

Hematobiochemical and Antioxidant Evaluation of *Aloe vera* Whole Leaf Extract on Fluoride Induced Toxicity in Wistar Albino Rats

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Received: February 09, 2016; Accepted: April 01, 2016; Published: April 16, 2016

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Abstract

Aloe vera whole leaf extract has a powerful antioxidant system by superoxide anion radicals scavenging, metal ion chelation, reducing power, hydroxyl radicals scavenging and total antioxidant activity in linoleic acid emulsion system. *Aloe vera* has been used worldwide both for pharmaceutical, food, and cosmetic industries due to the plethora of biological activities of some of its metabolites. *Aloe vera* also possesses anti-inflammatory, antitumor, anti-arthritis, antibacterial, antiulcer, hypoglycemic effects. Sub chronic administration of fluoride results in hematobiochemical alterations and *Aloe vera* amelioration in rats. Male Wistar albino rats that were randomly divided into four groups (n = 18). Sodium Fluoride was orally administered at 18mg NaF/ kg body weight to groups II and *Aloe Vera* at 200 mg /kg body wt. in distilled water was given along with Sodium Fluoride to group IV for 45 days to study its ameliorative effects. Group I and III were act as controls and treated with distilled water and *Aloe vera* respectively. The present data revealed reductions was recorded in the Hemoglobin (Hb) and Packed Cell Volume (PCV) and mean lymphocyte values whereas significant increase was noticed in TLC values and mean neutrophil count in NaF fed group. Serum total protein and calcium values were decreased significantly and serum creatinine and alkaline phosphatase levels were increased significantly in NaF treated rats. Oxidative damage indicators like SOD, Catalase and GPx levels were decreased in liver and kidney of all the NaF treated rats. In aloe ameliorated rats (Group IV), significant improvement was observed haematologically biochemically and antioxidant status when compared to the NaF treated group (Group II) due to direct or indirect hematoaugmenting properties, immunostimulatory effect and antioxidant properties of *Aloe vera*.

Keywords: Sodium fluoride toxicity; Hematology; Biochemical alterations; Antioxidant status; *Aloe vera* amelioration; Wistar rats

Introduction

Fluorosis is a major public health problem resulting from long-term consumption of water with high fluoride levels. In India, the states of Andhra Pradesh, Bihar, Chhattisgarh, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal are affected by fluoride contamination in water. This involves about 9000 villages affecting 30 million people [1]. Sources of

fluoride toxicity includes feed supplements and mineral mixtures with high fluoride containing rock phosphates, drinking water in fluoride rich soils (deep wells), consumption of forages by livestock grown in fluoride rich soils (stems and leaves accumulate fluoride), wire and cable insulations, pipe linings, rocket propellants, rodenticides, refrigerants, aerosol propellants, polymers for plastics, in the separation of uranium isotopes, and in the aluminum, beryllium, antimony, superphosphate fertilizer, electronic ceramics, fluorospar, the brick industries, industrial effluents, fumes from aluminium smelting factories, glass etching industries, acaricides, etc.,

Chronic fluoride poisoning is known to cause a variety of pathological changes in soft tissues. Structural and functional changes in muscle, liver, kidney, gastrointestinal tract, several reproductive and endocrine organs. Since, drinking water is the major source of excess fluoride, different techniques were developed for fluoride removal from water. However, most of them are unfit for clinical use because of chronic nature of the problem and associated side effects after their prolonged use. Therefore, nutritional intervention with antioxidant-rich substances is the ultimate goal as antidotes for combating with the health complaints arising from Fluorosis [2,3]. The toxic metal ions have been implicated in the generation of Reactive Oxygen Species (ROS) and Nitric Oxide (NO) and plant flavonoids could protect against oxidative damage.

In recent years, a considerable emphasis has been focused on the importance of the naturally available botanicals that can be consumed in an individual's everyday diet because of their antioxidant and anti-inflammatory properties [4]. Flavonoids may exert their beneficial effects either through their ability to lower oxidative stress and inflammation or directly by altering the signaling involved in neuronal communication, calcium buffering ability, neuroprotective stress shock proteins, plasticity, and stress signaling pathways [5].

Aloe vera has been used worldwide both for pharmaceutical, food, and cosmetic industries due to the plethora of biological activities of some of its metabolites. *Aloe vera* possesses

anti-inflammatory, antioxidant; antitumor, anti-arthritic, antibacterial, antiulcer, hypoglycemic effects [6]. *Aloe vera* has a powerful antioxidant system by superoxide anion radicals scavenging, metal ion chelation, reducing power, hydroxyl radicals scavenging and total antioxidant activity in linoleic acid emulsion system.

The experiment was carried out to study hematobiochemical and antioxidant evaluation of *Aloe vera* in sodium fluoride induced toxicity in male wistar albino rats with the following objectives.

1. To study the hematological and biochemical changes associated with sodium fluoride toxicity.
2. To study the oxidative damage associated with sodium fluoride toxicity.
3. To study the ameliorating effect of herbal product i.e., *Aloe vera* whole leaf extract in sodium fluoride induced toxicity.

Materials and Methods

Experimental animals

Male Wistar albino rats that were randomly divided into four groups (n = 18). During the experiment, the animals were kept in plastic cages in 12 light and dark cycles. The rats were randomized into four groups based on their body weights, so that the mean body weight of each group was comparable. Sodium Fluoride was orally administered at 18mg NaF/ kg body weight to groups II and *Aloe Vera* at 200 mg/ kg body wt. in distilled water was given along with Sodium Fluoride to group IV for 6 weeks to study ameliorative effects. Group I and III were treated with distilled water and *Aloe vera* respectively. Six rats from each group were sacrificed at fortnight interval. The experiment was conducted for 6 weeks with protocols approved by the institutional animal ethical committee.

Hematology

Blood was collected in 10% EDTA solution from all the groups at each sacrifice was used for the estimation of Total Leukocytes Count (TLC), Packed Cell Volume (PCV) by Microhematocrit method [7], Hemoglobin (Hb) by Sahli's method [8]. The blood smears were directly prepared and stained by Leishman's stain for Differential Leukocytes Count (DLC) by battlement method [5] and absolute counts were also calculated.

Biochemical profiles

Blood was collected from all groups at each sacrifice directly in to the sterile test tube and allowed to clot. The serum was collected and stored at 4°C until use and was used for the estimation of total serum proteins, calcium and alkaline phosphatase [ROBONIK (India) PVT Ltd] and creatinine [MEDSOURCE OZONE BIOMEDICALS Pvt. Ltd.].

Antioxidant status

At the end each sacrifice the liver and kidney were dissected and stored at -20°C until use. Tissue pieces of each organ were

homogenized in 0.05M ice cold phosphate buffer (pH 7.4). The homogenate was mixed with 10% trichloroacetic acid in the ratio of 1:1, centrifuged at 15,000rpm for 60min at 4°C and the supernatant obtained was used for estimation of super oxide dismutase [9], catalase [10] and glutathione peroxidase [11] in liver and kidney of all animals in all groups.

Statistical analysis

The data of both control and experimental groups were statistically analysed (one way ANOVA [12]) and significance was determined at results.

Results and Discussion

Hematology

The Mean and S.E values of Hematological parameters of different experimental groups (I, II, III & IV) are shown in Table 1. A significant ($P < 0.05$) decrease in Hb and PCV values was recorded in NaF treated rats (Group II) when compared to control rats (Group I). There was a significant increase in Hb and PCV values of amelioration group (Group IV) when compared to NaF treated group (Group II). There was a significant ($P < 0.05$) increase in TLC and mean neutrophil count values in NaF treated rats (Group II) when compared to the control rats (Group I). No significant decrease was noticed in TLC value of Group IV (*aloe vera* ameliorated rats) when compared to the Group II (NaF treated rats). Where as a significant ($P < 0.05$) decrease in mean lymphocytes count values in NaF treated rats (Group II) when compared to the control rats (Group I). Non significant improvement was noticed in mean lymphocytes count values of Group IV (*aloe vera* ameliorated rats) when compared to the Group II (NaF treated rats).

Biochemical alterations

The Mean and S.E values of Biochemical parameters and Antioxidant status of different experimental groups (I, II, III & IV) are shown in Table 2. A significant ($P < 0.05$) decrease in total serum proteins and calcium levels in NaF treated rats (Group II) when compared to control rats (Group I). Non significant increase was recorded in mean TSP and calcium values of *aloe vera* ameliorated rats (Group IV) when compared to the NaF treated rats (Group II). There was significant ($P < 0.05$) increase in serum creatinine and serum Alkaline Phosphatase (AKP) levels in NaF treated rats (Group II) when compared to the control rats (Group I). Non significant decrease was noticed in mean serum creatinine levels and a significant decrease in serum AKP levels of *aloe vera* ameliorated rats (Group IV) when compared to the NaF treated rats (Group II).

Antioxidant status

The Mean and S.E values of Antioxidant status of different experimental groups (I, II, III & IV) are shown in Table 3 respectively. Catalase, SOD and GPX overall mean values of liver and kidney of NaF treated rats (Group II) were significantly ($P < 0.05$) reduced when compared to control rats (Group I). Whereas non significant improvement was noticed in catalase and superoxide dismutase overall mean values of ameliorated

Table 1: Mean and Standard Error (SE) values of different hematological parameters in rats of different experimental groups.

Parameters Studied	Group I	Group II	Group III	Group IV
Hemoglobin concentration (g%)	13.73 ± 0.15 ^a	8.80 ± 1.27 ^b	14.47 ± 0.15 ^a	14.53 ± 0.18 ^a
Packed cell volume (%)	43.33 ± 0.88 ^a	26.00 ± 3.21 ^b	45.00 ± 0.58 ^a	42.33 ± 0.88 ^a
Total Leukocytes Count (thousands/ µl)	7.50 ± 0.18 ^b	13.73 ± 1.04 ^a	7.16 ± 0.12 ^b	8.48 ± 0.15 ^b
Neutrophils Count (%)	14.67 ± 0.88 ^b	29.00 ± 4.36 ^a	16.33 ± 0.88 ^b	17.67 ± 0.88 ^b
Lymphocytes Count (%)	83.00 ± 1.00 ^a	68.00 ± 4.36 ^b	80.33 ± 1.20 ^a	80.33 ± 0.88 ^a

Table 2: Mean and Standard Error (SE) values of different biochemical parameters in rats of different experimental groups.

Parameters Studied	Group I	Group II	Group III	Group IV
Total serum protein (g/dl)	9.58 ± 0.43 ^a	7.06 ± 0.88 ^b	10.54 ± 0.35 ^a	9.62 ± 0.40 ^a
Calcium (mg/dl)	61.82 ± 1.66 ^a	29.52 ± 3.43 ^b	63.59 ± 3.99 ^a	54.89 ± 3.73 ^a
Creatinine (mg/dl)	0.27 ± 0.03 ^b	0.58 ± 0.14 ^a	0.27 ± 0.03 ^b	0.32 ± 0.01 ^b
Alkaline Phosphatase (IU/l)	98.66 ± 3.07 ^c	179.28 ± 19.73 ^a	95.91 ± 5.32 ^c	136.95 ± 5.55 ^b

Table 3: Mean and SE values of Catalase, Superoxide Dismutase and glutathione Peroxide activity in liver and kidney of rats of different experimental groups.

Experimental groups	Catalase (nM of H ₂ O ₂ decomposed /min/mg of protein)		Superoxide Dismutase (SOD) (U/ min/mg of protein)		Glutathione Peroxide (GPx) activity (U/ min/mg of protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Group I	0.30 ± 0.01 ^a	0.22 ± 0.02 ^a	14.65 ± 1.02 ^a	25.34 ± 0.82 ^a	29.45 ± 0.62 ^a	30.00 ± 1.19 ^a
Group II	0.12 ± 0.04 ^b	0.12 ± 0.03 ^b	7.75 ± 1.79 ^b	13.40 ± 2.11 ^b	15.75 ± 3.17 ^b	16.74 ± 4.31 ^c
Group III	0.31 ± 0.01 ^a	0.22 ± 0.01 ^a	12.84 ± 0.47 ^a	26.34 ± 2.24 ^a	27.12 ± 0.53 ^a	26.67 ± 0.83 ^{ab}
Group IV	0.28 ± 0.01 ^a	0.18 ± 0.02 ^a	11.58 ± 1.62 ^{ab}	17.65 ± 1.26 ^b	20.99 ± 1.40 ^b	21.33 ± 2.69 ^{bc}

group (Group IV) when compared to sodium fluoride treated rats (Group II). There was a significant ($P < 0.05$) increase in GPx values of ameliorated group (Group IV) was observed when compared to NaF treated rats (Group II).

Significant decrease in PCV and Hb levels were observed in NaF treated group (II) when compared with the control group [13,14]. The decrease in haemoglobin concentration might be because of inhibition of globulin synthesis [15] or depression of erythropoiesis [16] or a decrease in the level of blood folic acid [17] or enhanced production of superoxide radicals and lipid peroxidation that lead to alterations in erythrocyte cell membrane function and structure [13]. Significant difference was noticed in between NaF and ameliorated groups which might be due to direct or indirect hematoprotective properties of *aloe vera* [18].

In the present study significant leucocytosis was observed in toxin treated group (Group II) when compared to the control. In ameliorated group (Group IV), non significant increase in TLC was noticed when compared to Group II rats which might be due to immunostimulatory activity of *aloe vera* [6].

In Differential leukocytes count, neutrophilia and lymphocytopenia were observed in fluoride treated rats (Group II) when compared to the control rats. This significant decrease in mean lymphocyte count observed in toxin treated group (group II) [14,19]. Lymphocytopenia might be due to depletion of lymphocytes in lymphoid organs like spleen and lymph nodes

as observed in the present experimental study. In ameliorated group (Group IV), non significant increase in mean lymphocyte count was noticed when compared to Group II rats, which might be due to immunostimulatory activity of *aloe vera* [6].

Significant decrease in the serum total protein was noticed in NaF treated rats (Group II) when compared to the control rats (Group I) [20-22]. The decreased levels of serum protein might be due to reduced feed intake and increased protein catabolism or hepato-renal damage as observed microscopically. In ameliorated group (Group IV), non significant increase in serum protein levels was noticed when compared to the NaF treated rats (Group II) which might be due to cytoprotective effect of *aloe vera* over hepatocytes [23].

Significant increase in the serum creatinine was noticed in toxin treated rats (group II) when compared to the control rats (group I) [24]. The increased levels of creatinine in NaF fed groups might be due to functional renal damage as evidenced by microscopical examination of kidney or urinary excretion of fluoride [25]. In ameliorated group, non significant decrease in serum creatinine levels compared to toxin treated group was observed and it might be due to cytoprotective and antioxidant effects of *aloe vera* [26].

In the present study, there was a significant ($P < 0.05$) decrease in mean serum calcium levels in NaF treated rats (Group II) when compared to control rats (group I) [27]. This decrease in serum calcium might be due to the binding of calcium

with fluoride to form calcium fluoride as evidenced by extensive histopathological changes in bones. In ameliorated group, non significant decrease in serum calcium levels compared to toxin treated group was observed and it might be due to antioxidant properties of *aloe vera* [6].

Alkaline Phosphatase (ALP) is the marker enzyme of fluoride toxicosis and bone pathology. An increase in serum alkaline phosphatase activity in animals treated with fluoride was observed in our study [28-30]. Increased activity of alkaline phosphatase might be due to the effect of fluorine intoxication on bone tissues or an increase in trabecular bone density which in turn might have lead to an increase in serum alkaline phosphatase [26] or binding of fluoride with magnesium ions, leading to inhibition of Mg-dependent enzymes, such as alkaline phosphatase [31]. In ameliorated group, non significant decrease in serum ALP levels compared to toxin treated group was observed and it might be due to cytoprotective and antioxidant effects of *aloe vera* [26].

In the present study a significant decrease in CAT, SOD and GPx was observed in liver and kidney of NaF treated animals when compared to control (Group I) [32-35]. The decrease in antioxidant enzymes activity in fluoride treated rats might be due to NaF induced generation of Reactive Oxygen Species (ROS) in liver and kidney. There was a significant ($P < 0.05$) increase in CAT, SOD and GPx values of ameliorated group (Group IV) when compared to NaF treated rats (Group II) were observed. This might be because of glycoprotein fraction of *aloe vera* that showed radical scavenging activity and isorabaichrome, an aloe derivative that has a potent antioxidant activity because of its caffeoyl group [36].

Acknowledgements

The authors are very much thankful to Sri Venkateswara Veterinary University, Tirupati for providing facilities to carry out Postgraduate research work in the Department of Veterinary Pathology, College of Veterinary Science, Tirupati.

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