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RESEARCH ARTICLE

ACUTE AND SUB-ACUTE ORAL TOXICITY STUDIES OF AQUEOUS EXTRACT OF *SECURIDACA LONGEPEDUNCULATA* FRESEN (POLYGALACEAE) ROOT BARKS IN RODENTS.

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Abstract

Securidaca longepedunculata Fresen, (Polygalaceae) is a multi-purpose plant mostly distributed in various tropical African countries, including Burkina Faso. To enable a safe use of the plant, acute and sub-acute toxicity tests of its aqueous root barks extract were undertaken on female NMRI mice and Wistar rats respectively, in accordance with Organization for Economic Cooperation and Development (OECD) test guidelines. In addition to general behavior, adverse effects and mortality recorded for up to 14 days during the acute study, biochemical and morphological parameters were also determined in subacute toxicity assays. In the oral acute toxicity assay, the aqueous extract of the plant root barks at dose of 2000 mg/kg b.w. induced mortality of mice. However, no mortality of mice were observed at dose of 300 mg/kg b.w. The LD₅₀ value estimated was 1000 mg/kg b.w. In the 28 days sub-acute oral toxicity study in Wistar rats, there was no mortality observed at doses of 50, 100 and 200 mg/kg/day. Furthermore, this subacute treatment did not induce any noticeable changes in body weight gain, food and water consumption and biochemical profiles in animals. In addition, no changes in macroscopical aspect of organs were observed. These results showed that acute administration of root barks aqueous extract of *S. longepedunculata* displays slightly toxicity in female mice, while subacute administration did not cause any toxic effect in Wistar rats. This suggested a safe use of the *S. longepedunculata* root barks based remedies for human or veterinary medicines.

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Introduction:-

Securidaca longepedunculata Fresen, (Polygalaceae) is mostly distributed in various tropical African countries, including Burkina Faso. It is a multi-purpose plant with a long history of use in African traditional medicine (Mongalo *et al.*, 2015). In Nigeria, many local uses of *Securidaca longepedunculata* (*S. longepedunculata*) were

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reported, i.e. abortion, constipation, cough, diarrhea, dislocated jaw, dysentery, fever, frequent stomach ache, headache, vaginal itches, malaria, piles, pneumonia, protection against evil spirit and witchcraft, sexual boost, skin cancer, skin infection, toothache and tuberculosis (Mustapha *et al.*, 2013). In Burkina Faso, it is traditionally used against diarrhea, dysentery, intestinal parasites, liver disease, bilious fever, hematuria, snake bite, malaria, bronchitis, stomachache, rheumatism, amenorrhea, dry colic, hiccups, rectal prolapse, intestinal obstruction, headache, constipation, shingles, poisoning, spell, and tumors (Nacoulma, 1996). This widespread use of the plant in traditional medicine (both human and animals) is supported by its pharmacological properties and its chemical composition. Indeed, pharmacological studies on *S. longepedunculata* showed some anti-inflammatory, antiulcer, antianemia and antiplasmodial properties of this species extracts (Ojewole *et al.*, 2008). Furthermore, phytochemical investigations of several parts of the plant revealed presence of saponins (Ndamitso *et al.*, 2013; Stevenson *et al.*, 2009), flavonoids (Auwal *et al.*, 2012; Muanda *et al.*, 2010), alkaloids (Wrobel *et al.*, 1996), steroids (Meli *et al.*, 2007), sucrose derivatives (De Tommasi *et al.*, 1993), phenolic acids (Muanda *et al.*, 2010) and volatile oils (Nebié *et al.*, 2004; Jayasekara *et al.*, 2005). Although a lot of medicinal plants are widely used and assumed to be safe, they could potentially be toxic (Nasri and Shirzad, 2013). For *S. longepedunculata*, acute toxicity studies have resulted in contradictory results. In a review of the literature, Mongalo *et al.* (2015) noticed a high variability in the results of acute toxicity studies. Moreover, oral LD50 values of roots aqueous extracts of the plant reported by different authors varied quite widely between 3160 mg/kg and 37 mg/kg in rat. The authors attributed this large variability to the differences in collection site, geographical area and the season of collection. In a repeated dose toxicity study, Etuk *et al.* (2006) found no mortality when administering per os doses of 300, 900 and 2700 mg/kg of aqueous root extract on a daily basis for a period of 28 days to Swiss albino mice. However, to our best knowledge, up to now there is no data in the literature on rat repeated dose toxicity study. Due to the widespread use of *S. longepedunculata* by rural communities and mainly vet-healers to treat several diseases, and to face discrepancy of acute toxicity findings and lack of sufficient informations on repeated dose studies, there is a need to further investigate its short and long-term toxicity manifestations.

Material and Methods:-

Plant material:-

Fresh roots of *S. longepedunculata* were collected in October 2015 around the city of Dédougou (savannah zone) in Burkina Faso. The plant sample was authenticated at “Herbier National du Burkina (HNBU)” located at Centre National de Recherche Scientifique et Technologique (CNRST), Ouagadougou (Burkina Faso) where the voucher specimen has been deposited under number HNB 8714.

Immediately after collection, the barks were extracted from the roots, washed with tap water and dried at ambient temperature under ventilation in the shade for two weeks. The dried barks were pulverized using an electrical blender. The powder obtain was labelled for easy identification and stored in dark tightly closed glass bottles until used.

Extract preparation:-

A portion of *S. longepedunculata* root barks powder sample was weighed (100 g) and macerated in 1 L of distilled water; the mixture was then shaken using electric shaker during 24 hours at room temperature. The suspension obtained was filtered using Whatman No.1 filter paper and the filtrate kept in deep freezer for 24 hours before lyophilization. The lyophilizate was then collected in dark tightly closed glass bottles and kept in a desiccator to avoid absorption of water until use.

Animals:-

Healthy males and females NMRI mice weighing between 28 and 32 g and Wistar rats (mean weight : 176±16g) were used in this study. The animals were procured from the “Institut de Recherche en Science de la Santé” (IRSS), Ouagadougou, Burkina Faso. They were housed in cages with free access to water and fed with standard laboratory pellet enriched with protein (29%). All animals were maintained in a controlled room temperature of 22-25°C with a 12 h dark/light cycle. The protocol of experimentation was carried out in accordance with protocols already validated by IRSS (Burkina Faso) and that meets international standards (Guidelines set by the European Union on the protection of animals (CEC Council 86/609) (Zimmermann, 1983 ; Meier *et al.*, 1986).

Acute toxicity test:-

Acute toxicity test was performed on female NMRI mice in accordance with Organization for Economic Cooperation and Development (OECD) test guideline 423, the acute toxic class method (OECD, 2001). After a 4-

hour fastening period, the extract was administered orally by gavage in single dose to the mice according to the sequential procedure. While conducting the test, 2000 mg/kg body weight (b.w.) of extract was chosen as the starting dose. Animals were observed individually during the 2 hours post-treatment to the end of which they were fed. They are then observed at least once daily for 14 days for mortality and signs of toxicity such as changes in skin and fur, eyes, mucus membranes, convulsion, salivation, diarrhea, lethargy, sleep and coma (Ouedraogo *et al.*, 2013).

Sub-acute toxicity test:-

The sub-acute oral toxicity study was carried out according to OECD guideline 407 (OECD, 2008). A total number of 40 Wistar rats of both sexes were randomly selected for that purpose. Females involved were nulliparous and nonpregnant. The rats were divided into four groups of 10 animals each (5 males and 5 females) ; males and females were kept in separate polypropylene cages. Group 1 served as control and received a daily administration of vehicle (distilled water). Groups 2, 3 and 4 as experimental males and females rats received extract doses of 50, 100 and 200 mg/kg body weight, respectively. The extract and vehicle were administered daily at the same time for 28 days. All animal's were closely observed for the first 1 and 4 hours of dosing to examine any adverse toxic signs, behavioural changes and at least twice a day for morbidity and mortality. Body weight and food consumption were recorded once weekly. Water consumption was monitored daily for each cage (5 rats per cage) up to 4 weeks. On the 29th day, after over-night fastening, all the rats were anaesthetized using ketamine. Blood samples were collected via cardiac puncture into dry tube(vacutainers) for each animal.

Blood analysis:-

The blood samples in dry vacutainers were centrifuged at 3000 rpm for 10 min using a table centrifuge (ROTOFIX 32A, Mettich Zenrifugen, Germany) ; the sera obtained were used for biochemical assays.

Blood chemistry tests were performed on an automatic biochemistry analyzer (Mindray BS-300, China). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREAT), total cholesterol (TC), total protein (TP), triglycerids (TRIG) glucose, calcium, chloride, phosphorus (PHOS), magnesium (MGN), total bilirubine (T-Bil) and direct bilirubine (D-Bil) were determined.

Effects on vital organs:-

After blood collection, internal organs including liver, heart, kidneys, lungs, and spleen were collected carefully from sacrificed animal in a Petri dish. The isolated organs were dried with cotton wool and weighed on a sensitive balance (Sartorius; precision 0.1 mg). Each weighed organ was standardized for 100 g body weight of each rat weighed to determine relative organs weights. After that, a gross examination (macroscopic analysis) of the target organs of the control and treated animals was done to check any significant change in texture and shape.

Statistical analysis:-

Results were expressed as means \pm standard deviation (SD). Means and standard deviations were calculated separately for males and females. The data were processed with Graph Pad Prism.5. The statistical significance of difference between treated and control groups were analyzed using one-way analysis of variance (ANOVA), followed by Dunett's multiple comparison tests. Differences were considered to be statically significant at $p < 0.05$.

Results:-

Acute toxicity study of of *S. longepedunculata* root barks aqueous extract in mice:-

In acute oral toxicity study, a single administration of aqueous extract of the plant at dose of 2000 mg/kg b.w. induced mortality of mice in the first step. However, no mortality of mice were observed at dose of 300 mg/kg b.w. (Table 1). According to the acute toxic class method, the aqueous extract of the plant tested is classified to the 4th toxicity class with a LD₅₀ value of 1000mg/kg b.w.

Main clinical symptom observed within 24 hours post-treatment in mice was somnolence at dose of 2000 mg/kg b.w.

Sub-acute oral toxicity study of *S. longepedunculata* root barks aqueous extract in rats:-

Effect on body weight gain:-

The means weekly body weight gains of control and daily treated rats with aqueous extract of the plant during 28 days are illustrated in figures 1 and 2. Globally, both treated and control rats showed a steady increase in the body weight during the study period. However, as show in figure 1, a significant difference ($p < 0.05$) in body weight gain between male treated rats with 200 mg/kg/day b.w. and controls were noticed at days 7, 21, and 28.

Effect on organs relative weight:-

Table 2 presents the mean relative weights of heart, lungs, liver, kidneys and spleen of control and treated rats. Any significant changes were noticed among different doses of treatment and control groups ($p > 0.05$).

Effect on rats water and food consumption in sub-acute toxicity study:-

The average water consumption of both treated groups were found to be unaffected by the treatment as there were no significant changes in the average water consumption when compared with the control as shown in table 3.

However, as presented in table 4, a reduce in food consumption was observed in group treated with 200 mg/kg b.w. of *S. longepedunculata* root barks aqueous extract when compared to control group.

Effect on blood chemistry value of rats in sub-acute toxicity study:-

The results of the various biochemical tests on the experimentally treated animals with the plant extract and control group are summarized in table 5. As shows in this table, *per os* administration of the plant extract at doses of 50, 100 and 200 mg/kg b.w. on rats did not result in significant changes in blood serum biochemical parameters such as ALT, CREAT, TP, TRIG, glucose, calcium, chloride, phosphorus, total protein, T-BIL and D-BIL levels when compared to control group. However, a significant rise of AST in treated group at dose of 50 mg/kg b.w. ($p < 0.01$ for male rats and $p < 0.05$ for female rats) and total cholesterol in female group treated with plant extract at dose of 200 mg/kg b.w. were noticed when compared to control.

Macroscopic effects on vital organs:-

The macroscopic examination of vital organs such as heart, lung, liver, kidney and spleen for gross defects revealed that treatment with extract were not adversely affected the different organs.

Table 1:- Mortality of female mice in acute oral toxicity study.

Doses level (mg/kg)	Mortality (72 hours)	
	First test	Second test
2000	2/3	Not tested
300	0/3	0/3

Table 2:- Mean relative organ weights of after 28 days treatment with *S. longepedunculata* root barks aqueous extract.

Organ	Sex	Dose (mg/kg b.w.)			
		0	50	100	200
Heart	M	0.35±0.03	0.36±0.06	0.34±0.02	0.35±0.02
	F	0.37±0.04	0.38±0.06	0.37±0.05	0.34±0.02
Lung	M	0.51±0.05	0.53±0.06	0.55±0.06	0.55±0.06
	F	0.60±0.06	0.61±0.08	0.65±0.08	0.60±0.15
Liver	M	2.81±0.20	3.06±0.26	2.98±0.26	2.96±0.06
	F	2.96±0.27	3.25±0.52	3.14±0.20	3.37±0.39
Kidneys	M	0.65±0.01	0.71±0.04	0.70±0.05	0.76±0.15
	F	0.68±0.07	0.75±0.10	0.71±0.06	0.68±0.06
Spleen	M	0.26±0.03	0.26±0.06	0.24±0.02	0.24±0.04
	F	0.25±0.03	0.30±0.05	0.29±0.07	0.29±0.05

Mean and standard deviation are presented (n = 10 ; 5/sex)

M = Male ; F = Female

Table3:- Mean daily water consumption (mL/day/rat).

Weeks	Sex	Dose (mg/kg b.w.)			
		0	50	100	200
D0-D7	M	39.29±1.89	36.43±2.44	42.57±5.13	34.29±4.50
	F	27.14±2.67	27.86±5.67	31.71±3.73	31.71±2.36
D8-D14	M	37.43±5.83	31.29±5.44	40.66±9.68	37.49±4.04
	F	33.57±4.76	32.86±7.20	35.43±7.25	31.14±5.90
D15-D21	M	43.00±6.68	34.29±4.50	37.29±8.67	40.86±6.74
	F	30.00±5.00	35.71±3.45	34.86±2.91	32.86±5.34
D21-D28	M	37.86±6.36	32.14±2.87	35.00±5.00	34.57±3.10
	F	29.29±4.50	30.57±3.26	33.57±3.78	33.93±3.34

Mean and standard deviation are presented (n = 10 ; 5/sex)

M = Male ; F = Female

Table 4:-Mean weekly food consumption (g/day/rat).

Weeks	Sex	Dose (mg/kg b.w.)			
		0	50	100	200
D0-D7	M	26.26	24.11	22.31	17.71
	F	18.31	15.66	17.54	14.80
D8-D14	M	27.00	25.57	25.66	24.26
	F	22.63	17.06	19.86	19.40
D15-D21	M	26.00	23.57	23.06	23.14
	F	17.37	15.49	20.14	16.25
D21-D28	M	27.56	23.37	26.34	23.31
	F	21.17	16.57	23.00	18.64

Mean and standard deviation are presented (n = 10 ; 5/sex)

M = Male ; F = Female

Table 5:-Biochemical parameters for rats after 28 days treatment with aqueous extract of root barks of *S. longepedunculata*.

Parameters	Sex	Dose (mg/kg b.w.)			
		0	50	100	200
ALT (U/L)	M	55.06±12.10	55.86±10.86	44.46±9.33	52.37±14.86
	F	46.78±16.36	46.63±6.73	49.87±19.48	61.83±14.90
AST (U/L)	M	91.49±13.92	130.57±16.64**	110.24±3.59	107.03±12.41
	F	98.25±13.71	198.26±39.76*	137.63±16.43	116.56±46.12
CREAT (mg/dL)	M	44.19±5.93	50.19±6.08	42.83±5.42	46.79±4.11
	F	42.44±2.41	50.79±7.95	47.32±4.57	47.59±6.63
TC (mg/dL)	M	32.86±5.79	34.28±7.72	28.33±3.32	31.24±5.80
	F	25.70±3.82	21.56±2.11	20.22±5.55	18.30±2.66*
TP(mg/dL)	M	59.08±3.48	62.90±5.07	61.25±1.19	60.74±5.31
	F	61.70±3.19	65.42±5.38	62.62±5.35	59.23±3.34
TRIG (mmol/L)	M	0.24±0.05	0.34±0.09	0.25±0.06	0.28±0.11
	F	0.26±0.05	0.32±0.05	0.30±0.07	0.23±0.05
Glucose (mg/dL)	M	4.74±0.45	5.46±3.12	4.60±0.35	5.72±1.19
	F	3.64±0.53	4.32±1.31	4.48±0.67	3.83±0.31
Calcium mmol/L)	M	2.08±0.11	2.12±0.08	2.05±0.13	2.18±0.13
	F	2.04±0.11	1.94±0.26	2.10±0.17	1.93±0.05
Chloride (mmol/L)	M	91.80±0.45	92.20±2.28	91.00±1.41	92.20±0.45
	F	91.80±0.84	92.80±1.01	91.00±1.00	91.50±1.00
PHOS (mg/dL)	M	1.94±0.21	2.24±0.43	1.83±0.17	2.34±0.23
	F	1.80±0.34	2.30±0.67	1.80±0.45	1.65±0.26
MGN (mmol/L)	M	0.61±0.06	0.84±0.14**	0.77±0.06	0.87±0.08**
	F	0.63±0.09	0.83±0.15	0.88±0.13*	0.79±0.06
T-BIL (mg/dl)	M	0.06±0.02	0.05±0.02	0.04±0.01	0.06±0.03
	F	0.06±0.02	0.05±0.02	0.04±0.02	0.03±0.02
D-BIL (mg/dl)	M	0.07±0.02	0.07±0.02	0.05±0.01	0.07±0.03
	F	0.07±0.02	0.07±0.02	0.05±0.00	0.05±0.02

Mean and standard deviation are presented (n = 10 ; 5/sex)

M = Male ; F = Female

*p<0.05 ; **p<0.01 versus control group

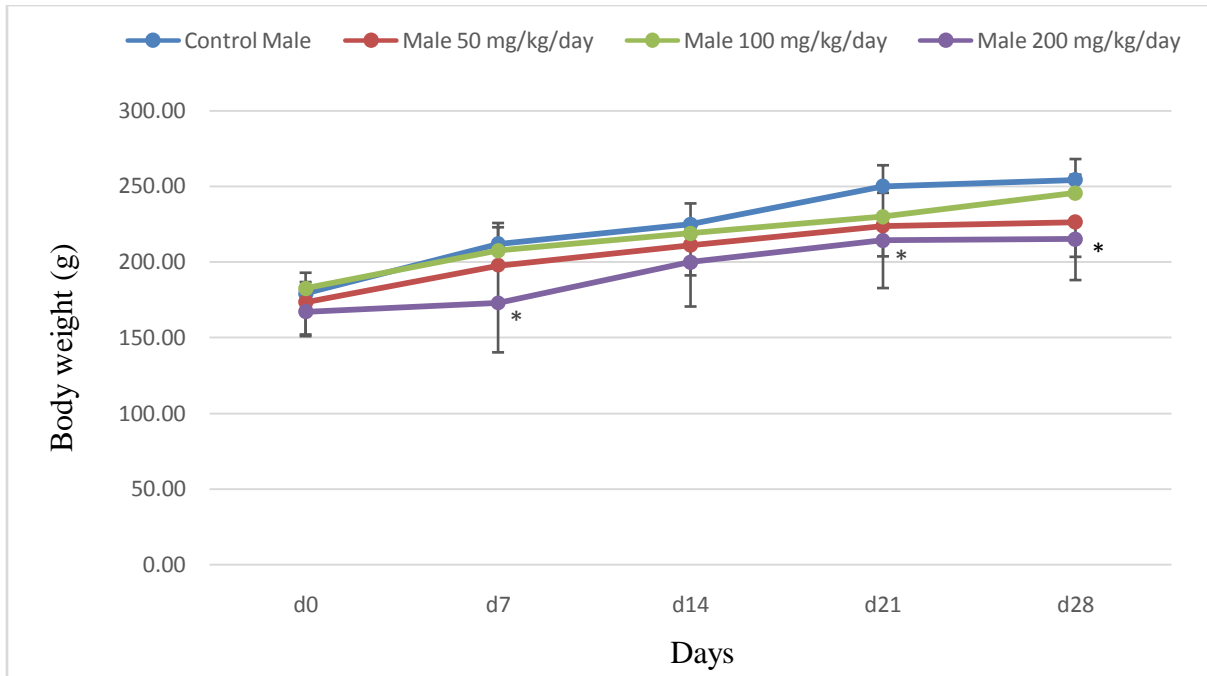


Fig 1:-Mean body weight (g) of control and treated male rats with different doses of *S.longepedunculataroot* barks aqueous extract.

Mean and Standard deviation are presented (n = 5)

*p<0.05 versus control group

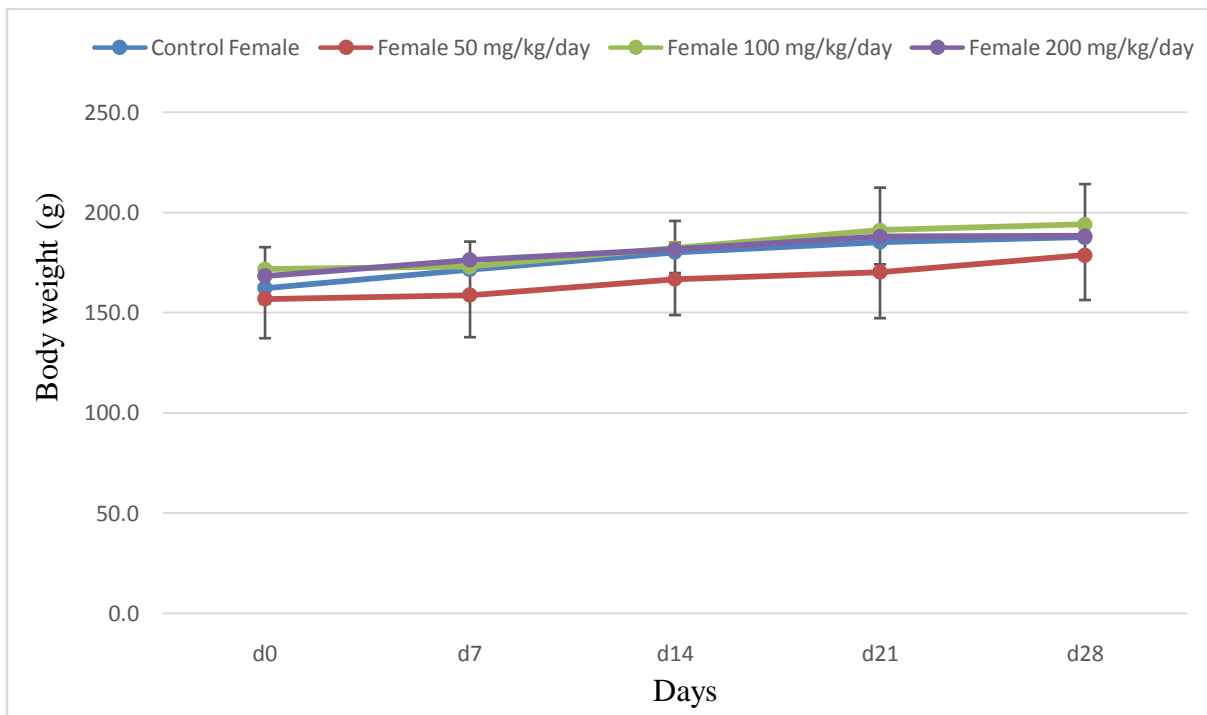


Fig. 2:-Mean body weight (g) of control and treated female rats with different doses of *S. longepedunculataroot* barks aqueous extract

Mean and Standard deviation are presented (n = 5)

Discussion:-

In general, acute and sub-acute toxicity tests are carried out in laboratory animals such as rodents to determine the degree of toxicity of substances for classification purposes. In the present study of acute oral toxicity of *S. longepedunculata* root barks aqueous extract, a single administration of plant extract at dose of 2000 mg/kg b.w. to mice causes some mortality and clinical signs such as somnolence. However, at dose of 300 mg/kg b.w. no mortality or behavioral change were not observed. Based on OCDE guideline 407, the LD₅₀% of plant extract is 1000 mg/kg. This LD₅₀% value suggest that plant extract tested is classified to the fourth toxicity class *ie* products wich have relatively a low oral acute toxicity (OCDE, 2001 ; Nations Unie, 2011). Our results are similar to those of Adeyemi *et al.* (2010) who obtained an LD₅₀ value of 1.740 g/kg (fourth toxicity class) for the whole root aqueous extract in mice by oral route. However, some other authors reached a LD₅₀ value of 370 mg/kg b.w. with the same root aqueous extracts administered orally to albino Sprague Dawley rats (Dapar *et al.*, 2007). The difference with our results could be explained by the animal used but also possibly the place (soil quality) where the roots were harvested. The mineralogical composition of the soils in the two sites could be different from one to another and have an impact on chemical composition of vegetals (Ouedraogo *et al.*, 2016).

The sub-acute toxicity study showed that the daily oral administration of *S. longepedunculata* root barks aqueous extract at doses of 50 ; 100 and 200 mg/kg/day b w. during 28-days did not cause any death nor clinical signs of toxicity.

During the study period, a steady increase in the body weight was observed in both treated and control rats with a slight decrease in body weight gain of male rats at dose of 200 mg/kg/day b.w. compared to controls. Also, the relative organ weights of treated groups were similar to control group. Body weight gain and relative organ weights are known to be sensitive indicators of toxic effects (Michael *et al.*, 2007). The results obtained during the 4 weeks of study concerning the weight gain suggest that aqueous extract of plant did not influence the animal's weight gain at doses of 50 and 100 mg/kg bw as they are an increase in body weight similar to control group. However at dose of 200 mg/kg/day b.w. a slight decrease in body weight gain was observed. This slight decrease could be attributed to the suppression of the animals' appetite by the extract leading to reduced in food intake (Ogbonnia *et al.*, 2010).

The biochemical parameters levels indicates physiological condition. The increase and decrease of biochemical parameters can convey indications regarding toxicity of specific organs (Das *et al* ; 2015). In the present sub-acute toxicity study, there were no significant change in most of the blood serum biological parameters analyzed in both treated and control groups. However, a significant increase in blood serum levels of transaminase AST in treated group at dose of 50 mg/kg/day b.w and total cholesterol (TC) in female treated rats with at dose of 200 mg/kg/day.b.w. were noticed.

Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) are largely used for the assessment of liver damage by drugs or any other hepatotoxin (Ramaiah *et al.*, 2011). In fact, ALAT and ASAT are serum enzymes markers synthesized in the liver. ALT is localised in the cell cytoplasm while AST is found both in mitochondria and cytoplasm (Prosper *et al.*, 2010). Then, as liver and heart releases ALT and AST in blood, an increase of their levels in plasma are strong indicators of liver and heart damage (Wasan *et al.*, 2001 ; Mythilypriya *et al.*, 2007). However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury. The no significant change observed in ALT values suggest that liver was not damaged.

Creatinine is a serum metabolite that is indicative of the kidney physiology (Whitby *et al.*, 1987). In this study, we didn't notice any significant change in creatinine values when comparing treated and control groups, meaning that these specific organs were not damaged.

Macroscopic examination of vital organs such as liver, kidney, heart, lungs and spleen as well revealed no treatment-related changes due to the administration of plant extract. This result is in accordance with the no change in organs relative weight in both treated and control groups.

Conclusion:-

Results obtain in this acute toxicity study suggest that *S. longepedunculata* root barks aqueous extract is practically safe when administered orally. The sub-acute toxicity study of the same extract revealed that its repeated oral administration does not significantly affect animals at lower doses; thus, when using doses above 100 mg/kg/day, one may face some toxic effects. However, further investigations such as chronic toxicity, effects on reproductive function and genotoxicity need to be undertaken for a more complete elucidation of the real safety profile of aqueous extractof *S. longepedunculata* root barks.

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