aloe vera, Sprague Dawley Rats, Sodium Retention, Water Retention.

INTRODUCTION

The popularity of complementary and alternative medicine (CAM) in contrast to modern or allopathic drugs cannot be denied. The reasons are the high cost of most modern drugs, their frustrating side effects and poor patient compliance particularly in the case of various chronic conditions.[1] However, herbal treatments that are a part of CAM, are not totally safe and also have their own set of adverse effects[2] but still there is no change in the practice of CAM in different countries of the world for more than a decade.[3] Patient satisfaction has been the main factor in case of complementary and alternative medicine especially in various chronic diseases.[4] There is a need for standardization of herbal medicine and validation of safety of popular herbal products used in many countries.[5] In the present study, *Aloe vera* was chosen as it is widely used herbal medicine all over the world particularly in Pakistan in those areas where medical facilities are inadequate, such as Cholistan and Pothwar.[6]

The beneficial value of *Aloe vera* leaf – gel for inflammatory diseases is well – established.[7-9] In the same sense, non-steroidal anti-inflammatory drugs (NSAIDs) are very popular in Pakistan and other countries because of their success in treating acute and chronic inflammatory conditions. However, these medicines have significant adverse effects. NSAIDs are associated with a relatively high incidence of adverse renal effects, including salt and fluid retention and hypertension.[10-12]

A case report shows that *Aloe vera* gel reduced joint pain successfully in a patient, but it also caused gross ankle edema. On the other hand, *Aloe vera* is being used for the treatment of hypertension in many parts of the world.[13] Looking at contrasting effects of these studies and observations, there is a need to rule out the effects of *Aloe vera* on fluid retention and electrolyte imbalance to validate its safety for oral consumption. Since *Aloe vera* has been found to be biologically very active with clear demonstration of activities against various pathological conditions,[8] it is highly unlikely that the plant does not have any toxic effects. This study uses established dose of *Aloe vera* gel, which shows anti-inflammatory activity, to see its effect on salt and water retention in healthy rats.

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METHODOLOGY

After approval from ethical committee, study was conducted at Post Graduate Medical Institute, Lahore for a duration of three months. Healthy male Sprague Dawley rats weighing 230 – 300 g were purchased from National Institute of Health (NIH), Islamabad. They were kept in the animal house for a week for acclimatization at constant room temperature (22°C – 24°C) with natural light-dark cycle and were fed rat chow, purchased from NIH with known sodium content of 0.11% as determined at P.C.S.I.R laboratories, Lahore.[14] Drinking mineral water with known sodium concentration from 7 – 30 mg/L, mean value 18.5 mg/L was given to the rats *adlibitum*.

For preparation of chloroform extract of *Aloe vera* gel, fresh *Aloe vera* leaves weighing about 25 kg were collected from the research field of the Institute of Agricultural Sciences, Punjab University, Lahore. The plants were not watered for two days prior to collection of the material. *Aloe vera* leaves were washed with tap water, cut longitudinally and thick epidermis was removed with a sterilized knife. The clear mucilaginous gel (12 kg) was blended using an electric blender. The blended gel sample was soaked in 12L of chloroform for 48 hours at room temperature. The mixture was then filtered using a muslin cloth and Whatmann No.1 filter paper. The filtrate was concentrated at 50°C in a rotary evaporator at reduced pressure which yielded a gummy crude extract. Water from the crude extract was removed with freeze drier. Dried extract weighed 12 g was transferred into clean and dried air-tight vials and stored at 4°C.

The rats were divided into two groups of six rats in each group. The first group was control (CTL) given 0.5 ml distilled water and the second group (AV) was given *Aloe vera* gel extract 200 mg/kg.[15] Dose of *Aloe vera* gel extract for each rat was weighed individually and dissolved in 0.5 ml of distilled water at time of administration. Both distilled water and extract were given orally by one ml syringe as a single morning dose.

Rats were weighed at start of the study and then throughout the experiment. Dose of extract was adjusted accordingly. Rats were kept individually in metabolic cages for 24 hours on day 0, then after two weeks on day 14 and finally on day 28. Urine was collected in glass container and sodium concentration was measured. After urine collection 1.5 ml blood sample was collected by cardiac puncture, using disposable syringe with 27 gauge needle,[16] out of which 0.5 ml was put in citrated vacutainers and used for determination of haematocrit by hematology analyzer (NIHON – KOHDEN) and one ml blood was put in serum vacutainers and placed at room temperature for 30 minutes. It was centrifuged at 3000 rpm to separate serum for determination of sodium and potassium concentration which was carried out in Chemical Pathology Laboratory of PGMI, Lahore by flame photometry.

Statistical Analysis

The grouped data was statistically evaluated with a computer software program SPSS, version 17. It was checked for normal distribution by Shapiro – Wilk test and homogeneity by Levene's test of equality of variance. Data was expressed as mean ± SD. Student's t-test (paired) was used for comparison between Day 0, 14 and 28 in either group. The independent sample t-test was applied to compare two groups at different times. The level of significance was set at $p < 0.05$.

RESULTS

Table 1 shows mean ± SD of individual parameters and comparison between two groups. Table 2 reveals comparison between day 0,14 and 28 within groups. The results show increased body weight in both control and *Aloe vera* groups. Increase was significant over time but difference between two groups was not significant. Urine sodium concentration fluctuated in both groups. Over time significant decrease was in AV group from day 0 – 14 only. Difference between two groups was not significant.

Table 1: *Sodium and water retention profile of animals in control and Aloe vera treated groups at various times (n = 6)*

Parameter	*Group*	*Day – 0*		*Day – 14*		*Day – 28*	
		Mean ± SD	*p-value*	*Mean ± SD*	*p-value*	*Mean ± SD*	*p-value*
Body Weight (g)	CTL	263.3 ± 9.5	0.46	270.3 ± 9.6	0.582	278.3 ± 8.5	0.715
	AV	271.7 ± 5.2		277 ± 6.7		282.5 ± 7.1	
Urine sodium concentration (mmol/l)	CTL	602.5 ± 162.0	0.247	650.5 ± 119.9	0.841	510 ± 90.5	0.153
	AV	819.2 ± 69.0		619 ± 95.6		723.3 ± 104.0	
Haematocrit %	CTL	37.5 ± 0.5	0.07	37.7 ± 0.5	0.061	37.8 ± 0.6	0.044
	AV	36.0 ± 0.6		36.1 ± 0.6		35.9 ± 0.6	

Parameter	*Group*	*Day – 0*		*Day – 14*		*Day – 28*	
		Mean ± SD	*p-value*	*Mean ± SD*	*p-value*	*Mean ± SD*	*p-value*
Serum sodium concentration (mmol/l)	CTL	143.8 ± 2.5	0.383	143.0 ± 1.7	0.155	139.3 ± 4.2	0.009
	AV	147.5 ± 3.1		148.7 ± 3.3		154.2 ± 1.9	
Serum potassium concentration (mmol/l)	CTL	4.7 ± 0.3	0.133	4.7 ± 0.4	0.088	4.7 ± 0.3	0.067
	AV	5.3 ± 0.3		5.5 ± 0.2		5.4 ± 0.2	

p-value ≤ 0.05 is significant

Table 2: *Comparison of parameters (p-value) among days in each group (n = 6).*

Parameter	*Group*	*Day 0 – Day 14*	*Day 14 – Day 28*	*Day 0 – Day 28*
Body Weight (g)	CTL	0.012	0.005	0.002
	AV	0.046	0.005	0.004
Urine sodium concentration (mmol/l)	CTL	0.537	0.074	0.469
	AV	0.029	0.174	0.203
Haematocrit %	CTL	0.268	0.762	0.379
	AV	0.677	0.189	0.476
Serum sodium concentration (mmol/l)	CTL	0.818	0.341	0.308
	AV	0.328	0.143	0.048
Serum potassium concentration (mmol/l)	CTL	0.788	0.908	0.876
	AV	0.408	0.296	0.848

p-value ≤ 0.05 is significant

Blood haematocrit remained almost the same in both groups, with slight fall in AV group at the end of study. Difference between two groups was significant at this time. Serum sodium level decreased a little in control and increased in *Aloe vera* group. There was no significant change recorded over time for both groups, except from day 0 – 28 in AV group. Difference between two groups was also significant on day 28. Study showed no significant change in serum potassium concentration in both groups.

DISCUSSION

A number of mechanisms are involved in the reduction of inflammation by *Aloe vera*. Some of these mechanisms include inhibition of arachidonic acid pathway as well as inhibition of inflammatory mediators such as histamine, prostaglandins and serotonin[15] due to presence of anti-inflammatory agents like lectins, Salicyclic acid, acemanan, isoaloeresin D, malic acid acylated carbohydrates, auxins, gibberellins, bradykinases and superoxie dismutase.[17,18] Since a number of compounds and agents found in *Aloe vera* leaf – gel are believed to contribute to its anti-inflammatory effects, the exact dose of *Aloe vera* containing tolerable amount of active ingredients, needs to be investigated.

So far, only a few adverse effects of this medicinal herb have been reported in literature. Sodium and water retention is not one of them. It has been revealed in the present study that *Aloe vera* caused slight fall in blood haematocrit towards end of study. Serum sodium concentration increased with time in AV group and it was higher than control group but value was within normal range.[19] This could be due to the fact that inflammation is controlled by this herb partly through COX inhibition, which decreases prostaglandin production and causes sodium and water retention. At dose used in present study, this inhibition was not significant enough to cause renal effects. In fact, low doses of this herb have hypotensive effect.[20]

No research is available for comparison of effect of *Aloe vera* gel extract on parameters studied. Oral *Aloe vera* has not been tried on humans in a lot of clinical trials yet, and therefore its safe dose for human consumption is still under investigation. In a randomized controlled trial which was conducted on type 2 diabetic hyperlipidemic patients, it was found that *Aloe vera* in a dose of 300mg twice daily did not produce any adverse effect and was tolerated well by these patients.[21]

Another trial by Langmead *et al,* has been conducted, in which hospital out-patients of ulcerative colitis were given oral *Aloe vera* gel twice daily for a period of 4 weeks in a dose ranging from 50 – 100 ml. According to the results of that study, this dose level appeared to be safe and did not cause any unexpected adverse effects. However, minor cases of abdominal bloating, sore throat, and pedal edema were reported, which were not any different from placebo.[22]

Most of animal experimental studies using *Aloe vera* have been conducted on disease models for preventive or therapeutic effect. A study which was conducted on normal rats suggested that the dose of *Aloe vera* gel above 200 mg/kg caused fluid accumulation and weight gain in testis[23]. In another study *Aloe vera* impaired renal handling of electrolytes with subsequent hyponatremia and hypercreatinemia.[24] In contrast to this it was found in our study that *Aloe vera* extract in a dose of 200 mg/kg neither increased body weight nor caused electrolyte imbalance.

Anti-inflammatory and analgesic effect of *Aloe vera* has been studied in dose dependent manner from 100 mg to 600 mg daily for 7 days[16] and 300 mg daily for 14 days[7] without causing any toxicity and major adverse effect in albino rats. Our study lasted for 28 days and there was no adverse effect and toxicity reported supporting the results of these studies.

In ***conclusion***, *Aloe vera* gel extract consumed orally in 200 mg/kg dose in rats does not cause any body weight gain or water retention in healthy rats, but slight fall in blood hematocrit and rise in serum sodium concentration cannot be ignored and further studies using increased doses of *Aloe vera* gel extract need to be conducted.

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Authors' Contribution

A. Q. A. Designing of experiment, collection of data, Interpretation of results, drafting of manuscript, made changes by reviewers for final version. **M. H.** Literature search, manuscript writing. **A. M.** Compiling of data, statistical analysis. **S. C.** Conceived idea, supervised research.

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