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Research Article

ANTI-NOCICEPTIVE ACTIVITIES AND TOXICOLOGICAL PROFILE OF AQUEOUS EXTRACT OF LEAVES OF *HIBISCUS ASPER*

TchoumbaTchoumiLilianeMireille, Mbiantcha Marius*,

Diffoum Jean Baptiste and Kamanyi Albert

Laboratory of Animal Physiology and Phytopharmacology, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon.
Department of Animal Biology, University of Dschang, Cameroon.

ABSTRACT

This work was been carried out with the objective of evaluating the anti-nociceptive activities and the toxicological profile of the aqueous extract of the leaves of *H.asper*. For acute and sub-chronic toxicity, no case of death was recorded and no modification of behavioral parameters. The relative body weight of rats showed a significant decrease. The weight of the heart of rats treated with all doses showed a significant decrease. The level of ASAT in serum of rats treated with 1000 mg/kg dose showed a significant increase, while the level of hepatic ASAT of rats treated with 250, 500 and 1000 mg/kg doses showed a significant decrease. The analgesic activity of extract on pain induced by acetic acid was significant at doses of 125, 250 and 500 mg/kg with maximum inhibition of 75.14% at the dose of 500 mg/kg. The extract at all doses significantly inhibited the two phases of pain induced by formalin. The analgesic effect of extract on pain induced by pressure was significant at 1h, 2h, 3h and 4h. The analgesic effect of extract on chronic inflammatory pain induced by formalin was significant on the 2^{nd}, 3^{rd}, $4^{th,}$ $5^{th}$and 7^{th} days of treatment. These results show that the aqueous extract of the leaves of *Hibiscus asper* can be considered as toxic, and that it possesses analgesic properties. The analgesic properties are at the same time peripheral and central; these analgesic properties could be due to flavonoids and polyphenols contained in the aqueous extract.

Keywords: *Hibiscus asper*, acute toxicity, sub-chronic toxicity, analgesic.

1-INTRODUCTION

Pain is a physiological alarm reaction that aims to protect the body against harmful stimuli that can cause tissue or organ damage[1]. Pain is a major health problem and is a major cause of consultation in the world it is the pattern of two out of three medical consultations[2]. Some medicinal plants are used in the treatment of pain ; *Hibiscus asper*is a herbaceous plant that is found in Cameroon its leaves are used in traditional medicine for the treatment of inflammation and Parkinson's disease. Phytochemical studies conducted on the aqueous extract of the leaves they revealed the presence of chemical compounds such as flavonoids and polyphenols[3]. In view of the interest aroused by the use of this plant for therapies such as: inflammation, infertility, yellow fever; there is no scientific study of the properties referring analgesics or treating the toxicity of this species. Inflammation and pain are related inflammation involves the release of compounds such as substance P or prostaglandins that stimulate the endings it is on this basis that born the importance of this work; So the main objective is to determine scientifically or appreciate the analgesic properties and the toxicological profile of the aqueous extract of Hibiscus asper leaves in mice and rats.

2-MATERIALSAND METHODS

2.1- Plant material and extraction

H. asper leaves were harvested in October 2011 in the city of Dschang(Cameroon). The botanical identification sheets had been made by the Dr. FochoDerreckofthe department of vegetal biology at the University of Dschang a specimen had been preserved in the National Herbarium at number Lucha034[3]. The leaves were sechees a shelter from the sun and then ground into a fine powder which was used for the extraction, Distilled water was used as an extraction solvent. 1000 grams of powder were soaked in 8 liters of distilled water and the mixture was left in a covered container for three days. The fourth day the mixture was filtered using a filter paper (SELECTA). The filtrate was evaporated in a door oven at 40 °C which yielded 122.62 g powder representing the aqueous extract a yield of 12.3 g.

2.2- Animals

White mice (*Musmusculus*) males and females weighing 20-30 g and male and female rats weighing on average 140 g were used.They came from the pet department of Animal Biology of the Faculty of Science of the University of Dschang, under the conditions of temperature and natural luminosity they had free access to drinking water and food.

2.3-Toxicity test

2.3.1- Acute toxicity test orally to rats

Healthy Wistar rats (3 months weighing on average 120 g) were divided into six groups of eight rats each (4 males and 4 females) males and females separately being lodges. The rats were placed in fasting (12 h) before treatment with free access to water.The aqueous extract was administered (single dose) by gavage (1 ml/100g) Group 1 was treated in distilled water; groups 2 to 6 were treated in the aqueous extract of the respective doses 4, 8, 12, 16 et 20 g/kg. Animals so treated have been deprived of food and water during the first three hours post-gavage during which the parameters such as: the aggressiveness (a screw attack rats vis the other), locomotion (displacement of animals in the cage), sensitivity to noise (reaction of a rat following the issuance of a noise), the sensitivity to pain (reaction of the rat after the tail pinch) and the state of the stool evaluated. After this delay the animals had free access to drinking water and food. The survivors were then guards for 7 days during which period the weight change was evaluated.

II.3.2- Subchronic oral toxicity in rats

Healthy Wistar rats (2.5 months weighing on average 100 g) were divided into six groups of 8 rats (4 males and 4 females), males and females separately being lodges. The acclimatized rats for 7 days in the laboratory were treated daily by gavage (1 ml/100 g)with doses of extract 125, 250, 500 and 1000 mg/kg body weight during a period of 4 weeks. The control group received distilled water. During this period of administration, the number of death, weight change, food and water were taken weekly ratings. One day after the administration of the 28th day the weight of all survivors was noted then they were anesthetized by intraperitoneal injection of Diazepan (10 mg/kg)a muscle relaxant which is followed by ketamine (10mg/Kg)10 min after the ketamine acts at the synaptic cleft by blocking the transmission of nerve messages. Blood was collected by cardiac puncture in dry tubes containing no anticoagulant and centrifuged at 3000 tr/min for 20 min and the supernatant retained levied a - 20 °C for biochemical analysis. Immediately after the collection of blood, liver, spleen, kidneys, heart and lungs were levied free of grease cleaned drained and weighed. Part of the liver and kidney were ground and then centrifuged 3000 tr/min for 20 min and the supernatant retained levied a - 20 °C for biochemical assays.

II.4- Analgesic activity

II.4.1-Antinociceptive activity

For each experiment animals were divided into 5 groups of 6 animals each. Group 1 was used as a control treated distilled water. Group 2 as a positive control treated to aspirin (20 mg/kg). Group 2 to 5 treated with the aqueous extract (125, 250 and 500 mg/kg).All animals were treated orally.

II.4.1.1- Acetic acid test inducing abdominal contractions

This test was carried out according to the method described by Vogel and Vogel (4).One hour after administration of each treatment the solution of acetic acid (1%) was administered by intra-peritoneal route (0.1 ml per 10 g body weight). After injection of acetic acid the mice were immediately placed in a transparent cage each and were observed for thirty minutes. The number of abdominal contraction accompanied stretching of hind legs (embodying the pain) was determined during the first 30 minutes after a lag time of 5 minutes. The percent inhibition (% I) was calculated by the following formula:

$$\% I = (N_C - N_t) / N_C \times 100$$

N_C: Mean number of contraction of control group

N_t: Mean number of contraction in the control group

II.4.1.2- Formalin test

The test was carried out according to the method described by Gaertner*et al.*,[5]. One hour after the administration of each treatment, 0.02 ml of formalin (2.5%) was injected under the soles of the right posterior paw of each mouse. The pain was materialized by the time licking the injected paw ; time licking the painful leg was stopwatch during the first 5 minutes the first phase corresponding to the pain and neuropathic pain between the 15th and 30th minute, second phase of the corresponding inflammatory pain after administration of formalin pain. The percentage of analgesic activity (% I) of each phase was calculated according to the formula:

$$\% I = (C - T)/C \times 100$$

C :Time licking the control group
T :Time licking the control group

II.4.1.3- Analgesimeter test

The test was carried out according to the method described by Nguelefack*et al.*,[6]. The reaction threshold of each rat the mechanical pain was measured with the aid of a brand analgesimeter like UgoBasile 37215 before treatment (0) and 30, 60, 120, 180 and 240 minutes after treatment. The percent inhibition (% I) in treated rats was calculated by the following formula

$$\% I = (T_t - T_0)\ \text{Control} - (T_t - T_0)\ \text{Test} / (T_t - T_0)\ \text{Control} \times 100$$

T_t: Threshold after treatment time
T_0: Threshold before treatment time

II.4.2- Antihypernociceptive activity

The test was carried out according to the method described by Cheng*et al* (2008)[7]. The reaction threshold of each rat the mechanical pain was measured on the first day of the experiment until the seventh day with analgésimétre (Ugo Basile-37215). One hour after oral administration of the test substances pain and inflammation were induced by injection of 0.1 ml of a solution of formalin (2%) in the plantar surface of the left rear leg of the animal. The threshold for reaction of the animal has the mechanical pain was postponed until 0.5 h to 168h. The second day of the seventh day handling the animals received daily the different test substances respectively according to their group after taking this threshold reaction values.

II.5- Statistical analysis

The results are presented as mean ± SEM. The ANOVA test by two waiht followed by Bonferroni and the test ANOVA one waiht and Turky post-test were necessarily used for statistical analysis. Significant differences were regarded the threshold probability of 0.05, 0.01 and 0.001.

II-RESULTS

Acute Toxicity

Up to a dose of 20 g / kg no deaths have been recorded which did not allow us to determine the numerical value of the LD_{100}. In the same logic, LD_{50}could not be determined by calculation or graphically. The general behavior of rats observed during acute treatment is recorded in Table 1, shows that the mobility and aggressiveness in animals treated at a dose 12 g / kg decreased slightly; while the other evaluated parameters do not vary in all treated animals at doses 0, 4, 8, 16 and 20 g/kg respectively.

The body weight of rats was measured during one week after administration of single doses of the extract. The aqueous extract causes no significant change in body weight of animals treated with different doses administered. Moreover the body weight of animals receipts extract increases relative to that of animals in the control group was treated with distilled water (figure 1).

Sub-chronic toxicity

The evolution of the average animal body weight food consumption and water consumption of animals during four weeks of treatment in sub-chronic toxicity are represented in figures 2, 3 and 4 respectively. The aqueous extract was the fourth week causes a significant decrease ($P<0.05$) in body weight of treated animals at doses 500 and 1000 mg / kg animal weight compared to the control group (figure 2).No significant change in food consumption in animals treated has been observed; nevertheless the food consumption of treated animals decreases at the 4th week (figure 3).Moreover the extract caused a significant increase ($P<0.05$) water consumption of animals treated at a dose 1000 mg / kg during the first week of treatment(figure 4).

The results of the effect of aqueous extract of leaves *H.aspers* on the relative weights of organs (liver, kidneys, lungs, heart, spleen) of animals sacrificed at the end of sub-chronic toxicity test were determined. No significant change in the

relative weight of liver kidney lung and spleen has been observed. Against, there was a significant decrease (P<0.05 et P<0.01) the relative weight of the heart of treated animals at doses 125 ; 250 ; 500 and 1000 mg/kg aqueous extract compared with that of control animals (figure 5).

The aqueous extract does not cause significant change in serum creatinine and renal. On the other hand renal creatinine decreased dose dependent manner in all treated animals but remains pupil compared to control animals. However the serum creatinine increased dose dependent manner in all treated animals but decreased at doses 125 and 250 mg/kg compared to the control group animals and increases at doses 500 and 1000 mg/kg compared to the control group (figure 6).

The aqueous extract causes a significant increase (P<0.01) the rate of serum protein in animals treated at a dose of 1000 mg / kg compared to the control group animals; whereas hepatic protein undergoes no significant change (figure 7).

The serum AST enzyme activity is significantly increased (P<0.05) in a dose of 1000 mg/kg. On the other hand we also noted that the hepatic activity of the enzyme AST is significantly reduced (P<0.05; P<0.01 and P<0.001) dose dependent at doses 250, 500 and 1000 mg/kg in animals treated animals compared to
the control group (figure 8).

Analgesic activity

Acetic acid test

Oral administration of aqueous extract of leaves of H. asper significantly reduced manner (p< 0.001)dose dependent and the number of abdominal contractions induced by acetic acid with the percent inhibition of 35.6, 53 and 75.14% respectively at doses of 125, 250 and 500 mg/kg. Aspirin used as reference material produced inhibition 65.32% (Table 2).

Formalin test

The aqueous extract of Hibiscus asper administered orally at doses 125 and 500 mg / kg introduced a significant analgesic effect (P<0.001) during both phases of the pain induced by the formalin. The maximum anti-nociceptive effect was observed at a dose 500 mg / kg with a percentage inhibition of 35.64 and 86.21% respectively the first and the second phase of pain. Aspirin used as reference material causes a significant inhibition (P<0.001) ofpain at the second phase (Table 3).

Analgesimeter test

Using a progressive force on the paw of the rat causes a pain which the animal reacted by pulling the paw. The aqueous extract was administered all doses significantly (P<0.05; P<0.01 and P<0.001) reduces the susceptibility of animals to pain induced by pressure from 1h. The dose of 125 mg / kg showed an inhibition of 73.39% one hour after administration of the extract; whereas at doses of 250 and 500 mg / kg the inhibitions are respectively 115.88 and 145.85% this was two hours after administration of the extract. Aspirin did not significantly protected animals against the pressure-induced pain (Table 4).

Hypernociceptive activity

Formalin test

The aqueous extract of the leaves of *Hibiscus asper* administered orally produces a significant inhibition of mechanical and chemical hypernociception induced by application of an increasing force and injection of formalin (2%) under the plantar surface of the left rear leg of the animal. At doses 250 and 500 mg/kg aqueous extract produced a significant antihypernociceptive activity has the 30th minute after administration, only the activity of the dose 500mg/kg has remained significantly maintained until the 4th time. On the second day treatment was resumed due to oral administration of the extract per day which makes the activity reappear antihypernociceptive significant on day 2 and this activity has remained maintained until day 7. Day 2 to Day 4 the effect is only significant (p <0.05; p < 0.01 and p<0.001) for dose 500 mg/kg. The 5th day of treatment the effect is significant (p < 0.05 and p < 0.01) for dose 125, 250 and 500 mg/kg. By day 7 the effect is only significant remaining(p < 0.05) for dose 500 mg/kg.

DISCUSSION

Oral administration of the aqueous extract of the leaves H.asper single doses of 4, 8, 12, 16 and 20 g/kg in the study of acute toxicity has caused no deaths during 7; the behavior of all animals treated all doses were normal. Mobility and sensitivity of animals treated at a dose of 12 g/kg slightly reduced and no change of behavior has been observed at doses of 4, 8, 16 and 20 g/kg. Values of inferior to 5 g / kg correspond to highly toxic substances and those of the DL_{50}superior to 5 g/kg to low-toxic[8,9],the results show that this extract has a relatively low toxicity but not negligible. The maximum tolerated dose 20 g/kg of the extract respectively represents about 40 times the therapeutic dose (500mg/kg) obtained in this study. In acute treatment administered extract

all doses has no behavioral impact parameter.

In subchronic toxicity body weight of the animals treated at doses of 500 and 1000 mg/kg for four weeks significantly reduced in the fourth week of body weight compared to control animals. Water consumption of the animals treated at a dose of 1000 mg/kg significantly increased in the first week of treatment compared with that of control animals. These results show that repeated administration of the extract inhibit receptors hunger which would explain a decrease in food intake leading to a loss of body weight. Similarly the repeated administration of the extract stimulates thirst receptors increasing water consumption of treated animals.

At the end of the fourth week of treatment the relative weights of organs such as the liver kidneys lungs and spleen of treated animals showed no significant change regardless of the administered dose; which could reveal the possibility of a lack of immunotoxic effects of this extract due to the fact that the spleen plays a major role in immunological mechanisms. We also noted a significant decrease in the relative weight of the heart at doses of 125, 250, 500 and 1000 mg/kg of extract,this could explain a possible cardiotoxic effect induced by the extract seems to be related to the adaptive atrophy that affects a priori hollow organs [10].The liver and kidneys are the main organs of detoxification and therefore the first target organ of toxic substances (xenobiotics). Thus these two bodies are very often affected in cases of toxicity.

The aqueous extract of leaves *H.asper* not result in significant changes in serum creatinine or creatinine in renal. Indeed the references values of creatinine are between 0.39-2.29 mg/dl[11]. Creatinine values obtained in this study were 0.72 mg/dl in the controls, 0.65;0.70; 0.72 and 1.01 mg/dl in treated with different doses animals, these different values are contained in the interval of reference. Therefore the observed increase in serum creatinine is not medically significant. Creatinine is a very useful molecule nephrology as it is an indicator of renal toxicity [12]. According to Haley and Bernard[13], an elevation in the rate of renal creatinine is an indication of kidney toxicity. During our test the animals treated with different doses have presented a non-significant decrease in renal creatinine levels compared to control animals.

The significant increase in serum protein is due to the amino acids contained in the food and causing an increase in protein synthesis given that plasma protein synthesis occurs in the liver [14]. Under normal conditions it is known that the rate of protein synthesis by the liver serum depends on the amino acid concentration in blood[15]. In case of increased plasma proteins able to refuel the blood amino acid total protein increases.

The activity of enzymes in the liver ALT decreases and increases in the blood while the enzyme activity of AST decreased significantly in the liver and significantly increased in the blood. This change reflects an impairment of liver cells because the evaluation of serum levels of ALT and AST enzymes is correlated with morphological damage to the liver[16]. Indeed the ALT and AST are the markers of hepatocyte lesion[17], are good indicators of liver function[18]. Levels are increased in the blood during the destruction of liver cells and this in all liver diseases (toxic or infectious). The liver damage was due to the activity of several oxidative hepatotoxic compounds contained in the extract given that the oxidizing activity of many substances is the main cause of liver poisoning[19]. These compounds would cause the reduction of the induction of tert-butyl hydroperoxide glutathione and lipid peroxidation thus contributing to cell damage [19].

At the end of the toxicological study it was found that acute treatment the aqueous extract of leaves of Hibiscus asper causes no deaths in treated rats up to a dose of 20g/kg. In subchronic toxicity the extract had very low toxic effects on body weight and heart weight. In view of all the foregoing Hibiscus asper leaves can be regarded as toxic and open field to the study of the pharmacological properties of the study or the analgesic properties of the extract.

Intraperitoneal injection of acetic acid causes pain characterized by abdominal contractions. This pain is associated in general has increased the rate of substances such as PGE2 in the peritoneal fluid and the release of mediators such as histamine and serotonin[6]. Local peritoneal receptors are solicited in this type of pain and are partly involved in the abdominal contraction[20]. The activity of the aqueous extract of leaves of Hibiscus asper significantly ($P<0.001$) inhibited the pain of 75.14% at a dose of 500 mg/kg. The aspirin dose of 20 mg / kg was also significantly ($P<0.001$) inhibited the pain of 65.32%. The analgesic effect of the extract on this model of pain could be due either to the inhibitory action of the extract on the nociceptive receptors sensitive to acid, or to the inhibition of the transmission of pain messages at the central level or to the inhibition of the production of algogenic. It is well known that the opioid the steroidal anti-inflammatory muscle relaxants antipyretics and antihistamines may inhibit this model of pain [21]. This shows that the acetic acid pain is a

nonspecific pain it is therefore difficult to give the mechanism of action of chemical compounds active in this model of pain.

In order to determine the mechanism of action of the extract the pain was induced by formalin and pressure. The formalin test is a valid and reliable model of nociception. The injection of formalin in the fascia of the plantar surface induced neurological intense and debuted immediately after injection of formalin pain and takes about 5 minutes; it probably would result from the activation of C-fibers with release of substance P or bradykinin[22] ; inflammatory pain which is moderate and begins at the 10th min and is ending at the 40th min this phase would depend has both a peripheral and central mechanism ; it is marked by the release of mediators such as substance P histamine serotonin PGE2 or bradykinin[23]. Therefore the formalin test helps to explain the possible mechanisms of action of the antinociceptive effect of the analgesic compounds[24]. Indeed peripheral analgesics such as aspirin indomethacin and paracetamol inhibit only the second phase of the pain while centrally acting substances such as narcotic analgesics or opioids inhibit indifferently both phases of this pain[25]. This extract tested on the pain provoked by the formalin pain that inhibits a very significant way ($P<0.001$) 35.64% and 86.21% in the first phase and the second phase respectively at a dose of 500mg/kg. This result shows that this extract has an effect similar to that of narcotic analgesics or opioids.This extract significantly inhibits the second phase of formalin-induced pain as well as suggesting the extract would possess peripheral analgesic activity. To verify that this peripheral analgesic activity is related or not inflammatory phenomenon this extract has been tested on pain induced by pressure.

Pain with pressure is a non-inflammatory peripheral pain in which the involvement of endogenous inflammatory mediators would be negligible[26]. The aqueous extract of the leaves of a *H.asper* significantly inhibited dose dependent manner and the pressure-induced pain. This allows to think that the active ingredients in this extract compounds possess effects on mecano-sensitive nociceptors. Aspirin does not present an analgesic activity in this model of pain. Although the central analgesic and peripherals inhibit contraction causes a number of chemical pain stimuli[27], only the peripheral analgesic reduce the sensitivity of animals in pain induced by pressure[28]. These results lead to confirm that the activity of the extract would be bound to a peripheral and central mechanism.

The antinociceptive activity of the aqueous extract of leaves of Hibiscus asper on different models of acute pain used in this study has led to evaluate the effects of the extract on chronic inflammatory pain. Indeed chronic pain differs from acute pain in terms of its persistence and compared to some adaptive changes such as neuroplasticity that has been described at various levels of the nervous system[29]. Chronic pain is refractory to most analgesic substances available.

To evaluate the properties of the aqueous extract of leaves of Hibiscus asper of chronic pain a model of chronic inflammatory pain (formalin) was used in this study. This model of persistent pain used in this study produces central sensitization in response to the production of several pro-inflammatory mediators which increase the sensitivity of peripheral and central sensory pathways[30]. The extract would possess significant anti hypernociceptive activity when administered in acute treatment this activity has duration until the 4th hour prolonged treatment the activity of the extract was significantly until day 7. During this painful process there has nociceptive mediators and production of proinflammatory cytokines[30]. The formalin used to induce this model of inflammatory pain produces inflammatory response that develops and persists in a few hours this response is due to activation of peripheral nociceptors[31], monitoring the release of various chemical mediators such as cytokines prostanoids serotonin glutamate nitric oxide histamine and immunological agents[32] that contribute to the initiation of inflammatory hyperalgesia[33]. The extract possesses significant activity in acute treatment antihypernociceptive (until the 4th time) and repeated treatment (up to 7th day). This result obtained leads us to think that antihypernociceptive activity of aqueous extract of leaves of Hibiscus as per be due to an inhibition of the inflammatory response. The effect of aqueous extract of leaves of Hibiscus as per in acute and repeated treatment in this model of chronic inflammatory pain could be tied to an interference of the extract in the mechanisms involved in the peripheral and/or central sensitization. All these analgesic effects are due to the presence of chemical compounds such as flavonoids and polyphenols in the aqueous extract[3].

Table 1: Behavioral manifestations of rats during the acute toxicity of the aqueous extract of leaves *Hibiscus asper*

DOSES (g/kg)	0	4	8	12	16	20
Total number of rats	8	8	8	8	8	8
Number of deaths	0	0	0	0	0	0
Mobility	N	N	N	D-	N	N
Sensitivity to pain	N	N	N	N	N	N
Sensitivity to noise	N	N	N	N	N	N
Aggressiveness	N	N	N	D-	N	N
States stool	G	G	G	G	G	G

N= normal ; D-= decreases slightly; G= granular.

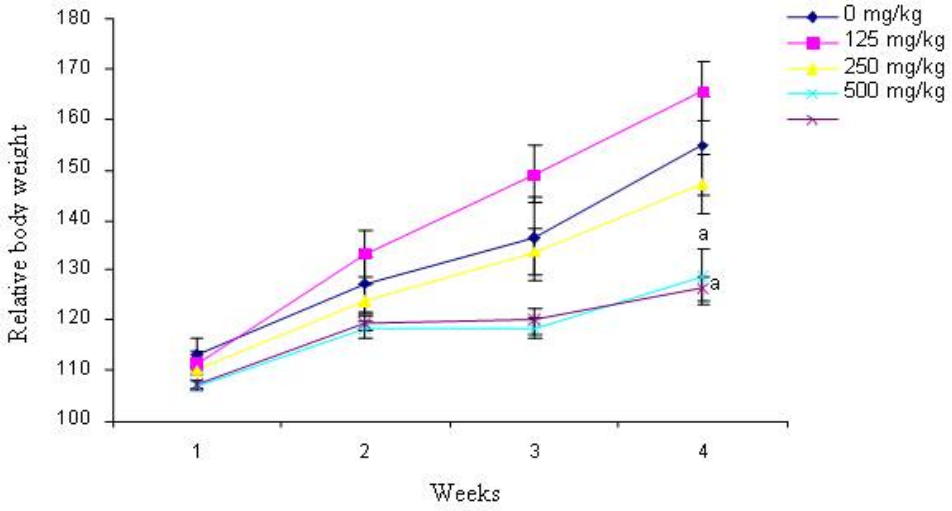

Fig. 1: Changes in body weight of rats as a function of doses of the aqueous extract of leaves of *Hibiscus asper* in acute toxicity.Each point represents the average of eight values ± SEM

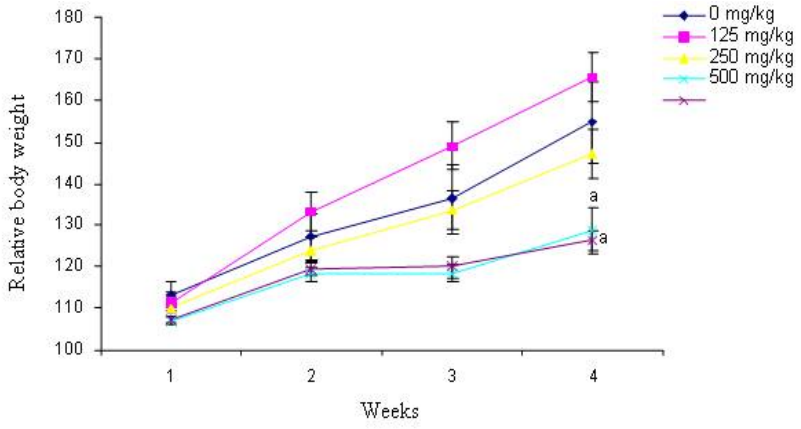

Fig. 2: Changes in body weight of rats as a function of doses of aqueous extract of *Hibiscus asper* leaves in sub-chronic toxicity. Each point represents the mean of eight values ± SEM. [a] P<0.05 ; statistically significant compared with the witness (0 mg/kg)

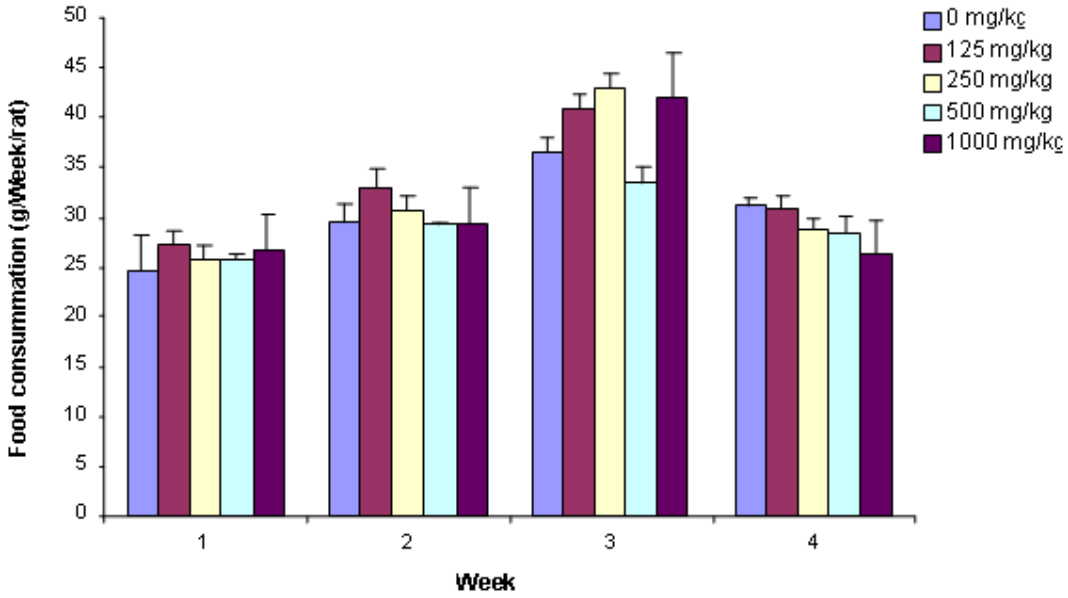

Fig. 3: Changes in food consumption according to the doses of aqueous extract of *Hibiscus asper* leaves in sub-chronic toxicity. Each bar represents the mean of eight values ± SEM

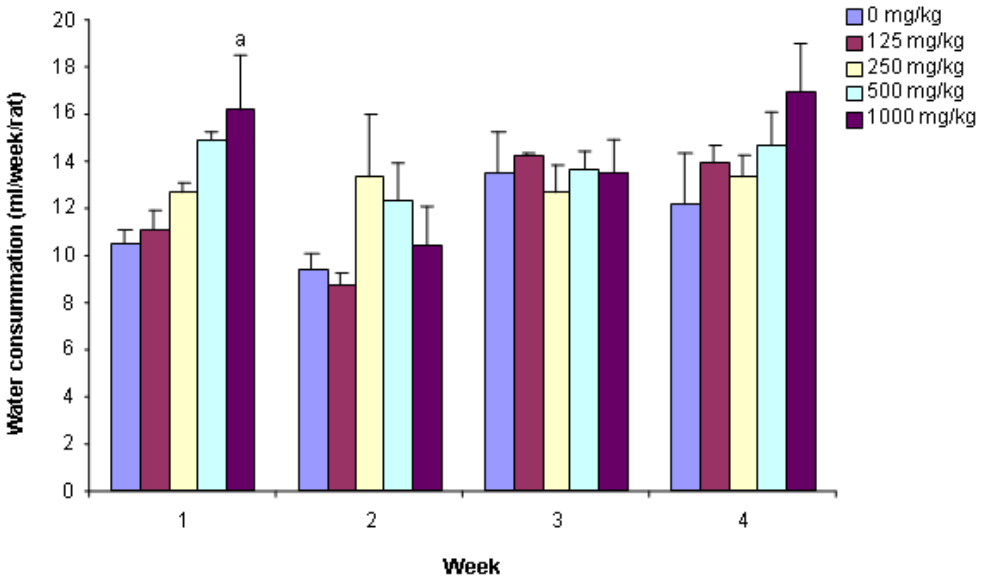

Fig. 4: Evolution of the water consumption based on aqueous extract doses of *Hibiscus asper* leaves in sub-chronic toxicity. Each bar represents the mean of eight values ± SEM. [a]p<0.05 ; significant difference compared to control group (0 mg/kg)

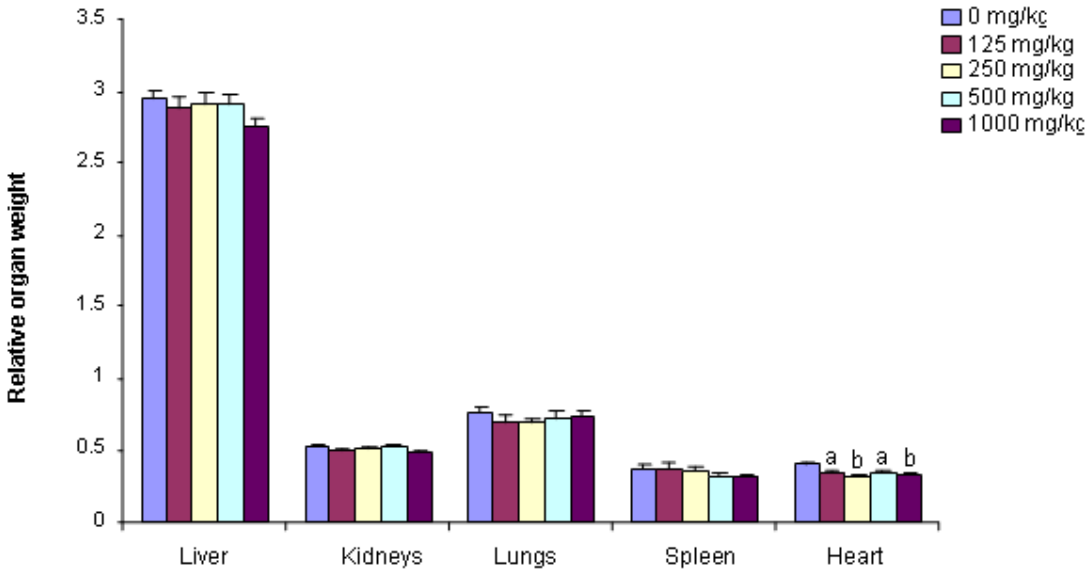

Fig. 5: Variation in organ weights based doses of aqueous extract of leaves of Hibiscus asper administered to rats in subchronic toxicity. Each bar represents the mean of eight values ± SEM. $^{a}P<0.05$;$^{b}P<0.01$; significant difference compared to control group (0 mg/kg)

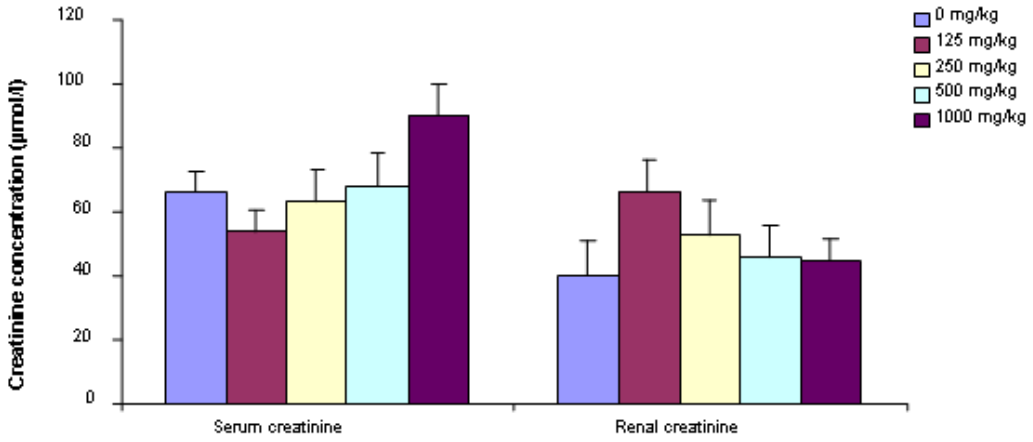

Fig. 6: Change in serum creatinine levels and kidney in treated rats at doses of leaf extract of *Hibiscus asper* in subchronic toxicity. Each bar represents the mean of eight values ± SEM

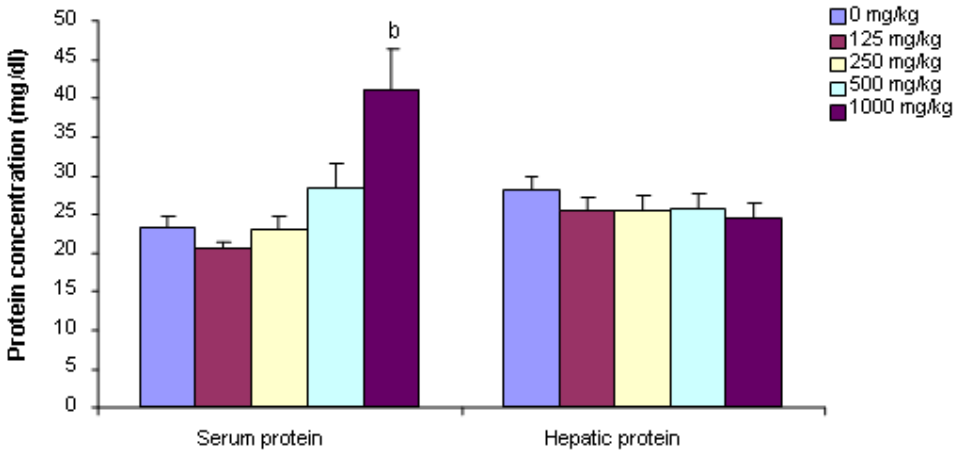

Fig. 7: Variation of serum protein and liver in rats treated with doses of aqueous extract of *Hibiscus asper* leaves in sub-chronic toxicity. Each bar represents the mean of eight values ± SEM. [b]P<0.01 ;significant difference compared to control group (0 mg/kg)

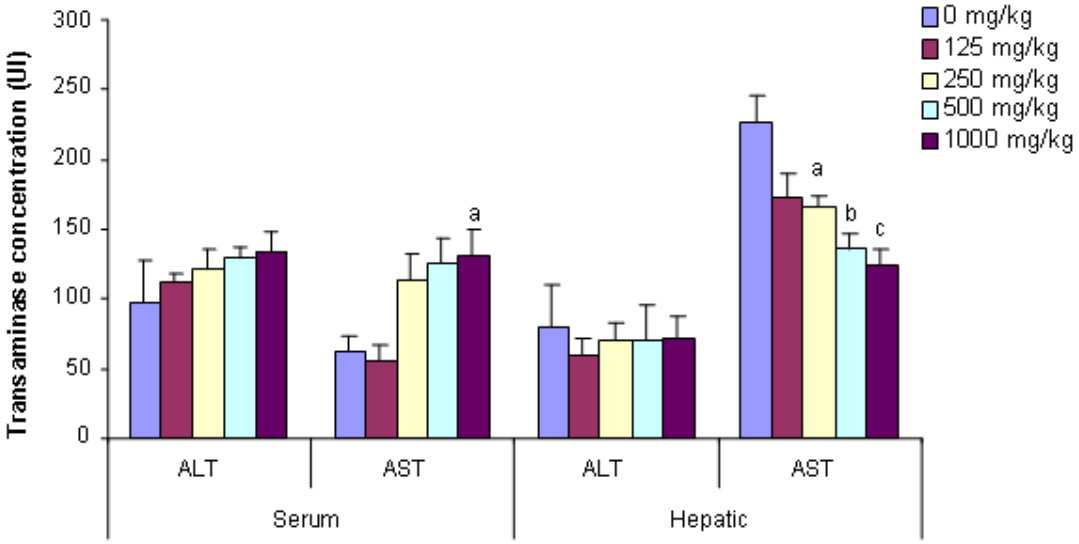

Fig. 8: Changes in serum levels and liver enzymes ALT (alanine amino transferase) and AST (aspartate amino transferase) in treated rats at doses of aqueous extract of Hibiscus asper leaves in sub-chronic toxicity.Each bar represents the mean of eight values ± SEM. [a]P<0.05 ;[b]P<0.01 and[c]P<0.001 ; significant difference compared to control group (0 mg/kg)

Table 2: Analgesic effects of the aqueous extract of leaves of *Hibiscus asper* on pain induced by intraperitoneal administration of acetic acid (1%)

GROUPS	DOSE (mg/kg)	NUMBER OF CONTRACTION	PERCENTAGE OF INHIBITION (%)
Distilled water	-	100.5 ± 2.88	-
Aspirin	**20**	19.5 ± 3.22[c]	65.32
Aqueous extract	**125**	27.17 ± 3.3[c]	35.60
	250	20.5 ± 3.4[c]	53.00
	500	12.5 ± 0.89[c]	75.14

Each value represents the mean ± SEM of 6 animals followed by the percentage of inhibition relative to the control group. [c]p< 0.001 statistically significant compared to control trafficking in distilled water.

Table 3: Analgesic effects of the aqueous extract of *Hibiscus asper* leaves on pain induced by subplantar injection of formalin (2.5%)

GROUPS	DOSE (mg/kg)	TIME LICKING (SECOND)		PERCENTAGE OF INHIBITION (%)	
		First phase	Second phase	First phase	Second phase
Distilledwater	-	89.5 ± 2.96	81.83 ± 4.5	_	_
Aspirin	20	64.33 ± 9.57	25.5 ± 3.38[c]	8,6	13.03
Aqueousextract	125	43.17 ± 10.52[c]	21.17 ± 3.34[c]	35,22	74.32
	250	32.67 ± 5.6[c]	18.33 ± 4.39[c]	35.44	77.40
	500	31.17 ± 3.2[c]	6 ± 2.3[c]	35.64	86.21

Each value represents the mean ± SEM of 6 animals followed by the percentage of inhibition relative to the control group.
[c]p< 0.001 statistically significant compared to control trafficking in distilled water.

Table 4: Time dependent effects of the aqueous extract of the leaves of *Hibiscus asper* on pain induced by pressure in the rat.

	Dose (mg/kg)	Sensitivity of animal (hour)					
		0 h	0.5 h	1 h	2 h	3 h	4 h
Distilled water	-	97.08 ± 9.14	111.25 ± 3.69	57.92 ± 8.86	66.17 ± 8.21	58.75 ± 8.70	42.08 ± 4.10
Aspirin	20	101.25 ± 21.44	112.50 ± 5.06 (20.61)	79.17 ± 11.30 (43.61)	60.33 ± 9.49 (- 32.38)	71.25 ± 13.61 (21.73)	47.50 ± 6.46 (2.27)
Extrait aqueux	125	112.92 ± 16.89	112.92 ± 11 06 (0)	102.50 ± 13.45[a] (73.39)	75.42 ± 8.65 (- 21.32)	84.08 ± 5.34 (24.76)	56.25 ± 8.47 (- 3.04)
	250	109.17 ± 15.26	130.42 ± 7.23 (- 49.96)	110.83 ± 9.44[b] (104.24)	114.08 ± 12.36[a] (115.88)	100.83 ± 15.71 (22.80)	84.08 ± 6.17[b] (54.38)
	500	108.33 ± 11.08	134.58 ± 14.60 (- 85.25)	118.33 ± 7.15[b] (125.54)	122.50 ± 9.01[b] (145.84)	111.67 ± 12.59[a] (108,71)	99.17 ± 6.57[c] (83.35)

Each value represents the mean ± SEM of 6 animals followed by the percentage of inhibition relative to the control group.
[a]p< 0.05, [b]p< 0.01, [c]p< 0.001 statistically significant compared to control trafficking in distilled water.

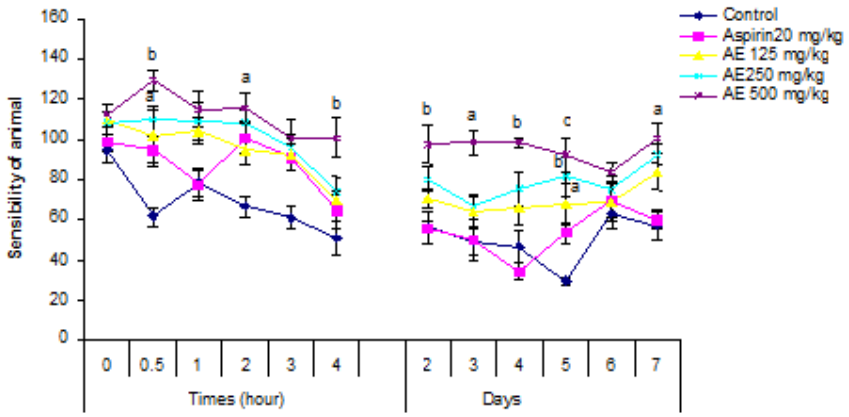

Fig. 9: Dependent time of oral administration of aqueous extract of the effect of *Hibiscus asper* leaves on mechanical hyperalgesia induced by analgesimeter. Each value represents the mean ± SEM of 6 animals followed by the percentage of inhibition relative to the control group. [a]p< 0.05, [b]p<0.01, [c]p< 0.001 statistically significant compared with the control

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