Safety Evaluation of Acute andChronic Treatment of Aqucous Calyx Extract of *Hibiscus sabdariffa* (L) in Rats

O.P. AJAGBONNA and +A.E. ADEBAYO
Department of Physiology and Pharmacology,
Faculty of Veterinary Medicine,
Usmanu Danfodiyo University, Sokoto
+Department of Chemical Pathology,
University of Maiduguri Teaching Hospital, Maiduguri, Nigeria

Abstract

Acute and chronic toxicity studies of the aqueous extract of the medicinal plant, *Hibiscus sabdariffa* (L) ('Soborodo') were carried out in rats. The oral and interaperitoneal route of administration of calyx aqueous exact were employed. Fatality was not recorded inn rat given 1000 mg to 2500 mg/100g body weight intra peritoneal but larger concentration of 3000 to 4000 mg/100g resulted in death. Mean lethal does of 3350mg/100g was obtained. In chronic toxicity tests in which the rates were given 50mg to 150mg/100g per day of aqueous extract to drink for four weeks, no fatality was recorded, no significant effect on both haematological and biochemical parameters measured and no significant damage to the body organs was observed. These data suggest a safe therapeutic benefit for *Hibiscus sabdariffa*.

Keywords: *Hisbiscus sabdariffa, safety, acute, chronic, studies, rat.*

Introduction

Various preparations of *Hibiscus sabdariffa* (HS) are used traditionally in the management of several diseases in man and animals (Oliver, 1960).Pharmacological investigations of calyx aqucous extract of the plant have demonstrated antihypertensive (Adegunloye, 1996), cathartic (Haruna, 1997), antibacterial and hyposlyceamic activities (Ajabonna and Oshagbemi 1997, Ajagbonna and Adebayo 1999),

However, despite the widespread use of this plant in folk medicine and as pasture for animals, very little have been done to ascertain its level of safety in animal and man. This study therefore examined the effects of acute and chronic infusion f HS in rats.

Materials and Methods

Plant Material

Dry red calyces of *Habiscus sabdariffa* purchased from Sokoto market was identified by a traditional medical practitioner and confirmed by a staff of the Botany Department of Usmanu Danfodiyo University, Sokoto. A voucher specimen is kept at the college Herbarium for reference purpose.

The extraction of the calyx was as previously described (Ajagbonna and Adegunloye, 1996). A portion (50g) of the dry calyces were boiled in 200 ml of distilled water at 100°C for 10 minutes. The cold decoction was filtered using Whatman No. 54 filter paper and the filtrate evaporated in aeration oven at 60°C . The dried residue was scrapped, placed in a capped bottle before storing in a dessicator. From the dried extract, a fresh stock solution was prepared on each day of the experiment.

Test Animals

Sprange Dawley rats (150-200g) of both sexes were purchased from the National Veterinary Research Institute, Vom, near Jos. The rats were kept in stainless steel cages for two weeks to adapt to laboratory conditions before use in the study. Water and feed (chick mash, Bendel feed ®, Sokoto Nigeria) were provided *ad libitum* throughout the period of study.

Acute Toxicity

To determine acute toxicity thirty (30) rats were divided into two equal groups of five each and treated intraperitonially (IP) with a suspension of the Hisbiscus sabdariffa extract (100, 1500, 2000, 2500, 3000 or 4000 mg/100g body weight. The rats were observed over a period of 24 hours for evidence of acute toxicity and death. LD50 was calculated using Arithmetic method of Karbar modified by Aliu and Nwude (1982).

Chronic Toxicity Study

Thirty two (32) Sprague Dawley rats were randomly divided into 4 groups of 8 rats each. Group 1 (control) rats were experimental groups 2, 3 and 4 received 50, 100 and 150 mg of the extract respectively per 100g body weight per orally twelve hourly in distilled water using water bottle.

After a period of two weeks, four rats were selected from the groups and blood collected from their tail

for the following analysis. The blood samples in EDTA bottles were sent to a diagnostic laboratory to determine the haemoglobin (Hb), red blood cell (RBC), packed cell volume (PVC) and while blood cell (WBC) counts using the methods described by Schlam *et al.* (1975) and Ajagbonna *et al.*(1999). Using the serum, activities of liver enzymes, alkaline phosphotase (ALP), aspartate amino transferase (GOT) and alanine aminotransferase (GPT) were estimated according to Rectman and Frankel (1957) using the Sigma diagnostic kits.

Determination of sodium, potassium, total protein, bilirubin and cholesterol were carried out using standard procedure. (Toro and Ackerman, 1995; Tilklan, 1979). At the end of four weeks feeding period, blood was also collected from the remaining rats in each group and all parameters measured earlier were again repeated.

Effect on Weight

The rats in each group were weighed for 5 days prior to the commencement of the experiment to obtain the pre-treatment body weight and following treatment with *Hibiscus sabdariffa*, body weight was measured every week for four weeks.

Gross Pathology

At the end of the four weeks period of treatment, the rats were sacrificed by decapitation and the major organs including the liver, kidney and heart were examined for any pathological change.

Statistics

The results are presented as mean SEM and statistical significance between groups was analysed using analysis of variance. Statistical significance was considered when $P<0.05$.

Results

Acute Toxicity

The rats were active after the administration of the HS extract for all the doses and no significant sign was observed before death. At the end of 24 hours, death was observed before death. At the end of 24 hours, death was observed in 20% of the rats treated with 3000mg/10g and 100% with rats treated with 4000 mg/100g. The LD_{50} was calculated to be 3350 mg/100g IP (Table).

Effect of HS Extract on Haemathology

The effect of various doses of the extract of HS, on RBC, PCV, Hb are presented in tables 2 and 3. Administration of the extract for four weeks did not significantly ($P<0.05$) change any of the measured values.

Effect of Extract on Serum Biochemistry

Administration of HS extract led to a significant ($P<0.05$) dose dependent fall in serum cholesterol at two weeks and four weeks of treatment (table 5). The other biochemical parameters however did not differ significantly ($P>o0.05$) from the control group (Tables 6 and 7).

Effect of HS Calyx on Rat Weight

Administration of HS Calyx to rats for 4 weeks did not produce any significant change in body weight in all the treatment groups when compared with the control group (Table 8).

Effect on Gross Pathology

There were no significant lesions in any of the organs examined.

Discussion

From the results obtained in the acute toxicity study, the LD_{50} of the extract in rats is 33350 mg/100g, showing that the extract has very low toxicity. According to Charke and Clarke (1977), any substances whose LD_{50} in rates is above 1,000 mg/kg is regarded as safe.

Blood is an important index of physiological and pathological status in animal and the parameters usually measured are haemoglobin, packed cell volume, white blood cell and red blood counts (Schlam *et al,* 1975).

The concentration of these indices can be influenced by ingestion of some toxic plants (Aatan and Arowolo, 1989); Ajagbonna *et al,* 1999). These parameters were all measured in this study after the treatment of HS for two and four weeks after the treatment of HS for two and four weeks without any significant changes from the control values suggesting some level of safety in the use of the extracts. This is in contrast to decrease in haemoglobin and red blood cell (anaemia) observed with the use of *Enantia chlorantha*, a plant known for its anti-malaria activity inman, (Agbaje and Anabanjo, 1994). Administration of HS for four weeks on this study did not affect most of the biochemical parameters measured except for the dose dependent decrease in the cholesterol level over the study period. It has been shown by previous workers that one way to check or reduce hypertension is to monitor and reduce total serum cholesterol level (Obatomi et al., 1956). The significant decrease in level of cholesterol obtained from this study is complimentary to our earlier reported anti-hypertensive property of HS which was reported to be by stabilizing the membrane and stimulating the Na^+ / K^+ ATPase system. (Adegunloye *et al.,* 1993). The lack of significant effect of HS extract on liver transminases is also remarkable.

It is known that many poisonous plants accumulate in the liver and so detoxification occurs here (Clarke and Clarke, 19777). A study of liver function tests may therefore prove useful in assessing especially

the toxic effects of medicinal plants on the liver. These tests involve mainly determination of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) (Tilkian, 1979) and any marked necrosis of the liver cells lead to escape of these enzymes into blood and therefore an increase in blood values. The lack of effect of the extract may not e toxic on the liver.

Also, examination of the liver of the test animals after two and four weeks of treatment with the HS extract did not reveal any lesion showing further lack of fatality in the use of this plant. Again , no reported cases of death following the use of HS in human and animal has been experienced according to the Nigerian traditional health practitioners who administer the plant drug regularly to their patients. The results of this stud', show that this extract may be safe for consumption. However, further research work is needed for confirmation of level of safety.

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TABLE 1: calculation of the LD_{10} for *Hibiscus sabdariffa*

Group (mg/100g)	Dose Diff	No. of Dead	Mean dead	Dose diff. x Mean dead	
1	1000	0	0	0	0
2	1500	500	0	0	0
3	2000	500	0	0	0
4	2500	500	0	0	0
5	3000	500	1	0.5	250
6	4000	1000	5	3	3000

$$LD50 = M \times dose - \frac{(Dose\ diff \times Mean\ dead)}{n}$$

$$= \frac{4000 - 3250}{n}$$

$$= 3350 mg/100g$$

TABLE 2: Effect of Calyx extract of *Hibiscus sabdariff* on packed cell volume (PCV) and Haemoglobin (Hb) in rats treated for four weeks.

Parameter	Dose of extract (mg/100g)	Treatment period (weeks)		
		0 -	2	4
PCV(%)	0	35±1.5	32±2.0	33±1.0
	50	34±1.2	3410	35±1.2
	100	35±0.8	36±1.0	36±1.4
	150	34±1.2	35±0.9	37±1.5
Hb (gm/dl)	0	13±1.2	13±0.4	12.5±1.0
	50	13±.6	13.505	13.0±1.0
	100	13.2±0.7	13.8±0.6	13.7±2
	150	13±0.8	14±0.4	14±1.5

TABLE 3: Effect of Calyx extract of *Hibiscus sabdariff* on red blood cell (RBC) and White blood cell count (RBC) in rats treated for four weeks.

Parameter	Dose of extract (mg/100g)	Treatment period (weeks)		
		0	2	4
RBC (x 10^6/L)	0	6.5±0.3	6.4±0.2	6.4±0.3
	50	6.3±0.1	6.6±0.3	6.9±0.6
	100	6.6±0.4	6.9±0.5	7.0±0.7
	150	6.6±0.4	6.8±0.5	7.0±0.7
WBC(x10^3/ml)	0	11.7±0.2	11.6±0.1	11.5±0.2
	50	11.8±0.3	11.7±0.3	11.3±0.1
	100	11.5±0.7	11.9±0.6	11.2±0.3
	150	11.8±0.6	12.0±5	11.6±0.6

TABLE 4: Effect of Calyx extract of *Hibiscus sabdariff* on total protein and albumin in rats treated for four weeks.

Parameter	Dose of extract (mg/100g)	Treatment period (weeks)		
		0	2	4
Total Protein (g/dl)	0	7.4±0.5	7.5±0.6	7.4±0.6
	50	7.4±0.5	7.4±0.5	7.0±0.3
	100	7.6±0.6	7.0±0.6	7.1±0.4
	150	7.3±0.3	7.0±0.6	7.2±0.3
Albumin (x10^3/ml)	0	3.3±0.3	3.2±0.4	3.3±0.4
	50	3.2±0.1	3.0±0.6	3.0±0.6
	100	3.2±0.5	3.4±0.7	3.4±0.6
	150	3.4±0.2	3.7±0.2	3.0±0.3

TABLE 5: Effect of Calyx extract of *Hibiscus sabdariff* on urea and cholesterol levels in rats treated for four weeks.

Parameter	Dose of extract (mg/100g)	Treatment period (weeks)		
		0	2	4
Cholesterol (mg/100ml)	0	80±5.0	81±4.5	80±7.5
	50	78±8.5	60±5.5*	55±4.0*
	100	82±7.5	57±4.2*	45±5.5*
	150	80±6.5	50±5.0*	40±6.5
Urea (μ/mol/L)	0	2.3±0.6	2.4±0.5	2.2±0.5
	50	2.4±0.2	2.5±0.8	2.5±0.3
	100	2.6±0.8	2.5±0.7	2.6±0.2
	150	2.4±0.3	2.5±0.6	2.4±0.2

Day 0 means immediately before extract treatment
*P<0.05 when compared with control.

TABLE 6: Effect of Calyx extract of *Hibiscus sabdariff* on serum enzyme activities in rats treated for four weeks.

Parameter	Dose of extract (mg/100g)	Treatment period (weeks)		
		0	2	4
ALP (iu/L)	0	23.2±1.8	23.4±1.5	23±2.0
	50	23.2±1.5	24.7±0.9	25±0.8
	100	23.6±1.5	25±0.1	26±0.5
	150	23±1.7	26±0.3	27±0.6
GOT(iu/L)	0	42.6±1.9	41.6±1.5	42.61.7
	50	40.5±1.0	44±1.4	421.6
	100	43.5±2.2	43±2.0	432.4
	150	41±1.5	41±1.6	431.6
GPT (iu/L)	0	22.3±1.2	22.3±1.4	23±1.2
	50	23.2±1.4	23±1.2	23.4±1.4
	100	22±1.4	22.6±1.3	22.6±1.6
	150	23±17	22.8±1.4	23±1.6

TABLE 7: Effects of Calyx extract of *Habiscus sabdariffa* on serum N^+ and K^+ levels in rats treated for four weeks.

Parameter	Dose of extract (mg/100g)	Treatment period (weeks)		
		0	2	4
Na^+ (mmol/L)	0	144±1.2	145±1.3	144±1.2
	50	143±2.5	145±2.5	144±2.0
	100	145±1.6	143±1.6	143±2.0
	150	144±3.0	146±2.5	149±1.7
K^+ (mmol/L	0	5.5±02	5.4±06	5.5±0.7
	50	5.3±04	4.9±0.7	5.0±0.6
	100	5.6±03	5.0±0.9	5.0±0.6
	150	5.5±0.6	5.5±0.5	4.9±0.9

TABLE 8: Effects of Calyx extract of *Hibiscus sabdariffa* on body weight (gm) of rats treated for four weeks.

Dose of extract (mg/100g)	Treatment period (weeks)				
	0	1	2	3	4
0	155±4	1604	174±3	180±5*	200±6.0*
50	160±3.5	165±1.0	170±5	185±5*	200±6.0*
100	1624±	170±9	185±6*	190±10*	200±8*
150	160±10	172±8	1858*	195±9*	200±7*