WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 3, 1434-1457. Research Article ISSN 2277– 7105

EFFECT OF AQUEOUS EXTRACT OF RAW *ALOE VERA* LEAVES: HISTOPATHOLOGICAL AND BIOCHEMICAL STUDIES IN INDIAN PALM SQUIREL (*FUNAMBULUS PENNATI*) AND *ANABAS*

Dr. Bishnu Charan Pradhan*[1] and Dr. Bharat Bhusan Mahapatra[2]

Dept. of Zoology
[1]Angul Mahila Mahavidyalaya, Angul Odisha. 759122 India.
[2]G.I.S.T, Gunupur, Rayagada, Odisha, 765022. India.

Article Received on 17 Jan. 2017,
Revised on 07 Feb. 2017,
Accepted on 27 Feb. 2017
DOI: 10.20959/wjpr20173-8029

***Corresponding Author**
Dr. Bishnu Charan Pradhan
Dept. of Zoology Angul Mahila Mahavidyalaya, Angul Odisha. 759122 India.

ABSTRACT

Aloe vera is used worldwide for several medical purposes as alternative medicine. There are positive and negative reports on the hypoglycaemic effects of this plant. Forty-five juvenile Anabas and 30 weanling Squirel (*Funambulus penniti*) exposed to water containing 50, 100 and 150ppm of aqueous extract of *Aloe vera* leaves for 96 hours and 28 days, respectively were used for this study. Fifteen Anabas fish and 10 Squirel (*Funambulus penniti*) exposed to clean water (0 ppm *A. vera*) served as controls. Clinical signs, mortality, gross and histologic organ pathology in the Anabas fish; weekly haematology, plasma biochemical parameters and organ pathology were monitored in the Squirel (*Funambulus penniti*). Anabas fish cultured in water containing *A. vera* exhibited erratic swimming patterns, rapid opercular movements, skin depigmentation and died within 24-96 hours. Gross and histologic tissue lesions in the test Anabas fish include skin depigmentation, pale and shriveled gills, dull, opaque and sunken eyes, stunting and clubbing of gill filaments, vacuolar degeneration and necrosis of gill epithelial cells, hyaline degeneration and necrosis of myofibrils, calcification of vasa vasori, hepatocellular vacuolar degeneration and necrosis. Haematologic and plasma biochemical changes in test Squirel (*Funambulus penniti*) include moderate to severe normocytic normochromic anaemia, hypoproteinaemia, increased AST levels, and decreased cholesterol and triglyceride levels. Gross and histologic tissue lesions include mild to moderate pulmonary congestion, flabbiness of the heart, hepatomegaly, mottling of kidneys, vacuolar degeneration and necrosis of hepatocytes, Kupffer cell hyperplasia, periportal fibrosis, glomerular and tubular degeneration and necrosis, matting and clubbing of small intestinal villi, catarrhal enteritis and goblet cell hyperplasia. The severity of these changes increased with increasing concentrations of *A. vera*. No mortality, gross or histologic changes were observed in both control fish and Squirel (*Funambulus penniti*) Results from this study show that consumption of water containing extracts of raw *A. vera* is very toxic to *Anabas* fish and Squirel (*Funambulus penniti*). The serious health implication for human consumption of raw *A. vera* is discussed.

KEYWORDS: *Aloe vera*, *Anabas*, Squirel (Funambulus penniti) alternate medicine, human health implications.

INTRODUCTION

Aloe vera (L), a member of the family *Lilieceae,* is a popular perennial succulent plant that is cactus - like in its characteristics (Tyler, 1993).[91] The plant has a long history as a multipurpose folk remedy (Reynolds and Dweck, 1999)[73] and has been associated with myth, magic and medicine since pre-biblical times (Balter, 1992).[6] Historical evidence indicates that *A. vera* originated in the warm, dry climate of southern and eastern Africa and was subsequently introduced into norther the mechanism of the reduction in mean glucose levels produced by *A. vera* has not been yet elucidated.

Aloe vera L. Burm. f. or *Aloe barbadensis* Miller (Liliaceae) is native of North Africa and also cultivated in Turkey. This miraculous plant has been used in the traditional medicinal practices of many cultures for a host of curative purposes (Capasso *et al*. 1998[19], Vogler & Ernst 1999[96]). Although oral hypoglycaemic agents are effective in controlling hyperglycaemia, they have prominent side effects (Rang *et al*. 1999).[69] This leads to increasing demand for herbal products plant with antidiabetic activity and fewer side effects (Shane-Whorter 2001[78], Grover *et al*. 2002[39], Vetrichelvan & Jegadeesan 2002[95]). In experimental diabetes, streptozotocin (STZ) causes selective degeneration of pancreastic β-cells thereby inhibiting insulin secretion. *A. vera* has also been shown to have antidiabetic and hypoglycaemic properties (Agarwal 1985[1], Hikino *et al*. 1986[43], Ghannam *et al*. 1986[34], Ajabnoor 1990[2], Beppu *et al*. 1993,[10] Okyar *et al*. 2001a[60]). It was postulated that hypoglycaemic effect of *A. vera* could be mediated through stimulation of synthesis and/or release of insulin from the β-cells of Langerhans (Ajabnoor 1990[2]). However.

Africa, the Arabian Peninsula, China, Gibraltar, the Mediterranean countries and the West Indies (Haller, 1990).[40] Hedendal (2000)[41] described *A. vera* as one of the most talked about, yet most misunderstood plants in history. Modern clinical use of *A. vera* began in the 1920s and claims now abound, in numerous research and commercial literature in journals and on the Internet, regarding its numerous therapeutic potentials when used both topically and parenterally. It is acclaimed to cure ailments ranging from mild fever, wounds and burns, gastrointestinal disorders, diabetes, sexual vitality and fertility problems to cancer, immune modulation and AIDS (Woo *et al*., 1981; Sebastian and Bhandari, 1984;[77] Agarwal, 1985[1]; Koo, 1994[50]; Kim and Lee, 1997[49]; Kemper and Chiou, 1999[47]; Kim *et al*., 1999[48]; Reynold and Dweck, 1999[73]; Davis, 1994[25]; Ritter, 2003[74]).

In India, there is a very strong cultural belief in herbal medicare, most often due to the latter's economic advantage and easier reach compared to the high cost of orthodox medicine. This is more compounded by low literacy levels and often epileptic and grossly inefficient orthodox healthcare delivery system. Since the sudden introduction and widely acclaimed megatherapeutic potentials of *A. vera* and its products (the *cure -all* craze) in the mid 1990s, and the highly expensive "processed" *A. vera* products, it is common site to see homestead *A. vera* "plantations" at every corner in most towns and villages. This has led to unrecommended and uncontrolled consumption of raw *A. vera* leaves by the lowly and mighty in the society. Conflicting reports on the therapeutic potentials of *A. vera* (Schmidt and Greenspoon, 1997[84]), its toxicity, especially when used parenterally (Brusick and Menge, 1997[18]; Balter, 1992[6]) and the fact that most advertised *A. vera* products have no specific approval for use by the Food and Drug Administration of the United States of America (FDA) (FLP, 2003[28]; Changes International, 2004[20]) necessitated this current research on the effects of aqueous extracts of raw *A. vera* leaves on rats and fish, as animal models. The implication of unguided human consumption of *A. vera* leaves in our healthcare system is discussed.

MATERIALS AND METHODS

Preparation of Aqueous Extract of Aloe vera

Aloe vera plant was obtained from the Department of Agronomy, District of Angul, Odisha, India. The leaves were washed with clean water, cut into pieces and weighed. 250gm of the cut leaves was pulverized in an electric blender (Philip Electrical, UK), soaked in 2 litres of water for 3 hours and later filtered through a 1mm mesh sieve. The filtrate was made up to 10 litres with water, making a working dilution of 25,000ppm of the water extract of A. vera.

Fish Experiment (Anabas)

Sixty juvenile Anabas fish, Oreochromis niloticus (mean length 9.5±1.5cm; body weight 30.0±5.0g) were purchased from Tropical Aquaculture Products Limited, a commercial fish multiplication center in Angul, Odisha, India. The fish were divided into four groups of 15 juveniles each in aerated four 40.5 litre plastic aquaria. Each of the aquaria contained 25 litres of water to which appropriate volumes of the working stock of the aqueous extract of A. vera were added to make 0ppm (no A. vera), 50ppm, 100ppm and 150ppm. Fish in the 0ppm tank served as the controls. Water temperature, dissolved oxygen and ammonia levels were maintained at acceptable levels (Boyd, 1981[16]; Moses, 1983[57]). The pH value of the water in each tank was determined using a pH probe (Gallenkamp, GmBh, Germany) and recorded. The fish were fed at 5% of their body weight, daily in two equal rations, in the morning (7.00hr) and evening (18.00hr). The feed was compounded to contain 28% crude protein and 3,400 calories. Immediately after the fish were placed in the tanks, observations of clinical signs (swimming patterns and response to stimuli), skin colouration and mortality were monitored every 6 hours in a 96-hour biostatic assay.

Squirel Experiment

Forty Squirel (*Funambulus penniti*), aged 8 weeks, purchased from the Experimental Animal Laboratory of the Faculty of Veterinary Medicine, University of Odisha, India were used for this study. The Squirel (*Funambulus penniti*) were stabilized for 1 week during which they were fed commercial rat pellets (Ladokun Feeds, Odisha, India) and clean drinking water ad libitum. At the commencement of the experiment, the Squirel (*Funambulus penniti*), were divided into four groups of 10 Squirels each and given drinking water containing 0ppm, 50ppm, 100ppm and 150ppm aqueous extract of A. vera, as described for the Anabas fish experiment. Clinical signs were monitored every 6 hours.

Two Squirel (*Funambulus penniti*), per group were randomly selected on days 0, 7, 14, 21 and 28 and mildly sedated in a diethyl ether fume chamber for 1-2 minutes. They were thereafter put on dorsal recumbency and the skin on the ribs and sternum was held taut using the left thumb and index fingers. Using a 22G needle attached to a 5ml syringe, 3ml of blood was aspirated from the heart into labeled tubes containing the sodium salt of ethylenediamine tetraacetic acid (Na-EDTA) anticoagulant and gently mixed to prevent clotting. Packed cell volume (PCV), haemoglobin (Hb) concentration, erythrocyte (RBC), total leucocyte (WBC) and differential leucocyte counts and corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined by standard techniques (Jain, 1986). After the centrifugation of the unclotted blood at 1,200g for 10 minutes, the plasma was carefully removed and used for the estimation of the following biochemical parameters: total protein, albumin, globulin, urea and creatinine as described by Ogunsanmi et al. (1994).[62] Others include activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT), measured by the procedures of Reitman and Frankel (1957)[72], alkaline phosphatase (ALP) by a modified method of Frajola et al. (1965)[32] and □-glutamyl transferase (GGT) by the method of Szas (1969).[87] The plasma total cholesterol and triglyceride were determined as described by Toro and Ackermann (1975)[90].

Thereafter, the Squirel (*Funambulus penniti*), were dissected and gross changes in the organs observed and recorded. Sections of the intestines, liver, lungs, kidney, heart and brain of each Squirel (*Funambulus penniti*), were harvested into labelled sample bottles containing 10% phosphate-buffered formalin fixative for 24 hours. They were thereafter, trimmed and dehydrated in graded concentrations of xylene, embedded in wax and sectioned at 5µ and fixed on to clean, grease-free glass slides. The thin sections were stained with haematoxylin and eosin (H&E) for histologic examination under the light microscope. Photomicrographs of organ/tissue lesions were taken with Authotek Camera (Leitz GmBh, Germany), attached to the light microscope and processed routinely.

Statistical Analyses

Data obtained were subjected to statistical analysis using 2-way analysis of variance (SAS, 1987)[75] and means were compared for significant differences (if any) using the Duncan's multiple range test (Duncan, 1959[27]).

RESULTS

Fish Experiment

No untoward clinical changes were observed in the control tank (0ppm A. vera) throughout the 96 hours of the experiment. Varying degrees of changes in swimming patterns, consisting of erratic swimming and reversal movements, attempts at jumping out of water, rapid opercular movements, gasping, staying for long periods of time under water and blanching (depigmentation) of skin were observed in *Anabas* fish in the test tanks. These changes were progressively more severe with increase in concentration of A. vera extract in tank water, that is, 50ppm, 100ppm and 150ppm, with the latter being most severe. The pH of the stock aqueous extract of A. vera was 5.8, while those of the water in the aquaria are Tank A (0ppm A. vera) – pH 7.2, Tank B (50ppm A. vera) – pH 6.9, Tank C (100ppm A. vera) – pH 6.7 and Tank D (150ppm A. vera) – pH 6.6. The mortality patterns of the test *Anabas* fish are as shown on Table 1. None of the control fish died throughout the period of study. Similarly, no deaths were recorded in all the *Anabas* fish tanks within 24 hours of the experiment. The first sets of mortalities (40%) were recorded after 24 hours in the Tank D (150ppm A. vera). 100% mortality was recorded in Tank C (100ppm) after 48 hours, while 80% of the *Anabas* fish in Tank B (50ppm) died after 72 hours.

Table 1: Mortality patterns in the control and test tilapia exposed to varying concentrations of aqueous extract of *A. vera*

	Concentration of	**Mortality**
	A. vera	**(n/15; %)**
Between 0 – 24 hours		
Tank A	0 ppm	(0%)
Tank B	50 ppm	(0%)
Tank C	100 ppm	(0%)
Tank D	150 ppm	(0%)
24 – 48 hours		
Tank A	0 ppm	(0%)
Tank B	50 ppm	(0%)
Tank C	100 ppm	(0%)
Tank D	150 ppm	(40%)
48 – 72 hours		
Tank A	0 ppm	(0%)
Tank B	50 ppm	(20%)
Tank C	100 ppm	15 (100%)
Tank D	150 ppm	(60%)
72 - 96 hours		
Tank A	0 ppm	0%)
Tank B	50 ppm	12 (80%)
Tank C	100 ppm	
Tank D	150 ppm	

No significant gross and histopathological changes were observed in the tissues and organs of control *Anabas* fish (0ppm *A. vera*) throughout the period of experiment. Table 2 shows the scores of gross and histologic changes in tissues/organs of the *Anabas* fish in the various experimental tanks. Generally, gross lesions include depigmentation of skin and pale and shriveled gills and dull, opaque and sunken eyes. Histologic changes include varying severities and spread of stunting and clubbing of gill filaments vacuolar degeneration and necrosis of gill epithelial cells and heterophilic infiltration of the gill submucosae (Plate 1). Others include hyaline degeneration and necrosis of myofibrils and calcification of vasa vasori, vacuolar degeneration and necrosis of hepatocytes and to a lesser degree, pancreatic cells and infiltration by melanomacrophages. There was severe villous atrophy, characterized by denudation and stuntingof villi, congestion, glandular degeneration, necrosis and lymphocytic infiltration in the submucosae of the intestines (Plate 2) and focal areas of neuronal degeneration, neuronophagia and gliosis in the cerebrum. The most severe histologic lesions were observed in *Anabas* fish in the tank containing 50ppm *A.vera*

Table 2: Pathology scores of control and test *Anabas* fish exposed to varying concentrations of aqueous extract of *A. vera*

Organ/Tissue Lesions	Tank A (0ppm)	Tank B (50ppm)	Tank C (100ppm)	Tank D (150ppm)
Skin and Eyes				
Blanched (depigmented) skin	-	+++	++	±
Sunken eyes	-	++	++	+
Gills				
Blanched and shriveled	-	++	++	±
Stunted, matted, clubbed gill	-	+++	++	+
filaments				
Vacuolar degeneration, necrosis of	-	++	++	+
epithelia cells				
Liver				
Hepatic degeneration and necrosis	-	+++	++	±
Heart				
Hyaline degeneration and necrosis of	-	++	+	±
myofibrils				
Calcification of vasa vasori	-	+	++	-
Small intestines				
Villous atrophy (matting and stunting)	-	+++	++	+
Goblet cell hyperplasia	±	++	++	+
Glandular degeneration and necrosis	-	+++	++	+
Brain				
Neuronal degeneration	-	++	+	±
Gliosis	-	+	++	+

- = No lesions observed;	± = Mild, focal lesions;	+ = Mild, multifocal lesions; ++ =	Moderately severe

- = No lesions±= Mild, focal = Mild, multifocal lesions; ++

observed; lesions; = Moderately severe diffuse lesions; +++ = Very severe diffuse lesions

Table 3: Haematology of Squirel (*Funambulus penniti*), given oral administration of the water extract of *A. Vera*

Days	Squirel Group	Haematological parameters/indices				
		PCV (%)	Hb conc. (g/dl)	RBC counts (x10 /µl)	MCV (fl)	MCHC (g/dl)
0	Group A	29.5±3.3[a]	8.5±0.7	4.3±0.1	67.5±1.9	29.3±2.3
	Group B	24.5±2.1[b]	10.2±4.0[a]	4.7±0.5[a]	63.6±2.2[a]	33.3±3.1[a]
	Group C	23.5±2.1[b]	10.3±2.3[a]	5.0±0.3[a]	65.6±6.7[a]	33.5±3.9[a]
	Group D	22.6±1.3[b]	8.7±1.1[ab]	4.5±0.2[a]	65.2±5.1[a]	30.2±3.0[a]
7	Group A	29.5±3.3[a]	9.8±2.1[ab]	4.9±0.6[a]	60.3±3.1[a]	33.2±2.6[a]
	Group B	24.5±2.1[b]	8.1±0.7[ab]	3.9±0.3[b]	62.8±1.2[a]	33.1±2.4[a]
	Group C	23.5±2.1[b]	6.9±0.5[b]	3.5±0.3[b]	67.2±1.3[a]	29.8±4.8[a]
	Group D	22.6±1.3[b]	6.7±1.1[b]	3.6±0.5[b]	63.1±3.2[a]	29.7±2.6[a]
14	Group A	28.0±2.7[a]	8.9±1.9[a]	4.1±0.6[a]	68.4±3.4[a]	32.1±1.1[a]
	Group B	22.5±0.7[b]	7.5±0.2[ab]	3.6±0.1[b]	62.6±2.0[a]	33.3±2.1[a]
	Group C	24.0±2.8[b]	7.9±0.9[ab]	3.6±0.8[a]	66.7±2.5[a]	32.9±2.9[a]
	Group D	23.1±1.4[b]	6.8±1.1[b]	3.4±1.3[b]	68.6±1.1[a]	29.8±2.0[a]
21	Group A	30.0±2.1	9.1±0.7	4.5±0.3	66.9±1.1	33.6±1.9
	Group B	23.6±2.3[b]	7.2±2.1[ab]	3.7±0.9[b]	64.4±1.5[a]	31.0±2.6[a]
	Group C	23.2±4.2[b]	7.4±1.4[ab]	3.5±0.5[b]	66.3±1.4[a]	31.7±1.0[a]
	Group D	22.4±2.2[b]	7.1±0.9[ab]	3.2±0.4[b]	66.9±0.9[a]	32.0±1.3[a]
28	Group A	31.5±0.7	10.1±0.2	4.5±0.6	70.1±3.5	32.4±3.9
	Group B	24.0±1.4[b]	7.0±0.8[ab]	3.4±0.3[b]	68.4±2.5[a]	29.3±2.0[a]
	Group C	23.5±2.9[b]	7.3±1.6[ab]	3.4±0.2[b]	69.1±1.3[a]	31.1±1.2[a]
	Group D	23.0±1.7[b]	7.2±1.8[ab]	3.3±0.5[b]	69.7±2.1[a]	31.4±2.3[a]

- *Squirel in Groups A, B, C and D received 0, 50, 100 and 150ppm aqueous extract of A. vera, respectively*

Table 4: Plasma proteins and enzyme activities of Squirel (*Funambulus penniti*), given oral administration of the water extract of *A. vera*

Days	Squirel Group	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	ALP (IU/l)	ALT (IU/l)	AST (IU/l)
0	Group A	6.6±0.3	3.1±0.1	3.5±0.3	177.5±11.8	38.5±2.8	22.0±1.0
	Group B	6.4±0.3[a]	3.2±0.1[a]	3.2±0.1[a]	145.0±7.7[c]	35.0±9.9[a]	23.5±1.2[e]
	Group C	6.4±0.2[a]	3.3±0.2[a]	3.1±0.2[a]	159.0±10.1[c]	34.0±1.4[a]	23.0±1.4[e]
	Group D	6.6±0.3[a]	3.3±0.2[a]	3.3±0.3[a]	166.3±9.6[b]	39.0±2.3[a]	22.3±2.0[e]
7	Group A	6.6±0.2	3.1±0.2	3.5±0.2	191.0±12.7	27.0±1.2	23.0±1.0
	Group B	6.1±0.2[b]	2.5±0.3[b]	3.6±0.3[a]	146.0±8.4[c]	28.5±4.9[ab]	26.9±2.4[d]
	Group C	6.0±0.1[b]	2.6±0.1[b]	3.4±0.2[a]	167.0±7.7[b]	26.5±3.5[b]	28.0±2.1[d]
	Group D	5.9±0.1[b]	2.4±0.2[b]	3.5±0.1[a]	155.9±8.6[c]	30.1±3.3[a]	26.9±2.2[d]
14	Group A	6.5±0.1	3.2±0.3	3.3±0.1	220.0±14.4	30.0±2.8	20.0±1.4
	Group B	5.6±0.1[c]	2.2±0.1[b]	3.4±0.2[a]	179.0±4.2[ab]	27.0±4.4[b]	40.0±2.7[c]
	Group C	5.7±0.2[c]	2.4±0.1[b]	3.3±0.2[a]	145.0±7.7[c]	30.3±2.3[a]	38.0±1.9[c]
	Group D	5.4±0.3[c]	2.2±0.2[b]	3.2±0.1[a]	201.2±3.3[a]	37.2±3.0[a]	52.3±6.3[b]

21	Group A	6.6±0.1	3.3±0.2	3.3±0.0	215.5±18.7	38.0±2.3	22.0±1.5
	Group B	5.6±0.0[c]	2.2±0.1[b]	3.4±0.0[a]	168.0±4.4[b]	29.0±1.4[a]	59.0±4.1[a]
	Group C	5.5±0.1[c]	2.3±0.2[b]	3.2±0.1[a]	137.0±7.8[c]	35.0±4.2[a]	63.0±6.2[a]
	Group D	5.6±0.2[c]	2.1±0.2[b]	3.4±0.2[a]	166.7±10.3[b]	29.6±4.5[a]	60.5±3.5[a]
28	Group A	6.7±0.2	3.5±0.1	3.2±0.1	187.5±10.6	34.5±3.9	25.0±2.4
	Group B	5.3±0.1[c]	2.1±0.0[b]	3.2±0.2[a]	222.0±16.7[a]	28.5±2.7[ab]	61.0±4.4[a]
	Group C	5.5±0.0[c]	2.3±0.0b	3.2±0.0[a]	149.0±8.4[c]	27.0±2.0[b]	67.5±3.5[a]
	Group D	5.4±0.1[c]	2.2±0.1b	3.2±0.1[a]	200.9±6.7[a]	31.2±2.2[a]	68.3±3.8[a]

Aloe Vera leaves: histopathologic and Biochemical effectsz.

Squirel (*Funambulus penniti*), *in Groups A, B, C and D received 0, 50, 100 and 150ppm aqueous extract of A. vera, respectively*

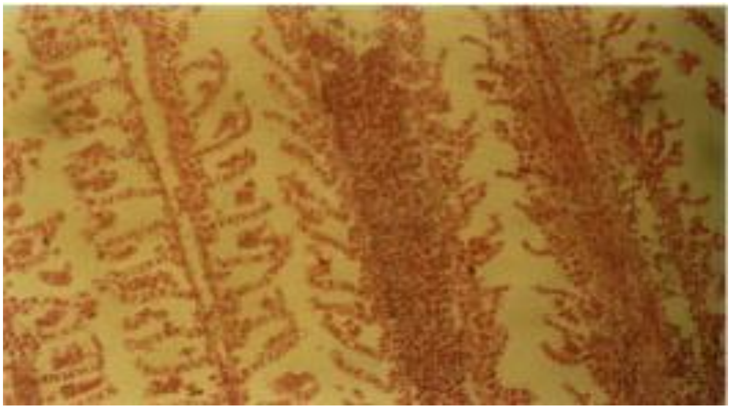

Plate:- 1: Photomicrograph of the gills of test fish in 50 ppm *Aloe* showing widespread vacuolar degeneration and necrosis of epithelia cells, haemorrhages and heterophilic infiltration. H & x600

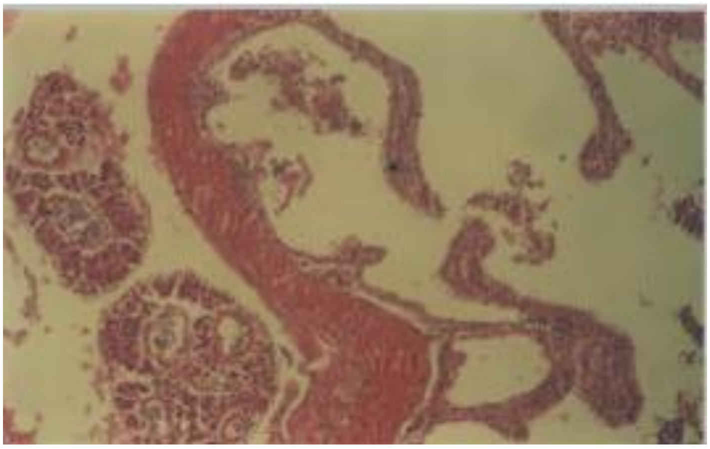

Plate:- 2: Photomicrograph of the intestine of test fish in 50 ppm *Aloe* showing severe haemorrhage, stunting and denudation of villi and glandular. degeneration H & E. x45

Squirel Experiment

in Squirel Groups B, C and 4Ddeveloped moderately severe normocytic normochromic anaemia ($p<0.05$) from day 7 onwards, while the control rats (Group 1; 0ppm *A. vera*) were not anaemic throughout the 28-day experimental period (Table 3). There were no notable changes in the leucogram of all the rats throughout the experiment (data not shown). Hypoproteinaemia, characterized by hypoalbuminaemia ($p<0.05$) developed in rats of Groups B, C and D, from day 7 onwards, with the latter being most severe (Table 4). Of the plasma enzymes, only AST increased considerably ($p<0.05$) in rats of Groups C and D from day 14 onwards. ALP and ALT levels showed inconsistent changes in the experimental rats throughout the experiment. Both cholesterol and triglyceride levels decreased ($p<0.05$) after day 7 onwards; while there were no significant changes ($p>0.05$) in plasma creatinine levels, changes in plasma urea levels were inconsistent (Table 5).

Table 6 shows the scores of gross and histologic changes in tissues/organs of the experimental rats. Apart from slightly congested lungs in one of rats on day 14 of the experiment, no observable gross lesions were observed in all the organs of the control rats (0ppm *A. vera*) throughout the 28-day period of the study.

Table 5: Plasma lipids and metabolites (mg/dl) of Squirel (*Funambulus penniti*), given oral administration of aqueous extract of *A. vera*

Days	Squirel Group	Cholesterol	Triglyceride	Creatinine	Urea
0	Group A	128.0±4.3	110.0±4.7	1.0±0.0	21.0±1.4
	Group B	124.5±4.9[a]	109.0±5.5[a]	1.1±0.2[a]	22.5±1.1[b]
	Group C	128.5±2.1[a]	110.5±7.5[a]	0.9±0.3[a]	20.0±3.5[b]
	Group D	126.5±2.3[a]	108.9±6.6[a]	1.0±0.2[a]	23.1±1.5[b]
7	Group A	129.0±2.4	109.5±2.0	1.0±0.1	22.5±0.7
	Group B	105.0±2.6[b]	95.0±1.2[b]	1.3±0.2[a]	27.0±1.9[a]
	Group C	110.0±4.4[b]	99.0±4.2[b]	1.2±0.3[a]	21.0±1.4[b]
	Group D	113.5±3.6[b]	98.6±3.5[b]	0.8±0.2[a]	25.3±2.1[ab]
14	Group A	125.0±1.7	111.0±2.0	1.1±0.1	21.0±1.6
	Group B	101.0±5.5[b]	96.0±1.8[b]	0.8±0.1[a]	16.5±2.1[c]
	Group C	88.5±2.2[c]	77.0±0.0[c]	0.7±0.2[a]	25.0±2.4[ab]
	Group D	100.2±3.1[b]	80.1±5.2[c]	1.2±0.3[a]	22.3±1.8[b]
21	Group A	124.0±2.7	108.0±1.7	1.5±0.5	29.0±1.4
	Group B	107.5±1.1[b]	80.0±2.8[c]	0.9±0.1[a]	26.5±2.1[a]
	Group C	90.0±2.8[c]	80.1±0.7[c]	1.0±0.2[a]	22.0±1.4[b]
	Group D	91.3±1.2[c]	78.8±1.1[c]	1.3±0.0[a]	22.5±1.6[b]
28	Group A	127.5±2.5	109.0±2.2	0.9±0.3	21.0±4.1
	Group B	92.5±2.2[c]	81.5±3.5[c]	1.5±0.3[a]	20.0±2.8[b]

	Group C	77.5±3.5[d]	65.0±1.3[d]	0.9±0.2[a]	26.5±2.2[a]
	Group D	78.3±2.2[d]	63.8±3.2[d]	0.8±0.2[a]	26.1±3.1[a]

* Squirel (*Funambulus penniti*), *in Groups A, B C and D received 0, 50, 100 and 150ppm aqueous extract of A. vera, respectively*

Table 6: Pathology scores of Squirel (*Funambulus penniti*), given oral administration of varying concentrations of the aqueous extract of A. *vera*

Organ/Tissue Lesions	**Group**	**A**	**Group**	**B**	**Group**	**C**	**Group**	**D**
	(0ppm)		**(50ppm)**		**(100ppm)**		**(150ppm)**	
Lungs								
Pulmonary congestion	-		++		++		++	
Thickened and peribronchiolar								
perivascular lymphocytic cuffs	±		+		++		++	
Liver								
Hepatomegaly	-		+		++		+++	
Hepatic degeneration and necrosis	-		+		+++		+++	
Kupffer cell hyperplasia	-		±		++		++	
Periportal fibrosis	-		-		±		+	
Heart								
Flabbiness	-		+		+		++	
Hyaline degeneration and necrosis of								
myofibrils	-		+		++		++	
Mononuclear cellular infiltration	-		±		±		+	
Kidney								
Mottling	-		+		++		++	
Glomerular and tubular degeneration								
and necrosis	-		++		++		++	
Small Intestine								
Congestion and haemaorrhage	±		+		++		+++	
Villous atrophy (matting and clubbing)	-		++		++		+++	
Catarrhal enteritis	-		++		++		++	
Glandular degeneration and necrosis	-		+		++		++	
Brain								
Meningeal and congestion	-		±		+		+	
haemorrhage	-		±		+		+	
Perivascular oedema	-		+		+		++	
Neuronal degeneration and gliosis								

Key to scores:-

- = No lesions observed;

+ = Mild, multifocal lesions;

+++ = Very severe diffuse lesions

± = Mild, focal lesions

++ = Moderately severe diffuse lesions

Similarly, no gross lesions were observed on the lungs, hearts, brain, intestines, spleen and kidneys of Groups B and C Squirel (*Funambulus penniti*), up till 14 days of the study. However, there was a progressive increase in the paleness and sizes of the livers of the in Squirel (*Funambulus penniti*), Groups B, C and D from day 7 of the study onwards. Hepatomegaly was most severe in the Squirel (*Funambulus penniti*), in Groups C on day 14. There was a moderate flabbiness of the heart and increasing mottling of the kidneys of Squirel (*Funambulus penniti*), in Groups C and D. These were most severe in rats of Group D on the 28th day. No gross lesions were observed on the brains and intestines, except that the intestinal contents became progressively pasty and later watery in of all the rats of Groups B, Cand D from day 7 till the end of the study.

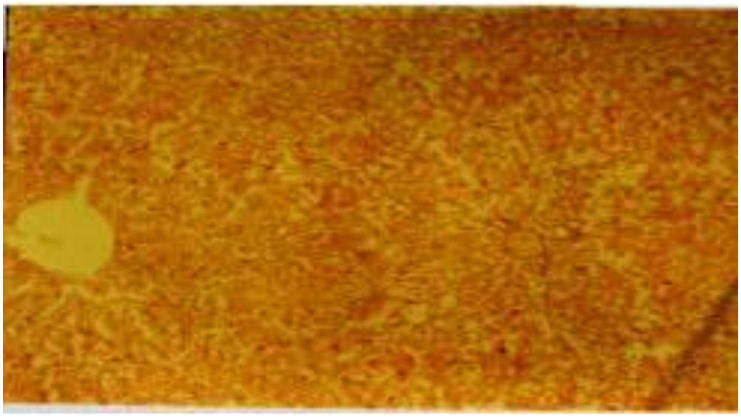

Plate. 3: Photomicrograph of the liver of a Group D Squirel (*Funambulus penniti*), (150 ppm *Aloe*) on day 7 showing diffuse periportal and centrilobular vacuolar degeneration of hepatocytes H & E. x450

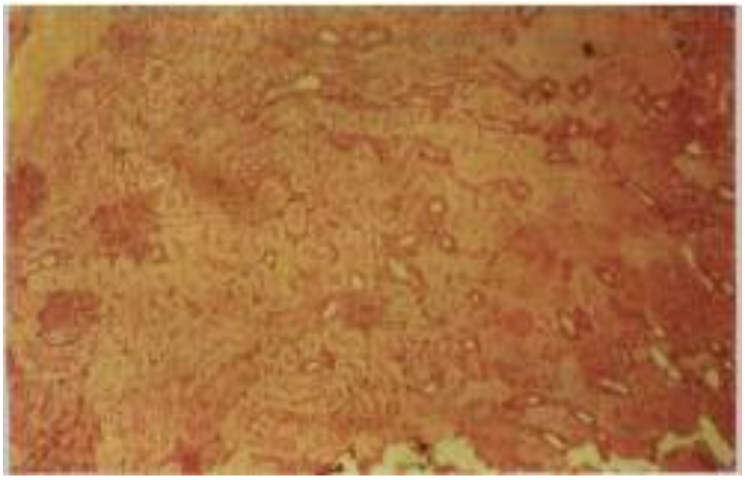

Plate 4: Photomicrograph of the cortex of the kidney of a Group B Squirel (*Funambulus penniti*), (50 ppm *Aloe*) on day 14 showing severe degeneration and necros is of glomeruli and tubules H & E. x600

Histopathological changes include very mild to moderate pulmonary congestion, mild peribronchiolar and perivascular lymphocytic cuffings and thickened interalveolar septa in the lungs, varying severities of vacuolar degeneration and necrosis of hepatocytes. (Plate 3), Kupffer cell hyperplasia and mild periportal fibrosis, especially on day 28 in squirels of Group D. There was moderate to severe glomerular and tubular degeneration and necrosis (Plate 4). Intestinal lesions include matting and clubbing of villi (Plate 5), catarrhalenteritis and goblet cell hyperplasia. There were focal areas of hyaline degeneration and necrosis of myofibrils and mononuclear cellular infiltration in rats of Groups B and D on day 28. Brain lesions include meningeal congestion and haemorrhages, perivascular oedema, neuronal degeneration and moderate gliosis.

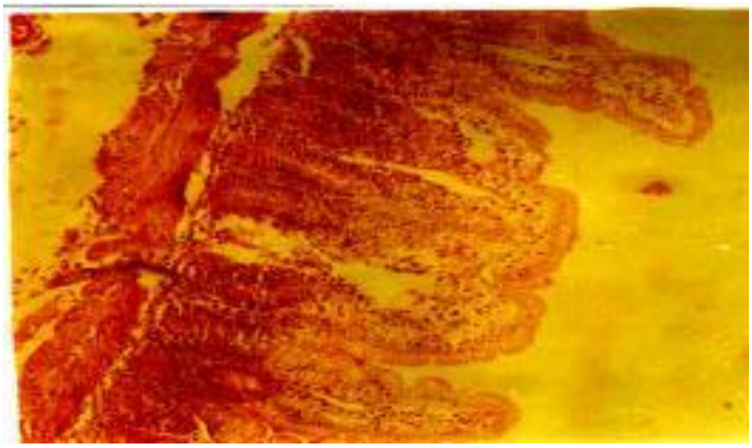

Plate 5: Photomicrograph of the small intestine of a Group C Squirel (*Funambulus penniti*), (150 ppm *Aloe*) on day 7 showing matting and stunting of villi H & x450.

Indian Palm squirels

DISCUSSION

Essentially two products can be extracted from *A. vera* leaves; the clear gel that forms naturally in the leaf's hollow interior is used to treat skin irritation and it is an active ingredient in hundreds of skin lotions, toilet soaps, sun blocks and cosmetics (Grindlay and Reynolds, 1986[38]; Kemper and Chiou, 1999[47]; Foster, 2004[29]; Moody *et al*., 2004[56]). The resin canal cells found in thick leaf epidermis produce a yellow juice (latex) that is used as a laxative and disinfectant (Tyler, 1993[91]; Ghazanfar, 1994; Foster, 2004[29]) and in experimental and folklore medicine for liver complaints, piles, emetic, anti-pyretic, enlarged spleen, cooling agent, skin diseases, tuberculosis, fungal diseases, peptic ulcers, reduction of blood glucose levels, asthma, AIDS, anti-cancer, and as an immunomodulator (Shah *et al*., 1989; Kemper and Chiou, 1999[47]; Reynolds and Dweck, 1999[73]; Hedendal, 2000[41]). In fact the list is endless. The terms "gel" and "juice" are not clearly defined by manufacturers and often are confused by consumers. The mechanical separation process is not always complete, so *A. vera* latex can be found in some *A. vera* gels; hence it is desirable to make the gels as pure as possible, because *A. vera* latex contains the anthraquinone glycosides - aloin A and B, which are potent laxatives (Tyler, 1994).[92]

The results obtained in this study have shown the water extract of *A. vera* as a very potent toxic substance to *Anabas* fish and whe Squirel (*Funambulus penniti*), when consumed. It is especially lethal to Anabas fish at as low as 50ppm in water causing 100% mortality within 96 hours. There was severe depigmentation, destruction of the gills, intestines, liver and less so the kidneys and brain, with the worst organ damage occurring in fish with longer exposure of the *A. vera* extract (50ppm) over 96 hours. The organ and tissue damage in the experimental fish may be due to the direct toxicity of the *A. vera* extract on organs such as the gills, intestines, liver and heart. Damage, especially to the gills, will affect oxygen exchange and tissue respiration, culminating in organ and tissue hypoxia, degeneration and necrosis. The pH of the various concentrations of the water with *A. vera* extract in the aquaria are slightly in the acidic range (pH 6.6 – 6.9). *A. vera* gel is 99% water with a pH of 4.5 (Kemper and Chiou, 1999).[47]

Even though, no mortality was recorded in the experimental rats in this study, diarrhoea, catarrhal enteritis, villous atrophy, liver, kidney and heart damage were the hallmarks of the intoxication of with *A. vera* extract and these were more severe as the concentration of the extract increased. The normocytic normochromic anaemia and significantly increased plasma activity of AST in the experimental are the direct consequencies of several organ damage, especially of the liver, heart and kidneys (Jubb *et al*., 1995).[46] The hypoalbuminaemia may be related to the profuse diarrhoea and protein-losing gastroenteropathy, as well as hepatic damage (Jubb et al., 1995).[46] One interesting finding however is the lowering of plasma lipids in Squirel (*Funambulus penniti*) that consumed the aqueous extract of *A. vera*. This could be interpreted to mean that *A. vera* may be useful for treating artherosclerotic problems and reduction of plasma lipids. This claim however, needs to be further examined.

The major objective of this research was to investigate the effect of unguided and often unrecommended oral administration of *A. vera* leaves in human, using the Anabas and Squirel (*Funambulus penniti*) as animal models. This is very important because of the current craze, in India, by middle and low income, literate and especially illiterate, old and young men and women who consume often large amounts of raw *A. vera* leaves due to spurious advertisements and belief in the plant as a "cure all" and "magic" plant, all in the name of "naturopathy" and "alternate" health methods. It is to be noted that most advertisers and users of this plant (and probably so for numerous other herbs) are oblivious of the fact that the parenteral administration of raw *A. vera* plant is not approved by the FDA (Smith and Struck, 1997[86]; FLP, 2003.[28]

Changes International, 2004).[20] Death has been reported of four cancer patients who were treated with *A. vera* intravenously by a physician whose license was subsequently revoked after investigation and trial by the Maryland Police (Smith, 1997a[82]; Smith and Bloom, 1997[85]; Smith and Lipton, 1997[84]; Smith and Struck[86], 1997). Brusick and Menge (1997)[18] expressed concern over the safety of *A. vera* stating that genotoxicity studies showed that *A. vera*-containing laxatives pose cancer risks to human when used as directed. Acute toxicity with *A. vera* gel caused severe cramping, diarrhoea and nausea, while long-term ingestion has been reported to lead to potassium deficiency, muscle weakness and cardiac arrhythmias (Kemper and Chiou, 1999[47]). Some of the known contraindications of products containing *A. vera* include intestinal obstruction or stenosis, atony, severe dehydration with electrolyte depletion, chronic constipation inflammatory intestinal diseases, cardiovascular diseases, pregnancy or lactation, in patients with cramps, colic, haemorrhoids, nephritis, or any undiagnosed abdominal symptoms such as pain, nausea, or vomiting (Hedendal, 2000).[41] While folk medicine has its roots in the very annals of human history, caution must be exercised in the use of some of these herbs and that claims such as have been attributed to *A. vera* must be backed by unequivocal scientific evidence. There are many areas of contradictions in most of the advertised therapeutic potentials of *A. vera*, especially when used parenterally. A few of the notable contradictions include: its use as an emmenagogue and abortifacient (Saha *et al*., 1961; Singh *et al*., 1979[81]; Nath *et al*., 1992)[58] vis-à-vis its use for the prevention of miscarriage (Bhattharai, 1992)[7] and its anti-oxytotic activity (Andrade *et al*., 1996)[4]; the use of *A. vera* gel in the treatment of peptic ulcers (Blitz *et al*., 1963)[11] vis-à-vis the ineffectiveness of both the exudate and gel components of *A. vera* in the treatment of gastric and duodenal ulcers experimentally induced in rats (Parmar *et al*., 1986)[63]; and its acclaimed use in lowering blood glucose level in diabetics (Ghannam *et al*., 1986[34]; Ajabnoor, 1990[2]; Yongchaiyudha *et al*., 1996[93]), contradicted by Koo (1994)[50] who reported not only the ineffectiveness of the *A. vera* gel in lowering blood glucose levels of alloxan-treated rats, but that it actually seemed to have caused an increase.

Most of the Indians who consume raw *A. vera* leaves have very strong belief in its alleged megatherapeutic efficacy (personal communication), but are unable to purchase the numerous commercially available “processed” products because of their prohibitive costs, as they belong to the low income group. They then resort to the unguided and continuous consumption of the raw leaves, oblivious of its implications on their health and productivity, not only in the short, but long term. There is no unified recommended dosage information for most *A. vera* products, whether raw or “processed” and provision of dosage information by most marketers and advertisers of *aloe* products does not constitute a recommendation or endorsement (Kemper and Chiou, 1999).[47] Avila *et al*. (1997)[5] confirmed that aloin and a low molecular weight fraction of the *A. vera* leaf (*Aloe*-emodin) are cytotoxic and should be removed from products processed for commercial use. A high molecular weight fraction of *A.*

vera was shown to deplete complement, while the low molecular weight fraction interfered with processes in activated polymorphonuclear leukocytes, leading to the production of oxygen free radicals and subsequent cytotoxicity (t'Hart *et al*., 1988[88], 1990[89]). Very many undiagnosed illnesses, inexplicable deaths and conditions in humans, especially in the under-developed and developing economies of the world, may be related to the consumption of unapproved herbal preparations or products such as from *A. vera*.

While discussing the need for alternative medical practitioners to give evidence for their acclaimed "alternative" health methods, Smith (1997b[83]) stated that the scientific approach to drug or medicant testing is designed to weed out ideas that users wish were true, but most often are not, or in most cases unproved. Herbalists, naturopathics and even drug companies want their products used and sold, but they have to prove that these drugs work and are safe enough (Smith, 1997b[83]). Skeptics may not be willing to accept the plausibility of most paranormal claims, especially in the case being discussed here, unless the evidence is extremely strong, hence the saying that "extraordinary claims demand extraordinary proof" (Gracely, 1998)[37] is justifiable.

CONCLUSIONS

In conclusion, the oral consumption of raw *A. vera* leaves, especially when unrecommended, should be discouraged (or at best handled with utmost caution) because of its potential deleterious effects on human and animal health and productivity. It is therefore recommended that the appropriate Food and Drug Regulatory bodies in the country should closely monitor and regulate the administration of *A. vera*, in any form. The dumping into our land and water bodies of *A. vera* "wastes" by those who cultivate and "process" the plant should be discouraged in order to prevent the bioaccumulation and magnification along food. chains of *A. vera*'s toxic constituents and hence cause mass mortality and morbidities in man and animals. Mass education and the provision of adequate and accessible basic medicare to the people are also very imperative. It is to be noted that the areas (or situations) where alternative therapies seem to have most appeal is in the very ones where conventional therapies are either not available, or are not able to satisfy the expectations of the consumer (Alcock, 2001). Even when available, it may be too expensive and unaffordable by the less privileged.

REFERENCES

1. Agarwal, O.P. (1985). Prevention of artheromatous heart disease. Angiology 36: 485-492.

2. Ajabnoor, M.A. (1990). Effect of aloes on blood glucose levels in normal and alloxan diabetic mice. J. Ethnopharmacol. 28: 215-220.
3. Al-Awadi FM & Gumaa KA (1987) Studies on the activity of individual plants of an antidiabetic plant mixture. *Acta Diabetologica Latina* 24: 37-41.
4. Andrade, A.A.B.D., Araujo, A.L., Diniz, M.F.F.M., Oliveira, R.A.G., Mendonca, F. and Mendonca, D. (1996). Use of medicinal plants during the puereral gravid cycle. J. Brasilia Ginecol. 106: 177-185.
5. Avila, H., Rivero, J., Herrera, F. and Fraile, G. (1997). Cytotoxicity of a low molecular weight fraction from *A. vera* (*A. barbadensis* Miller) gel. Toxicon 35: 1423-1430.
6. Balter, T. (1992). Aloe Vera. InnerSelf Publications, Altamonte Springs, FL. Also on Bhattarai, N.K. (1992). Folk use of plants in veterinary medicine in Central Nepal. Fitoterapia 63: 497-506.
7. Bailey CJ & Day C (1989) Traditional plant medicines as treatment for diabetes. *Diabetes Care* 12: 553-564.
8. Beppu H, Koike T, Shimpo K, Chihara T, Hoshino M, Ida C & Kuzuya H (2003) Radical- scavenging effects of *Aloe arborescens* Miller on prevention of pancreatic islet β-cell destruction in rats. *Journal of Ethnopharmacology* 89: 37-45.
9. Beppu H, Nagamura Y & Fujita K (1993) Hypoglycaemic and antidiabetic effects in mice of *Aloe arborescens* Miller var. *natalensis* Berger. *Phytotherapy Research* 7: S37-S42.
10. Blitz, J., Smith, J.W. and Gerard, J.R. (1963). *Aloe vera* gel in peptic ulcer: preliminary report. J. A m. Osteopathic Assoc. 62: 731-735.
11. Bolkent S, Yanardag R, Tabakoglu-Oguz A & Ozsoy-Sacan Ö (2000) Effects of chard (*Beta vulgaris* L. var. *cicla*) extract on pancreatic β-cells in streptozotocin-diabetic rats: a morphological and biochemical study. *Journal of Ethnopharmacology* 73: 251-259.
12. Bonner-Weir S, Trent D, Honey RN & Weir GC (1981) Responses of neonatal rat islets to streptozotocin: limited β-cell regeneration and hyperglycaemia. *Diabetes* 30: 64-69.
13. Bolkent S, Akev N, Ozsoy N, Sengezer-Inceli M, Can A, Alper O, Yanardag R. Effect of Aloe vera (L.) Burm. fil. leaf gel and pulp extracts on kidney in type-II diabetic rat models. Indian J Exp Biol. 2004; 42(1): 48-52.
14. Boudreau MD, Beland FA (2006) "An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera". Journal of environmental science and health. Part C, Environmental carcinogenesis & ecotoxicology reviews; 24(1): 103-54.

15. Boyd, C. (1982). Water Quality in Warmwater Fish Ponds. C. Boyd, ed., Auburn University Agricultural experimental Station, Auburn, Alabama. Craftmaster Printers Inc., Opelika, Alabama, 354pp.
16. Burke A, Smyth EM, Fitzgerald GA.(2006.) Analgesic-Antipyretic Agents; Pharmacotherapy of Gout. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gillman's The Pharmacological Basis of Therapeutics. 11th ed. New York: Mcgraw-Hill; p.683.
17. Brusick, D. and Menge, U. (1997). Assessment of the genotoxic risk from Laxative Senna Products. Environ. Mol. Mutagen. 29: 1-98.
18. Capasso F, Borrelli F, Capasso R, Di Carlo G, Izzo AA, Pinto L, Mascolo N, Castaldo S, & Longo R (1998) Aloe and its therapeutic use. *Phytotherapy Research* 12: S124-S127.
19. Changes International (2001). Complete Health And Nutrition Gives Everyone Strength Chalaprawat M (1997) The hypoglycaemic effects of aloe vera in Thai diabetic patients. *Journal of Clinical Epidemiology* 50: 3S.
20. Chithra P, Sajithlal GB & Chandrakasan G (1998) Influence of aloe vera on the healing of dermal wounds in diabetic rats. *Journal of Ethnopharmacology* 59: 195-201.
21. Das AV, Padayatti PS & Paulose CS (1996) Effect of leaf extract of *Aegle marmelose* (L.) Correa ex Roxb. on histological and ultrastructural changes in tissue of streptozotocin-induced diabetic rats. *Indian Journal of Experimental Biology* 34: 341-345.
22. Davis RH Kabbani JM, Moro NP. (1987)Aloe Vera and wound Healing. Journal of the American Podiatric Medical Association.; 77(4): 165-169.
23. Davis R H, Donato JJ, Hartman GM, Haas RC(1994);. Anti-inflammatory and wound healing activity of a growth substance in Aloe vera. *J-Am-Podiatr-Med-Assoc*. 84(2): 77-81.
24. Dray A. (1995); Inflammatory mediators of pain. Br J Anaesthesiol 75: 125-31.
25. Duncan, R.G. (1959). Multiple range and multiple F tests. Biometrics 11: 1-42.
26. FLP (2003). Forever Living Products 2003. Catalogue.
27. Foster, S. (2004). *A. vera*: the succulent with skin-soothing, cell-protecting properties. Herbs for Health Magazine.
28. Eshghi F., Hosseinimehr SJ, Rahmani N, Khademloo M, Norozi MS, Hojati O(2010);. Effects of Aloe vera cream on Posthemorrhoidectomy Pain and Wound Healing: Results of a Randomized, Blind, Placebo-Control Study. The Journal of Alternative And Complementary Medicine, 16(6): 657-650.

29. Ernst E. (2000) Adverse effects of herbal drugs in dermatology. The British Journal of Dermatology; 143: 923-9.
30. Fields Howard L., Martin Joseph B. Pain(2008)Pathophysiology and management. In: Harrison's principles of internal medicine. 17th ed. New York: Mcgraw-Hill,. p. 81-82.
31. Frajola, W.J., Williams, R.D. and Austad, R.A. (1965).The kinetic, spectrophotometric assay of serum alkaline phosphatase. A m. J. Clin. Pathol. 43: 261-264.
32. Ghannam, N., Kingston, M., Al Meshaal, I.A., Tariq, M., Parman, N.S. and Woodhouse, N. (1986). The antidia.
33. Ghosh MN(2008). Fundamentals of Experimental Pharmacology. 4th ed. Hilton and Company p.163. betic activity of aloes: preliminary clinical and experimental observations. Hormone Res. 24: 288-294.
34. George KA, Eric W, David DO, George AK. (2009); Antinociceptive effects of _*ewboulia loveis* stem bark extract in a rat model. Phcog Mag 17: 49-54.
35. Gracely, E. (1998). *Phactum.* A Newsletter of the Philadelphia Association for Critical Thinking (PhACT). Hahnemann School of Medicine in Philadelphia. Also available on.
36. Grindlay, D. and Reynolds, T. (1986). The *A. vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. J. Ethnopharmacol. 16: 117-151.
37. Grover JK, Yadav S & Vats V (2002) Medicinal plants of India with anti-diabetic potential. *Journal of Ethnopharmacology* 81: 81-100.
38. Haller, J.S. (1990). A drug for all seasons, medical and pharmacological history of *A. vera*. Bull. New York Acad. Med. 66: 647-659.
39. Hedendal, B.E. (2000). Whole- leaf *A. vera*, almost a panacea: An overview of one of the most accepted,yet misunderstood, medicinal plants in history.
40. HLA T, Nielson K (1992). Human cyclooxygenase-2 cDNA. Proc Natl Acad Sciences USA; 89: 7388-89.
41. Non-steroidal Anti-inflammatory Drugs and Antipyretic-Analgesics. In: Tripathi KD. Essentials of Medical Pharmacology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers; 2008; 184-186.
42. Hikino H, Takahashi M, Murakami M, Konno C, Mirin Y, Karikura M & Hayashi T (1986) Isolation and hypoglycaemic activity of Arborans A and B, glycans of *Aloe arborescens* var. *natalensis* leaves. *International Journal of Crude Drug Research* 24: 183-186.
43. Ivorra MD, Paya M & Villar A (1989) A review of natural products and plants as potential antidiabetic drugs. *Journal of Ethnopharmacology* 27: 243-275.

44. Jain, N.C. (1986). Schalm's Veterinary Haematology. Jain, N.C., ed., 4th Edition, Lea and Ferbiger, Philadelphia.
45. Jubb, K.V.F, Kennedy, P.C. and Palmer, N. (1995). Pathology of Domestic Animals. 3rd edition, Academic Press Inc., New York.
46. Kemper, K.J. and Chiou, V. (1999). *A. vera*. The Longwood Herbal Task Force (and The Center for Holistic Pediatric Education and Research Revised July 29, 1999.
47. Kim, H.S., Kacew, S. and Lee, B.M. (1999). *In vitro* chemopreventive effects of plant polysaccharides (*A. barbadensis* Milller, *Lentinus edodes*, *Ganoderma lucidum* and *Coriolus versicolor*). Carcinogenesis 20: 1637-1640.
48. Kim, H.S. and Lee, B.M. (1997). Inhibition of benzo-[a]pyrene- DNA adduct formation by *A. barabdensis* Miller. Carcinogenesis 18: 771-776.
49. Koo, M.W.L. (1994). *A. ver*a: Antiulcer and antidiabetic effects. Phytotherapy Res. 8: 461- 464.
50. Kern ST & Engerman RL (1987) Kidney morphology in experimental hyperglycaemia. *Diabetes* 36: 244-249. Koo MWL (1994) *Aloe vera*: anti-ulcer and antidiabetic effects. *Phytotherapy Research* 8*:* 461-464.
51. Langmead L, Feakins RM, Goldthorpe S, Holt H, Tsironi E, De Silva A, Jewell DP, Rampton DS. Randomized, double-blind, placebo-controlled trial of oral aloe vera gel for active ulcerative colitis. Aliment Pharmacol Ther. 2004; 19(7): 739-47.
52. Maenthaisong R, Chaiyakunapruk N, Niruntraporn S, KongkaewC. (2007); The Efficacy of aloe vera used for burn wound healing: a systematic review. *Burns* 33(6): 713-8.
53. Mossa JS (1985) A study on the drude antidiabetic drugs used in Arabian folk medicine. *International Journal of Crude Drug Research* 23(3): 137-145.
54. Mwale M, Masika PJ.(2001.) Analgesic and anti-inflammatory activities of *Aloe ferox Mill*. Aqueous extract. African Journal of Pharmacy and Pharmacology; 4(6): 291-297.
55. Moody, J.O., Adebiyi, O.A. and Adeniyi, B.A. (2004). Do*Aloe vera* and *Ageratum conyzoides* enhance the anti-microbial activity of traditional medicinal soft soaps (Osedudu)? J. Ethnopharmacol. 92: 57-60.
56. Moses, B.S. (1983). Introduction to Fisheries Studies in the Biology of Africa. B.S. Moses, ed., Ibadan University Press, Ibadan. Nigeria, pp. 58-74.
57. Nath, D., Sethi, N., Singh, R.K. and Jain, A.K. (1992). Commonly used Indian abortifacient plants with special reference to their teratologic effects in rats. J. Ethnopharmacol. 36: 147-154.

58. Neef H, Declercq P & Laekeman G (1995) Hypoglycaemic activity of selected European plants. *Phytotherapy Research* 9: 45-48.
59. Okyar A, Can, A, Akev, N, Baktir, G & Sütlüpinar, N (2001b) *Aloe vera* yapraklarinin tip II diabetik sicanlarin kan sekeri düzeyine etkisi (kronik deneyler) (Turkish), in *XIII. Bitkisel Ilac Hammaddeleri Toplantisi*, Proceedings (Marmara University Press, Istanbul), 281.
60. Okyar A, Can A, Akev N, Baktir G, Sutlupinar N (2001a) Effect of *Aloe vera* leaves on blood glucose level in type-I and type-II diabetic rat models. *Phytotherapy Research* 15: 157-161.
61. Ogunsanmi, A.O., Akpavie, S.O. and Anosa, V.O. (1994). Serum biochemical changes in West African dwarf sheep experimentally infected with *Trypanosoma brucei*. Rev. Elev. Med. Vet. pays Trop. 47: 195-200.
62. Parmar, N.S., Tariq, M., Al-Yahya, M.A., Ageel, A.M. and Al-Said, M.S. (1986). Evaluation of *Aloe vera* leaf exudate and gel for gastric and duodenal anti-ulcer activity. Fitoterapia 57: 380-383.
63. Palanichamy S, Nagarajan S & Devasagayam M (1988) Effect of *Cassia alata* leaf extract on hyperglycaemic rats. *Journal of Ethnopharmacology* 22: 81-90.
64. Parinandi NL, Thompson EW & Schmid HHO (1990) Diabetic heart and kidney exhibit increased resistance to lipid peroxidation. *Biochimica and Biophysica Acta* 1047: 63-69.
65. Perez G RM, Zavala S MA, Perez G S & Perez G C (1998) Antidiabetic effect of compounds isolated from plants. *Phytomedicine* 5: 55-75.
66. Portha B, Blondel O, Serradas P, Mc Evoy R, Giroix M-H, Kergoat M & Bailbe D (1989) The rat models of non-insulin dependent diabetes induced by neonatal streptozotocin. *Diabete et Métabolisme (Paris)* 15: 61-75.
67. Rahmy TR & El-Ridi MR (2002) Action of *Anastatica hierochuntica* plant extract on islets of Langerhans in normal and diabetic rats. *Egyptian Journal of Biology* 4: 87-94.
68. Rang HP, Dale MM, & Ritter JM (1999) Pharmocology. 4th ed. Churchill Livingstone pp393-98.
69. R. N. Chopra. Chopra’s Indigenous Drugs Of India. 2nd ed. Calcutta: Academic Publishers; 1994; 61-62.
70. Ramachandra CT, Rao PS. Processing of Aloe Vera Leaf Gel: A Review. American Journal of Agricultural and Biological Sciences 2008; 3(2): 502-510.
71. Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. A m. J. Clin. Path. 28: 56-62.

72. Reynolds, T. and Dweck, A.C. (1999) *A. vera* leaf gel: A review update. J. Ethnopharmacol. 68: 3-37.
73. Ritter, L. (2003). *A. vera*. A to Z. HSU & Co. Saha, J.C., Savini, E.C. and Kasinathan, S. (1961). Ecbolic properties of Indian medicinal plants. Part 1. Indian J. Med. Res. 49: 130-151.
74. SAS (1987). Statistical Analysis System. Users' Guide, Ver. 6.03, SAS Institute Inc., Cary, North Carolina, USA.
75. Schmidt, J.M. and Greenspoon, J.S. (1993). *Aloe vera* dermal wound gel is associated with a delay in wound healing. Obst. Gynaecol. 78: 115-117.
76. Sebastian, M.K. and Bhandari, M.M. (1984). Medico-ethnobotany of Mount Abu, Rajasthan, India. J. Ethnopharmacol. 12: 223-230.
77. Shane-Whorter L (2001) Biological complementary therapies: a focus on botanical products in diabetes. *Diabetes Spectrum* 14: 199-208.
78. Shelton MS.(1991) Aloe Vera, its chemical and therapeutic properties. International journal of dermatology.; 30: 679-683.
79. Shukla R, Sharma SB, Puri D, Prabhu KM, & Murthy PS (2000) Medicinal plants useful in diabetes. *Indian Journal of Clinical Biochemistry* 15: (Suppl) 169.
80. Singh, M.P., Malla, S.B., Rajbhandari, S.B. and Manandhar, A. (1979). Medicinal plants of Nepal - Retrospects and Prospects. Econ. Botany 33: 185-198.
81. Smith, L. (1997a). Judge rejects case against doctors Aide: man allegedly gave *A. vera* injections. Washington Post, Nov 18, 1997: Sec B, 3:2.
82. Smith, G.L. (1997b). Common Questions about Science and "Alternative" Health Methods.
83. Smith, L. and Lipton, E. (1997). Panel revokes the licence of VA physician. Doctor's *A. vera* therapy suspected in 3 deaths. Washington Post, Sept. 27, 1997; Sec A, 1:2.
84. Smith, L. and Blum, J. (1997). Police probe death of secondary patent treated by Manasses doctor. Washington Post, Sept 26, 1997: Sec. B, 1:2.
85. Smith, L. and Struct, D. (1997). The *A. vera* Trial. Investigation of four patients' death lead officials of FDA to company based in Maryland. Washington Post, Oct. 11, 1997: Sec C, 1:2
86. Szas, G.A. (1969). Kinetic photometric method for serum glutamly transferase. J. Clin. Chem. 15: 124-136.

87. t'Hart, L.A., van Enckevort, P.H., van Dijk, H., Zaat, R., de Silva, K.T.D. and Labadie, R.P. (1988). Two functionally and chemically distinct immunomodulatory compounds in the gel of *Aloe vera*. J. Ethnopharmacol. 23: 61-71.
88. t'Hart, L.A., Nibbering, P.H., van den Barselaar, M.T., van Dijk, H., van den Berg, A.J., Labadie, R.P. (1990). Effects of low molecular constituents from *Aloe vera* gel on oxidative metabolism and cytotoxic and bactericidal activities of human neutrophils. Int. J. Immunopharmacol. 12: 427-434.
89. Toro, G. and Ackermann, P.G. (1975). Practical Clinical Chemistry. 1st ed., Little Brown and Co. Inc., Boston, USA.
90. Tyler, V. (1993). The Honest Herbal: A sensible Guide to Use of Herbs and Related Remedies. Third Edition. Binghamton; N.Y: Pharmaceutical Products Press.
91. Tyler, V. (1994). Herbs of Choice: The Therapeutic Use of Phytomedicinals. Binghamton, NY: Pharmaceutical Products Press.
92. Yongchaiyudha, S., Rungpitarangsi, V., Bunyapraphatsara, N. and Chokechaijaroenporn, O. (1986). Antidiabetic activity of *Aloe vera* juice. I. Clinical trial in new cases of diabetes mellitus Phytomedicine 3: 241-24.
93. Vazquez B, Avilla G, Segura D, Escalante B(1986). Antiinflammatory activity of extracts from Aloe vera gel. Journal of Ethnopharmacology 1996; 55(1): 69-75.
94. Vetrichelvan T & Jegadeesan M (2002) Anti-diabetic activity of alcoholic extract of *Aerva lanata* (L.) Juss. Ex Schultes in rats. *Journal of Ethnopharmacology* 80: 103-107.
95. Vogler BK & Ernst E (1999) *Aloe vera*: a systematic review of clinical effectiveness. *British Journal of General Practice* 49: 823-828.
96. Vogel HG. (2002) In: Drug Discovery and Evaluation. Pharmacological Assays. 2nd ed. New York: Springer;). p. 717.
97. Vogel HG. (2002). In: Drug Discovery and Evaluation. Pharmacological Assays. 2nd ed. New York: Springer; p. 694.
98. Yanardag R, Bolkent S, Tabakoglu-Oguz, A & Ozsoy-Sacan, O (2003) Effects of *Petroselinum crispum* extract on pancreatic β-cells and blood glucose of streptozotocin-induced diabetic rats. *Biological and Pharmaceutical Bulletin* 26: 1206-1210.
99. Yusuf S, Agunu A, Diana M.(2004). The effect of Aloe vera A. Berger (Liliaceae) on gastric acid secretion and acute gastric mucosal injury in rats. J Ethnopharmacol.; 93(1): 33-7.