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Full Length Research Paper

Acute and subacute toxicity of aqueous extract of leaves mixture of *Aloe buettneri* (Liliaceae), *Dicliptera verticillata* (Acanthaceae), *Hibiscus macranthus* (Malvaceae) and *Justicia insularis* (Acanthaceae) on Swiss mice and albinos Wistar female rats

Pone K. B.[1,2]*, Telefo, P. B.[2], Gouado, I.[3] and Tchouanguep, F. M.[2]

[1]Central Institute of Medicinal and Aromatic Plants, P. O. CIMAP, Kukrail Picnic Spot Road, 226015-Lucknow, Uttar Pradesh, India.
[2]Department of Biochemistry, Faculty of Science, University of Dschang. P. O. Box 67 Dschang, Cameroon.
[3]Department of Biochemistry, Faculty of Science, University of Douala, P. O. Box 24157, Douala, Cameroon.

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***Aloe buettneri* (Liliaceae), *Justicia insularis* (Acanthaceae), *Hibiscus macranthus* (Malvaceae) and *Dicliptera verticillata* (Acanthaceae) (ADHJ) are medicinal plants generally found in tropical and subtropical areas. The leaf mixture of these plants is used in the Western Region of Cameroon to increase fertility, regularize the menstrual cycle and to treat dysmenorrhoea or cases of infertility in women. In order to evaluate the toxicity of the leaf mixture extract of these plants, the values of their LD_{50} and LD_{100} were determined in Swiss mice and the subacute toxicity studied in albinos Wistar female rats. The herbal drug induced changes in the physiological (body and vital organ weights), toxicological [alanine aminotransferase (A.L.T), aspartate aminotransferase (A.S.T), creatinine] and biochemical (total proteins) parameters. The LD_{50} and LD_{100} were 27 and 32 g/kg in male mice, respectively, whereas in female mice the values were 18 and 24 g/kg, respectively. The leaf mixture of the plants has significantly increased the reproductive organ weights of treated female rats. The serum, uterine and ovarian proteins as well as the creatinine levels was increased significantly, while the hepatic protein level was decreased. The rate of AST remained unchanged whereas that of ALT increased when animals were treated at the dose of 100 mg/kg. These results suggests on one hand that aqueous extract is not short-term poisonous but presents unfavourable effects in the long run (60 days) and on the other hand, the aqueous extract have a direct action on the reproductive organs and cause disturbances on cellular metabolism.**

Key words: Toxicity, mice, female rats, toxicological and biochemical parameters.

INTRODUCTION

Medicinal plants are those which contain active substances on living organisms and are used as precursor in drug synthesis (Abayomi, 1984). Some of these plants contain secondary metabolites which can affect human and mammal reproduction (Butenandt and Jacobi, 1933). The leaf mixture of *Aloe buettneri, Dicliptera verticillata, Justicia insularis* and *Hibiscus macranthus* are vegetal species used in the Western region of Cameroon to increase fertility, regularize the menstrual cycle and treat dysmenorrhea or some cases of infertility in women. Burkill (1982) and Chopra (1933) have shown that the

*Corresponding author. E-mail: pon2812@yahoo.fr. Tel: (+91)9005600574.

decoction of *J. insularis* alleviates the childbirth pain during the last month of pregnancy. Telefo et al., 1998 have reported that, different doses of aqueous extracts from the leaves of *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus* and *Dicliptera verticillata*, when given daily to 22 day old rats for 5, 10, 15, 20 and 25 days by gastric intubation increases the serum oestradiol level attesting the presence of some oestrogenic compounds in the plant extracts. Furthermore, the aqueous extract of the leaf mixture of *Aloe buettneri, Dicliptera verticillata, Justicia insularis* and *Hibiscus macranthus* given by oral route to the immature female rats at the doses of 13, 49 and 94 mg/kg/day for 15 days induced a significant increase in ovarian and uteri weight as well as serum and ovarian oestradiol (Telefo et al., 2001). Guemo (2002) have shown that the decoction of the leaves of *A. buettneri, D. verticillata, J. insularis* induce the uterus contraction, whereas *H. macranthus* releases it.

Despite the various pharmacological data on the leaf mixture extract of these plants, no data on their toxicity is available in the literature. Therefore, the aim of the present study is to identify the adverse effects of the leaf mixture of these plants on Swiss mice and Wistar albino female rats. To attain our objective we have evaluated specifically: the values of LD_{50} and LD_{100} of the aqueous extract (AE); the effect of the aqueous extract on the body and reproductive organ weights (liver, kidney, ovary and uterus) and the effect of aqueous extract on toxicological (alanine aminotransferase [ALT], aspartate aminotransferase [AST], creatinine) and biochemical parameters (total proteins).

MATERIALS AND METHODS

Leaf samples of *A. buettneri, D. verticillata* and *J. insularis* were collected in March and May 2005 in Dschang region, while the leaf sample of *H. macranthus* was collected in Batoufam area (West-Cameroon) in May and June 2005. The aerial part of each specimen were air-dried in shadow at room temperature and reduced into fine powder using an electric grinder (Moulinex).

Extract preparation

The powders were mixed in the proportion of 25% *A. buettneri, D. verticillata, H. macranthus* and *J. insularis* (ADHJ). Then, the crude aqueous extract (AE) of leaves was prepared by adding 1000 ml of distilled water to 100 g of mixture leaf powder and kept at 100°C for 30 min. The extract was filtered and then concentrated in rotary evaporator at temperature lower than 45°C. The yield of the dry residue was 26.56% w/w.

Experimental animals

Adult male and female Swiss mice (weighing 20-30g) and 21 day old female Wistar rats (weighing 22-35g) were used. They were bred in the animal house of the Faculty of Science at the University of Dschang-Cameroon under approximately 20°C. They were receiving food and water *ad libitum*.

Toxicity testing

Acute toxicity

The mice were treated orally with doses ranging from 2 to 32 g/kg of the crude extract. The animals were observed for the clinical signs of toxicity (locomotion, sensibility to pinch, agressivity, tail and faeces aspects) during 24 h. Furthermore, the LD_{50} values were calculated according to Berhens and Karber method (1983).

Sub acute toxicity

The extract was administered to rats by force-feeding at the rate of 0; 12.5; 50 and 100 mg/kg doses for 40 (group 1) and 60 (group 2) days. All the animals were observed for appearance of toxicity signs or behavioural alterations during the experimental period. At the end of the treatment, the animals were anaesthetized with chloroform vapours and the blood was collected through cardiac puncture using sterile syringes. The animals were sacrificed and the uterus, ovary, spleen, liver, suprarenal gland and kidneys were collected, cleaned and weighed.

Preparation of the homogenates

After weighing the ovaries and uteri, they were grinded separately in mortars containing fresh phosphate buffer (1/150 M; pH 7.0) to obtain 1% homogenate. The liver homogenate was prepared by the same procedure but at 10%. The homogenates were centrifuged for 20 min at 4°C and 2000 rpm. The supernatants were separated from the pellets and stored at -20°C until use.

Serum preparation

After the sacrifice of the animals by decapitation, the blood was collected in the test tubes and allowed to rest for 2 h at 2°C. Further, all the tubes were centrifuged for 20 min at 4°C and 1500 rpm. The supernatants (serum) were collected using the micro-pipette and kept at -20°C.

Biochemical analysis

Serum and hepatic total proteins were measured by Biuret method described by Gornall et al. (1949), while uteri and ovaries total proteins were assessed by Bradford method (1976). Measurements of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine levels were done with their respective kits.

Statistical analysis

The differences among the experimental and control groups were determined using the ANOVA test and comparison of average performed with Paraphenylenediamine (PPD)-Fischer test. Level of significance was set at $p<0.05$.

RESULTS

Acute toxicity of AE of (ADHJ)

Diarrhoea, decrease of the activity and difficulties in the locomotion were observed within the first 6 h of treatment in the groups receiving the extract at doses varying

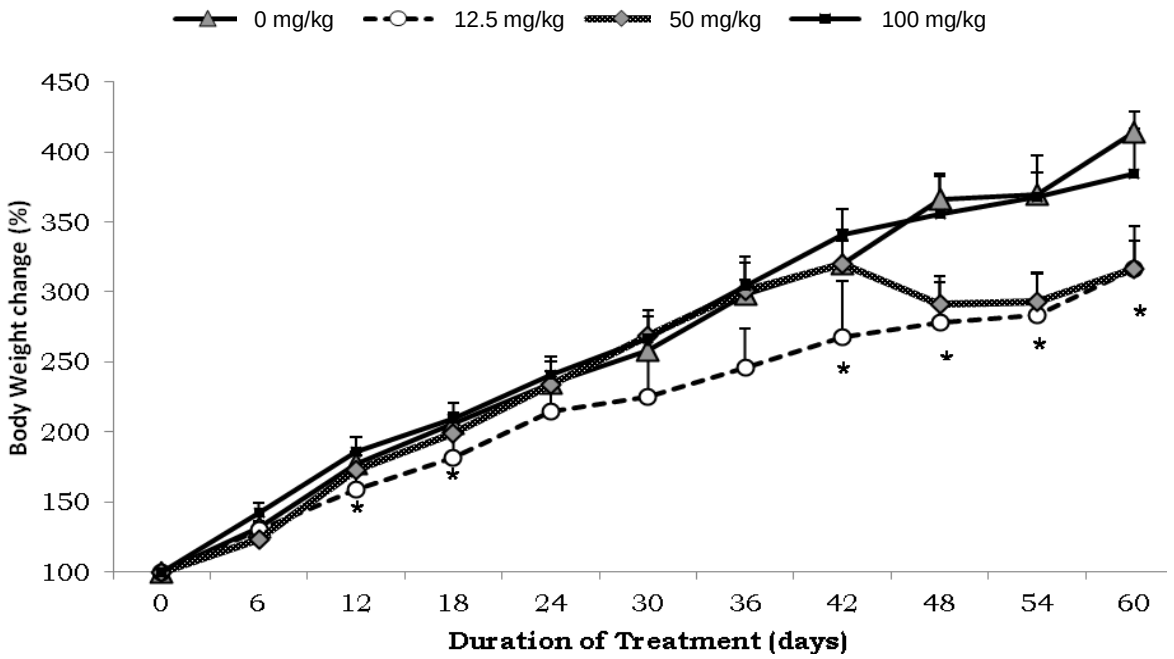

Figure 1. Effect of AE of ADHJ on body weight of rats after 40 and 60 days treatment.

Table 1. Effect on liver weight, hepatic and serum proteins after oral administration of AE of ADHJ during 40 and 60 days in Wistar female rats.

Dose (mg/kg)	Treatment duration					
	40 days			60 days		
	Liver weight (g/ 100 g of bw)	Serum protein (mg/ml)	Hepatic protein (mg/g of bw)	Liver weight (g/ 100 g of bw)	Serum protein (mg/ml)	Hepatic protein (mg/g of bw)
0	5.43 ± 0.21	132.03 ± 2.32	12.27 ± 0.57	4.05 ± 0.16	160.67 ± 4.90	15.17 ± 0.48
12.5	5.97 ± 0.30	177.84 ± 4.61***	14.01 ± 0.52*	4.09 ± 0.24	173.92 ± 11.09*	11.86 ± 0.84*
50	4.80 ± 0.18	171.28 ± 1.12***	13.39 ± 0.46*	3.73 ± 0.06	178.65 ± 10,43*	12.92 ± 0.69*
100	5.86 ± 0.16	180.73 ± 2.23***	19.67 ± 0.09**	3.70 ± 0.09	216.89 ± 23.78**	11.98 ± 1.34*

The values represented Mean ± ESM of liver weight, hepatic and serum proteins levels in each group (n=6). *p<0.05; **P<0.01; ***P<0.001 (PPD-Fischer test). Bw = Body weight.

between (16 to 32 g/kg). No abnormalities were observed in treated groups at varying doses (between 2 to 4 g/kg) compared to the vehicle control. After 48 h, the death of all the animals was recorded at the doses of 24 and 32 g/kg, respectively for female and male mice. The LD_{50} values were 18 and 26.8 g/kg for male and female mice, respectively.

Subacute toxicity of AE of (ADHJ)

Effect of AE of ADHJ on body weight

The body weights were linearly proportional to the period of treatment and the values were increased at the end of the treatment in comparison to the initial values. This is materialized by the appearance of the curves from 0 to 60 days, but as compare to the vehicle control the weights of the rats treated with 12.5 mg/kg had decreased significantly (Figure 1).

Effect of AE of ADHJ on hepatotoxic biochemical parameters

No variation was found in relative liver weight (Table 1). The hepatic protein levels decreased in the groups treated during 60 days, while the values were increased in the groups treated during 40 days. The administration of AE of ADHJ did not change the AST level. However, at the rate of 12.5; 50 and 100 mg/kg, the ALT levels were decreased compared to the vehicle control (Figure 2) in rats treated during 40 days. The ALT level was also increased when the animals were treated during 60 days

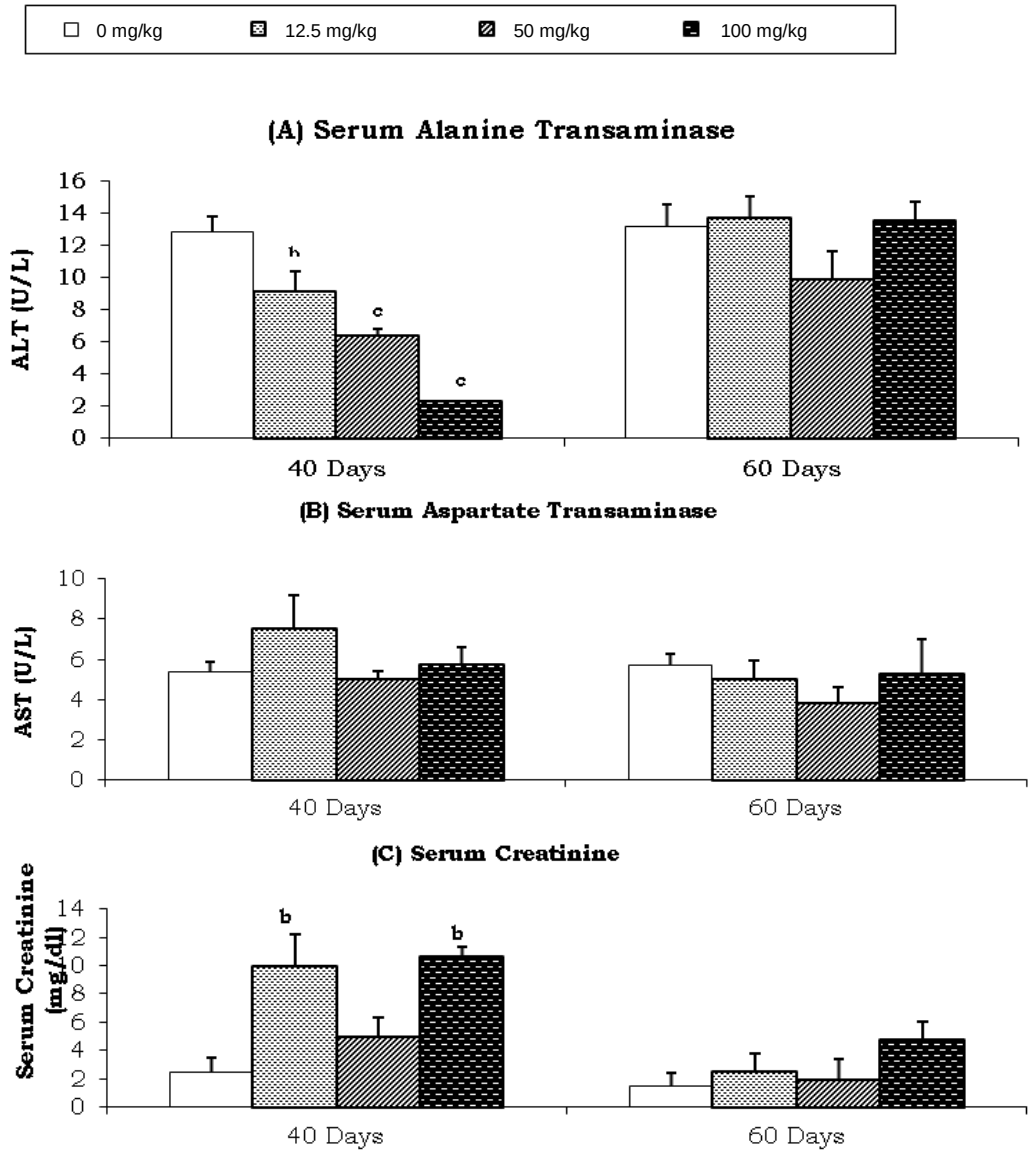

Figure 2. ALT (A), AST (B) and creatinine (C) levels in the serum of treated rats during 40 and 60 days. The values represented Mean ± ESM of liver weight, hepatic and serum proteins levels in each group (n=6). *p<0.05; **p<0.01; ***p<0.001 (PPD-Fischer test).

at 100 mg/kg (Figure 2).

Nephrotoxicity of AE of ADHJ

The administration of the plant mixture extract has induced a significant increase of the kidney weights in the rats treated during 40 days whereas in those treated during 60 days the values remain unchanged, all compared to the vehicle control (Table 2). The serum creatinine level significantly increased in all the groups treated during 40 days with the leaf mixture AE (Figure 2).

Effect of AE of ADHJ on uterus and ovary of treated female rats

The administration of AE of ADHJ did not change ovarian weight but increased uterine weight, corpus luteum number, ovarian and uterus protein levels (Table 3).

DISCUSSION

Acute toxicity of AE of ADHJ

Generally, the oral administration of AE of ADHJ to male

Table 2. Effect of AE of ADHJ on relative organ weights of kidneys, spleen and suprarenal glands on Wistar female rats.

Dose (mg/kg)	Treatment duration					
	40 days			60 days		
	Kidney weight (g/ 100 g of bw)	Spleen weight (g/ 100 g of bw)	Weight of surrenal gland (g/ 100 g of bw)	Kidney weight (g/ 100 g of bw)	Spleen weight (g/ 100 g of bw)	Weight of surrenal gland (g/ 100 g of bw)
0	0.83 ± 0.05	0.66 ± 0.05	0.031 ± 0.003	0.96 ± 0.03	0.46 ± 0,09	0.0439 ± 0.0017
12.5	0.99 ± 0. 06*	0.61 ± 0.09*	0.033 ± 0.003	0.94 ± 0.04	0.42 ± 0.09	0.0378 ± 0.002
50	1.15 ± 0.07**	0.47 ± 0.02*	0.035 ± 0.002	0.93 ± 0.02	0.48 ± 0.018	0.035 ± 0.0032**
100	1.01 ± 0.02**	0.50 ± 0.05*	0.032 ± 0.001	0.99 ± 0.03	0.48 ± 0.02	0.0307 ± 0.015***

The values represented Mean± ESM of relative organ weights of kidneys, spleen and suprarenal glands in each group (n=6). *p<0.05; **P<0.01; ***P<0.001 (PPD-Fischer test).

Table 3. Effect of AE of ADHJ on corpus luteum number, ovarian and uterus protein levels and weights.

Treatment duration (days)	Dose (mg/kg)	Ovarian weight (mg/100 g bw)	Ovarian protein (µg/mg)	Uteri weight (mg/100 g bw)	Uteri protein (µg/mg)	Corpus luteum number
40	0	36.8 ± 3,10	20.51 ± 1.48	65.10 ± 6.00	23.46 ± 1.34	0.00 ± 0.00
	12.5	28.00 ± 3.70	32.06 ± 3.02**	69.00 ± 12.00	27.06 ± 1.29*	2.67 ± 2.67*
	50	45.10 ± 2.30	31.18 ± 1.46**	105.50 ± 25.30	25.00 ± 0.46*	2.33 ± 2.33*
	100	38.00 ± 3.10	31.03 ± 2.97**	99.40 ± 32.00	30.57 ± 1.50***	10.00 ± 0.00***
60	0	41.80 ± 1.90	14.64 ± 1.23	65.00 ± 6.60	14.64 ± 10.23	2.00 ± 1.26
	12.5	53.60 ± 4.30	25.00 ± 0.59***	88.90 ± 6.55	25.00 ± 0.59	17.00 ± 4.60**
	50	42.40 ± 4.00	29.33 ± 1.17***	82.70 ± 9.10	29.33 ± 1.17	14.17 ± 6.77**
	100	44.90 ± 2.80	29.69 ± 2.13***	78.30 ± 7.90	29.69 ± 2.13	11.17 ± 2.44**

The values represented Mean ± ESM of corpus luteum number, ovarian and uterus protein levels and weights in each group (n=6). *p<0.05; **P<0.01; ***P<0.001 (PPD-Fischer test).

and female mice at the doses of 16 – 32 g/kg induced difficulties in locomotion and agressivity. These signs may have resulted from the attack of the nervous system (Bep and Bever, 1986) owing to the high intake of the herbal drug.

Similar signs have also been recorded in the study of toxicological evaluation of the aqueous extract of *Allium sativum* bulbs on laboratory mice and rats (Gatsing et al., 2005). The low reaction of the mice after pinching the tail and decreased sensibility in touch may result from the low level of prostaglandins induced by the treatment with the extract. Prostaglandins are hormones that regulate the perception of pain (Lehninger, 1982). Prostaglandins are not stored, and their release is dependent on biosynthesis. Evidently, various medications that prevent the perception of pain inhibit the conversion of arachidonic acid by inhibiting the release of prostaglandins synthetase or by interfering in some other way with the synthesis of prostaglandins (Eisenhauer et al., 1998). In spite of the above side effects, the LD_{50} values higher than 5g/kg permit to classify the AE of ADHJ among the non toxic substances according to the Hodge and Steiner criteria (Delongeas et al., 1983; Schorderet, 1992).

Subacute toxicity of AE of ADHJ

The AE of ADHJ induced various degrees of activities on some biochemical parameters of toxicological study. The oral administration of AE of ADHJ during 40 days increased body and organs (suprarenal glands, uterus ovary and kidneys) weights. The increase in reproductive organ weight gain was certainly due to the fixation of estrogenic compounds present in the AE on uterus and ovary receptors (Jensen and Desombre, 1972; Katzenellenbogen et al., 1979). Similar results were obtained by Ettebong et al. (2011) during the study of the contraceptive, estrogenic and anti-estrogenic potentials of methanolic root extract of *Carpolobia lutea* in rodents where in very low doses the plant extract has increased the uterine wet weight. Meanwhile, the standard error of the uteri weights were high and might be due to the fact that the uterus mass was affected by womb fluid collection. The decrease of the body weights in rats treated with the plant mixture at the dose of 12.5 mg/kg might be due to the less food and water intake as reported by Joseph et al., 1989. The significant increase of ALT serum levels in rats treated with the AE of ADHJ

(100 mg/kg) compare to that of the control group suggested that the extract might be harmful to the liver as described by Kakeno et al. (1997) and Emerson et al. (1993). The ALT in blood increases when the hepatic cellular permeability varies or when necrosis and cellular injury occur. The significant ($p<0.01$) increase of creatinine level in rats treated with AE during 40 days might be due to their release in urine during the excretion. Moreover, the creatinine value was higher than 0.04 g/L; consequently a nephrotoxicity may be suggested with reference to the work done by James and Kathleen (1992).

Conclusion

Despite the fact that the AE of ADHJ is not short term poisonous, the increase of ALT and creatinine serum levels in treated rats at the rate of 100 mg/kg during 40 days and 12,5, 50, 100 mg/kg during 60 days is a proof that this extract may exhibits some adverse effects in the long run. Thus, considering the important and widespread traditional use of the plants in combination, for the sake of the safety of populations, the histopathological analyses need to be performed in order to study the manifestations on the liver and kidneys through microscopic examinations.

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