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ACUTE ORAL TOXICITY AND NEUROBEHAVIOURAL TOXICOLOGICAL EFFECTS OF METHANOLIC EXTRACT OF *CHRYSOPHYLLUM PERPULCHRUM* IN RATS

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Keywords:

Acute toxicity, Male rats, *Chrysophyllum perpulchrum*, Behavior, Biochemical and histological parameters

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ABSTRACT: This study aimed to investigate the acute toxicity of methanolic extract of *Chrysophyllum perpulchrum* on biochemical, behavioral and histopathological variation in male Wistar rats. Acute toxicity of the methanol extract of *Chrysophyllum perpulchrum* was carried out on 36 male rats using different doses of the extract ranging from 500 to 2000 mg/kg body weight. Behavioral tests were used to determine the effect of the extract on cognitive performance such as anxiety, memory, and locomotor activity. Biochemical analyses of (ALAT, ASAT, glycemia) were carried out on the plasma, while pathological changes in the kidneys, liver were examined histologically. Our results showed that the LD_{50} value indicates that the methanolic extract of the *C. perpulchrum* administered orally, is slightly toxic and can induce some slight behavioral disturbances related to anxiety whereas the learning and memory capacities remained unaffected. The acute oral treatment of rats with methanolic extract of *Chrysophyllum perpulchrum*, in general, did not induce significant modifications of the biochemical profile when compared to the control group. However, the histopathological evaluation indicated that the extract has some adverse effect on the morphology of the tissues in the liver and kidneys. In conclusion the methanolic extract of *Chrysophyllum perpulchrum* possesses a wide spectrum of CNS activity. Further studies are necessary to verify the potential effect of this plant to modulate the cognitive levels especially mood and anxiety based on the molecule content of this plant, dose and the method of administration.

INTRODUCTION: *Chrysophyllum perpulchrum* (Sapotaceae) is used in the traditional Ivory Coast pharmacopeia to cure fevers, a bark decoction have been used traditionally for the treatment of jaundice, asthma, and other respiratory complaints, and also madness [1].

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Other Authors have shown that *C. perpulchrum* extracts possess antioxidant and anti-diabetic activity [2, 3].

According to Philipe *et al.*, [5] the treatment of diabetic rats with total methanolic extract of *C. perpulchrum* corrects these disruptions and improves resistance against diabetes. This action is related to its chemical composition characterized by the presence of flavonoids and alkaloids [2]. Even though the stem bark of *C. perpulchrum* is well reputed for its therapeutic activity in folklore, there are few data on the dose-response, in part because

of the lack of identification of the specific compound associated with the toxic effects.

The toxicity of *C. perpulchrum* has been evaluated with some human bronchial epithelial cells. Philippe *et al*., [4] have performed the acute, subacute toxicity and cytotoxicity of *C. perpulchrum* in mice at doses ranged from 0 to 4000 mg/kg of body weight (bw) and he found that the maximal tolerated dose (MTD) which is 500 mg/kg of bw, 50% lethal dose of 1259 mg/kg of bw whatever the method used. *C. perpulchrum* is moderately toxic and would entail light damage of kidney. On the other hand, this substance is not toxic to the human bronchial epithelial cells. In another study, he reported that no evidence of toxicity was observed in rat receiving an aqueous extract of *C. perpulchrum* at dose levels 100, 2000 and 5000 mg/kg of body weight (bw) [5].

View the lack of data and also the ecological importance of this plant in the Ivorian pharmacopeia, and given the rarity of this plant which seems to have therapeutic virtues for human health. Toxicological data are essential in animals under various conditions to establish the safety and the efficacy level of the compounds of this plant which may help to decide whether a new drug is adopted for clinical use or note. This led to the present study where we investigated the acute toxicity of methanolic extract of *Chrysophyllum perpulchrum* on biochemical, behavioral and histo-pathological variation in male Wistar rats.

MATERIALS AND METHODS:

Animals: Thirty sex Adult Male Wistar rats, weighing between 153- 205 g, aged 3 months, were used and kept in the animal house of the Biological Department - Faculty of Sciences- Ibn Tofail University–Kénitra, Morocco. The animals were kept in plastic cages (430*290*210 mm) at an animal house in an air-conditioned environment with 3 to 4 in each cage and maintained at room temperature of (25 ± 2) °C . (60% ± 10%) under 12 h night and light cycle. Animals have free access to food (standard diet: standard pellet diet, Kawt - Temara Morocco) and water and acclimatized for at least a week before the commencement of the experiment. Animals were housed and handled according to The Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, USA 1996, the Moroccan Communities Council Directive for the Care and Use of Laboratory Animals is an ongoing project). The minimal number of animals was used to achieve statistical significance, and all efforts were made to minimize their suffering.

Preparation of Extract: Extraction of total polyphenols from *Chrysophyllum perpulchrum* is obtained by solid-liquid / liquid-liquid extraction and based on the solubility difference of polyphenols in organic solvents. The stem barks of the *Chrysophyllum perpulchrum* were sun-dried and crushed into powder using a mortar and Culatti micro-crusher. The powder obtained (50 g) was macerated in 300 mL of hexane during 48 h at room temperature and filtered using a Whatman Millipore filter. Then, the marc was solubilized in 1.5 L of methanol at 96%. The filtrate was lyophilized at -40 °C. The powder obtained was stored at 5 °C until further use.

Acute Toxicity Study: The oral acute toxicity study of methanolic extract of *Chrysophyllum perpulchrum* was evaluated according to Organization for Economic Cooperation and Development (OECD) guideline 423, where the limit test dose of 2000 mg/kg was used. All the animals were kept at overnight fasting before every experiment with free excess to water. The animals were divided into six groups, each comprising 6 animals. The 1st group served as a negative control, while 2nd, 3rd, 4th,5^{th,} and 6th was considered as tested groups received orally *Chrysophyllum perpulchrum* extract at the dose of 500 mg/kg, 600 mg/kg, 1125 mg/kg, 1500 mg/kg and 2000 mg/kg. Before dose administration, the body weight of each animal was determined, and the dose was calculated according to the body weight. The animals were observed for any toxic effect for the first 4 h after the treatment period. Further, animals were investigated for 3 days for any toxic effect. Behavioral changes and other parameters such as body weight, urinations, food intake, water intake, respiration, convulsion, tremor, temperature, constipations, changes in eye and skin colors, *etc*.

Behavioral Tests:

Open-Field Test (OF): To assess possible effects of plant extract on spontaneous locomotor activity and the ability to respond to a novel environment

rats were evaluated in the open-field test during 10 min. Apparatus consisted of topping open wooden bow (100 × 100 × 40) covered by white, consistent plastic. The floor of the arena was divided into 25 squares unit by black lines and lit in the center with halogen lamps of 60 W installed in the ceiling [6, 7]. The frequencies of line crossing with the four paws (locomotion) and several rearing in exploratory activity (anxiety level), were recorded by a video camera positioned above the OF.

Elevated Plus-Maze (EPM): To measure the degree of anxiety-related behavior, we use the elevated plus-maze. The apparatus is made of wood and consisted of two enclosed arm (29 × 5 × 15) and two open arms (29 × 2.5 × 15), placed at a right angle crossing in a common central platform (5 × 5). The central platform is illuminated with halogen lamps of 60 W offer rat an aversive condition spatial. Each animal is placed onto platform facing the open arm, and the following behaviors are recorded during 5 min. The time spent in each arm and the numbers of entries in the open and close arm are scored from a video sequence. The level anxiety of rat is assessed by the time spent on the open arm divided by total time, and the number of open-arm entries divided by a total number of arm entries [8].

Novel Object Recognition (NOR) Task: The apparatus and procedures for NOR training have been described elsewhere [9, 10]. The task took place in a 40 × 50 cm^2 open field surrounded by 50 cm high walls, made of plywood covered by black fine plastic layer. All animals were given a habituation session where they were left to freely explore the open field for 5 min. No objects were placed in the box during the habituation trial. Twenty-four hours after habituation. NOR training was conducted by placing individual rats for 5 min into the field. in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. In a long-term retention test given 24 h after training. The same rats explored the field for 5 min in the presence of a familiar object (A) and a novel object (B). A single set of three objects was used for all animals. All objects presented similar textures: colors and sizes, but distinctive shapes. The index of recognition memory was defined as the ratio of object B exploration number and the sum of object A and B exploration number. Between trials, the objects were washed with 10% ethanol solution. Exploration of an object was defined as directing the nose to the object at a distance # 1 cm and touching it with the nose; conversely, turning around or sitting on the object was not considered as exploratory behavior. NOR procedures were conducted in the presence of luminescent source (60 W) from 1 m in the ceiling.

Preparation of Biological Samples: At the end of the experiment (D19), the animals are weighed and sacrificed by decapitation after chloral anesthesia (0.5 ml per 100 g body weight) after 12 h of fasting. The blood is immediately collected in dry tubes for biochemical tests (transaminases ASAT and ALAT, glycemia). The organs (brain, liver, heart, spleen, stomach, lungs, kidneys, adrenal glands and testes) are carefully removed and weighed and then stored in paraformal aldehyde.

Effect of Plant Extract on Serum Biochemical Parameters: The biochemical analysis was done on plasma after centrifugation of collected blood and the following parameters like aspartate transaminase (AST), alanine transaminase (ALT). Glycemia was determined for both control and extract treated groups. All analyses were determined on using Biosystem BTS 310 photo-colorimeter and standard biosystem reagents (Biolab, Casablanca, Morocco).

Histological Examination: Samples of kidneys and liver were fixed in 10% buffered formalin. embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin and examined under light microscopy. The histological analysis aimed to assess the tissue integrity of the organs. The parameters analyzed were: degeneration, necrosis, apoptosis, leukocyte infiltration…

Statistical Analyzes: Data obtained was expressed as Mean ± SEM. To evaluate the differences between control and treated groups, the one-way ANOVA and the repeated one-way ANOVA parametric test were used. A p-value of less than 0.05 were considered to reflect a statistically significant difference.

RESULTS:

Assessment of Acute Toxicity: After feeding the plant extract *Chrysophyllum perpulchrum* at different doses (500 mg/kg, 600 mg/kg, 1125

mg/kg, 1500 mg/kg and 2000 mg/kg body weight). The clinical signs of toxicity presented in the rats after 5 min are: the acceleration of the respiratory rhythm, decrease of the locomotor activity, straightening of their hair, and sometimes nosebleed was observed (epistaxis). A dose-lethality curve, representing percentages of mortality relative to the logarithms of doses, is plotted for the extracts tested **Fig. 1**. Our results showed that the LD_{50} is therefore estimated at 1500 mg/kg.

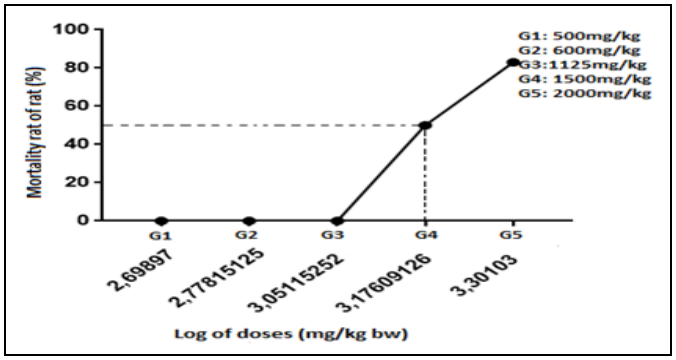

FIG. 1: MORTALITY RATE OF RAT AS A FUNCTION OF LEVELS OF TOTAL METHANOLIC EXTRACT OF *CHRYSOPHYLLUM PERPULCHRUM*

Effect of the Plant on the Variation of the Body Weight, Food Intake, Water Consumption and Rectal Temperature: All rats gained weight during the study, but the weight gains of rats in the G6 (2000 mg/kg) were significantly higher (38.45%) than those of the control animals ($p<0.05$) **Table 1**.

Dietary intake was low in the all studied groups from 500 mg/kg to 2000 mg/kg groups; this decrease is respectively 84%, 44%, 43%, 48%, and 77% ($p<0.05$). Also. This consumption increased at the end of the experiment to reach 100%. However, no significant variation was noted in the other groups compared to the control group at the end of the experiment ($p>0.05$) **Table 1**.

Similarly to food intake, we found that all the groups receiving the extracts of the plant at different doses consume at the starting time more water compared to the control group notably the group of 500 mg/kg, 1125 mg/kg and 1500 mg/kg whereas the group of 600 mg/kg and 2000 mg/kg consumes less water than control. These two groups showed an elevated consumption at the end of the experiment compared to the beginning and compared to the control group. However, we didn't show any changes in water consumption between all the treated groups ($p>0.05$) **Table 1**.

There was a decrease in rectal temperature in all groups receiving the plant extract at different doses compared to the control group at the end of the experiment. However, this decrease was statistically indifferent in **Table 1**.

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF *CHRYSOPHYLLUM PERPULCHRUM* ON BODY WEIGHT, FOOD CONSUMPTION, WATER INTAKE, AND RECTAL TEMPERATURE

		Control group	G2: 500 mg/kg	G3: 600 mg/kg	G4: 1125 mg/kg	G5: 1500 mg/k g	G6: 2000 mg/kg
body weight (g)	Initial	181.27 ± 3.33	190.03 ± 3.4	176.83 ± 2.8	176.1 ± 2.8	183.90 ± 3.37	185.61 ± 3.41
	Final	199 ± 3.65^{a}	191.71 ± 3.5^{a}	201.76 ± 3.7^{a}	202.34 ± 3.7^{a}	198.97 ± 3.65^{a}	224.06 ± 4.11^{b}
	Gain (%)	17.73	1.68	24.93	26.24	15.07	38.45
Rectal temperature (°C)	Initial	35.5 ± 0.07	35.6 ± 0.08	35.2 ± 0.05	35.4 ± 0.13	35.43±0.16	35.03 ± 0.04
	Final	35 ± 0.065	34.2 ± 0.18	33.5 ± 0.13	34.02 ±0.6	33.75 ± 0.43	33.6 ± 0.01
Food intake (g)	Initial	100 ± 0.0^{a}	17 ± 0.02^{b}	56 ± 0.01^{c}	57 ± 0.02^{c}	52 ± 0.156^{c}	23 ± 0.08^{b}
	Final	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Water intake (ml)	Initial	17 ± 1.01^{a}	30 ± 1.74^{b}	3 ± 1.45^{c}	36 ± 2.15^{b}	38 ± 1.31^{b}	10 ± 0.27^{a}
	Final	40 ± 0.17	39 ± 0.014	33 ± 0.23	58 ± 0.13	43 ± 0.670	35±0.09

Values are presented as Means ± SEM. n = 6 in each group. Values that do not share the same letter(s) (a, b, c) are significantly ($p<0.05$) different from each other

Effects of Acute Toxicity of *Chrysophyllum perpulchrum* on Cognitive Behavioral Performance: Locomotor Activity: The oral administration of plant extract into rats, showed a significant decrease in the number of crossed squares observed in groups G2 (500 mg/kg) and significant increase of total visited squares in group G5 (1500 mg/kg) compared to control rats ($p<0.05$). However, no significant difference between the control group and the remaining groups (600. 1125 and 2000) was recorded ($p>0.05$) **Fig. 2.**

Anxiety: The frequency of entries into the open arm is significantly altered in treated groups with methanolic extract of *Chrysophyllum perpulchrum*

(p<0.05) when compared to control **Fig. 3A**. We noted that group G2 (500 mg/kg) and group G6 (2000 mg/kg) visit less frequently the open arm, those of the group G3 (600 mg/kg), group G4 (1125 mg/kg) and group G5 (1500 mg/kg) showed a significant increase in the total number of entries into open arm compared with other treated groups (p<0.05) but without any differences with control male rat. Moreover, the rats' behavior in EPM showed that plant extract exposure at both doses (500 and 2000 mg/kg) exhibited anxiety-like response **Fig. 3B** represented by the reduced time spent in the open arms and increased time spent in the enclosed arm (p<0.05).

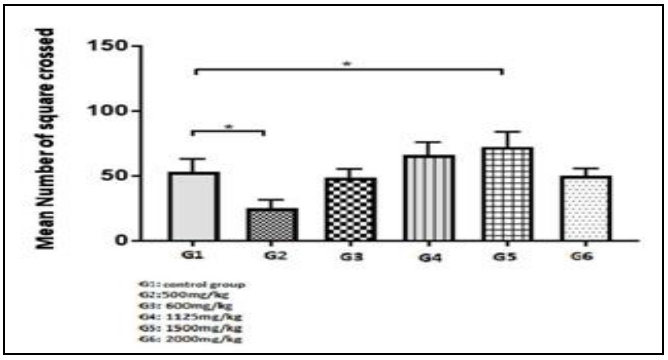

FIG. 2: EFFECT OF *CHRYSOPHYLLUM PERPULCHRUM* ON THE TOTAL NUMBER OF TILES VISITED. The results are expressed as mean ± SEM. * Significant difference at the 0.05 level of significance

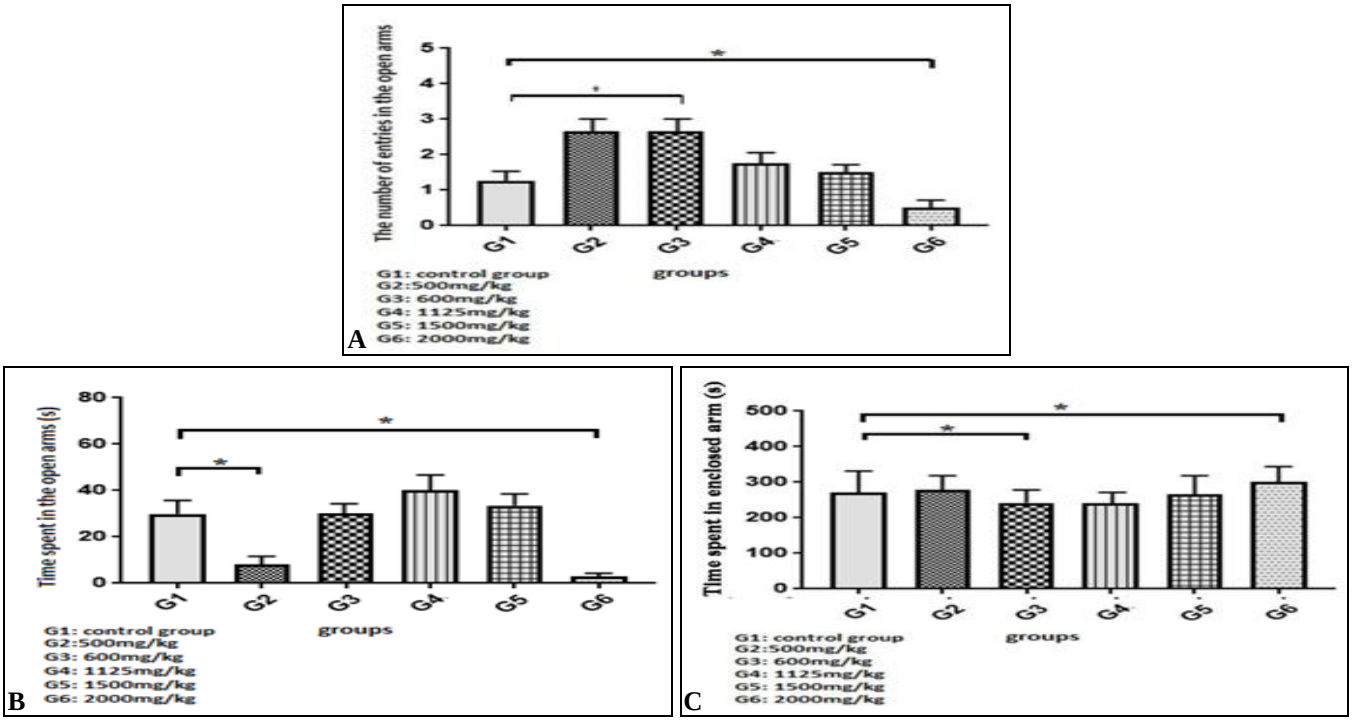

FIG. 3: EFFECT OF *CHRYSOPHYLLUM PERPULCHRUM* ON LEVELS OF ANXIETY ASSESSED BY THE ELEVATED ARM TEST (EPM), A: NUMBER OF INPUTS IN THE OPEN ARMS B: TIME SPENT IN THE OPEN ARMS, C: TIME SPENT IN THE ENCLOSED ARMS. The results are expressed in Mean ± Standard Mean Error (SEM). * Significant difference at the 0.05 significance level

Object Recognition Memory:

Short-Term Memory (STM): During the retention session, all rats were able to differentiate between the two objects (the familiar and the new). Indeed all the rats explore the new object more. However, there is no significant difference in the time of exploration of the new between the different groups tested in comparison with the control group (p>0.05) **Fig. 4A**. Similarly, the object recognition index does not seem to be altered by the treatment (p>0.05). Indeed, control rats and treated rats explore the same manner the new object during the retention session **Fig. 4B**.

Long-Term Memory (LTM): Our results showed that all rats explore more the new object during the retention session than the old one (after 24 h). The exploration time was significantly higher in groups 3, 4 and 6 (600, 1125 and 2000 mg/kg) compared to the control group (p<0.05). However, this increase remains without apparent significance in group 5 (1500 mg/kg) compared to the control (p>0.05). Nevertheless. A significant decrease in the exploration time of the new object was noted in group 2 (500 mg/kg) compared to the control one (p<0.05) **Fig. 4C**.

In STM phase, the mean of RI (Recognition Index) is above the threshold of object recognition (50%) in the control group. However, all treated groups display similar performance on object exploration time. During LTM recognition testing, rats of G2 group (500 mg/kg) have reduced their RI compared to that performed in the STM phase. In contrast, the RI remains sensibly unchanged in rats of different groups 2, 3, 4 and 5 (600, 1125, 1500 and 2000 mg/kg), from STM to LTM session **Fig. 4B**, **4D**.

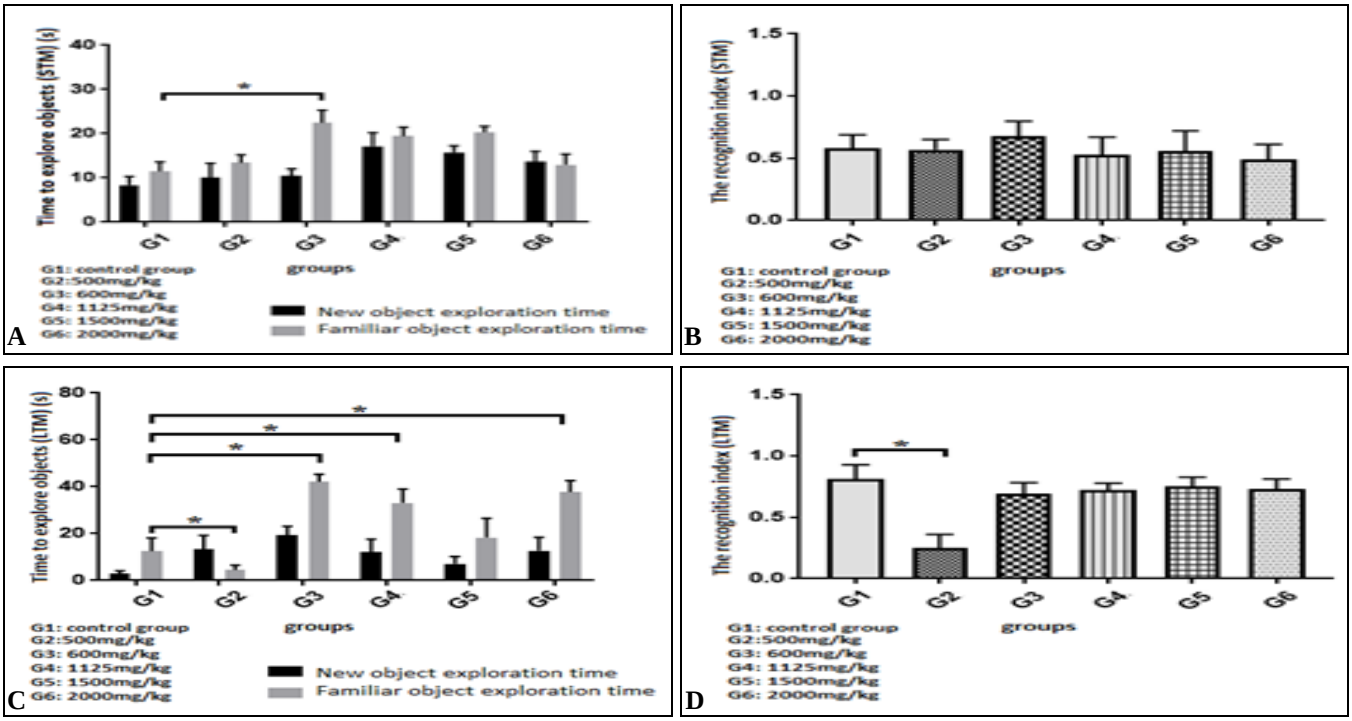

FIG. 4: EFFECT OF METHANOLIC EXTRACT OF *CHRYSOPHYLLUM PERPULCHRUM* ON. A: THE TIME OF EXPLORATION OF THE OBJECTS OF THE SHORT-TERM MEMORY (STM); B: THE OBJECT RECOGNITION INDEX IN (STM), C: OBJECT SCANNING TIME FOR LONG-TERM MEMORY (LTM), D: THE OBJECT RECOGNITION INDEX FOR LONG-TERM MEMORY (LTM). The results are expressed as mean ± SEM. * significant difference at 0.05 significance level

Relative Weights of Different Organs: The effect plant tested extract on principal organ weights relative to body weight are shown in **Table 2**. Changes in organ weight were observed in liver, kidney, lung, stomach, and testes of treated groups. Significant increases were noted in liver's groups of 500 mg/kg, 600 mg/kg and 2000 mg/kg ($p<0.05$), stomach's groups of 1125 mg/kg 1500 mg/kg and 2000 mg/kg ($p<0.05$) and lung's group of 500 mg/kg and 600 mg/kg ($p<0.05$) compared to controls. A decrease in kidney's weight was observed in all treated groups against controls. The extracted plant did not induce significant changes to the relative weight of the following organs: spleen, brain, and heart.

TABLE 2: EFFECT OF ORAL ADMINSTRATION OF METHANOLIC EXTRACT OF *CHRYSOPHYLLUM PERPULCHRUM* ON RELATIVE ORAGN WEIGHT (g) OF RATS

Organ	Control	500 mg/kg	600 mg/kg	1125 mg/kg	1500 mg/kg	2000 mg/kg
Brain	1.76 ± 0.03	1.82 ± 0.03	1.81 ± 0.03	1.87 ± 0.03	1.68 ± 0.03	1.85 ± 0.03
Heart	0.86 ± 0.02	0.77 ± 0.01	0.86 ± 0.02	0.81 ± 0.01	0.81 ± 0.01	0.93 ± 0.02
Liver	6.62 ± 0.14 [a]	8.02 ± 0.15 [b]	7.21 ± 0.13 [b]	6.69 ± 0.12[a]	6.45 ± 0.12[a]	8 ± 0.146 [b]
Kidney	0.91 ± 0.02	0.86 ± 0.02	0.70 ± 0.01	0.70 ± 0.01	0.83 ± 0.02	0.86 ± 0.02
Spleen	0.62 ± 0.01	0.63 ± 0.01	0.74 ± 0.01	0.47 ± 0.01	0.62 ± 0.01	0.70 ± 0.01
Lung	1.97 ± 0.04 [a]	2.7 ± 0.05[b]	2.21 ± 0.04[b]	1.54 ± 0.03[a]	1.73 ± 0.03[a]	1.78 ± 0.03[a]
stomach	5.08 ± 0.09[a]	3.86 ± 0.07[c]	3.13 ± 0.06[c]	6.51 ± 0.11[b]	7.05 ± 0.13[b]	8.63 ± 0.16[b]
Testes	2.12 ± 0.04 [a]	2.01 ± 0.04 [a]	2.03 ± 0.04 [a]	2.44 ± 0.04 [a]	2.29 ± 0.04 [a]	1.73 ± 0.03 [b]

Values are presented as Means ± SEM, n = 6 in each group. Values that do not share the same letter(s) (a, b, c) are significantly ($p<0.05$) different from each other.

Biochemical Parameters:

Transaminase ASAT and ALAT: The acute oral treatment of rats with methanolic extract of *Chrysophyllum perpulchrum*, in general, did not induce significant modifications of the biochemical profile when compared to the control group. However, a decrease in AST and ALT serum blood levels were observed in the animals that received the three doses (600, 1125, 1500 or 2000 mg/kg) in comparison to the control group **Fig. 5A**, **5B**.

Glycemia: Blood glucose levels (mg/dl) in the treated groups are shown in **Fig. 5C**, the result showed significant increases in glucose levels of rats receiving the plant extracts compared to the control group.

These increase reached successively 54.90%, 49.80%, 55.29%, 41.18% and 47.65% in groups receiving 500, 600, 1125, 1500 and 2000 mg/kg compared to control one.

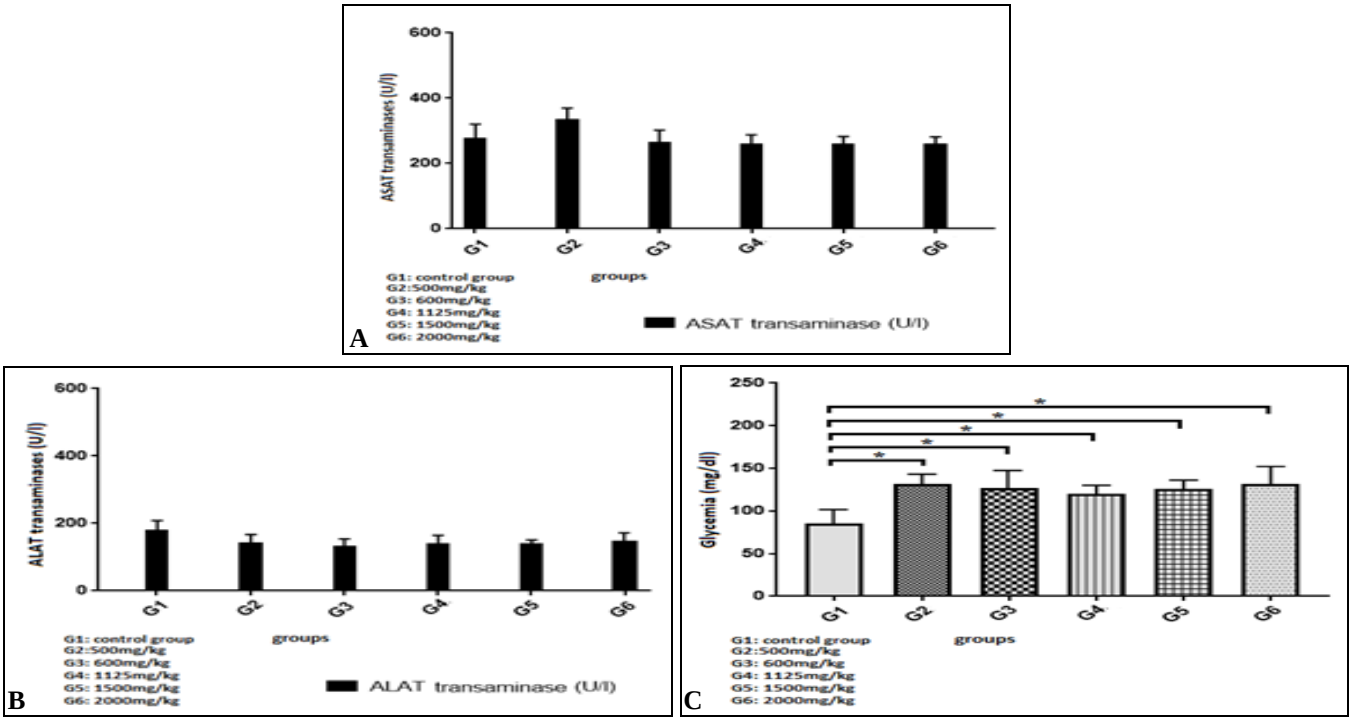

FIG. 5: EFFECT OF *CHRYSOPHYLLUM PERPULCHRUM* ON BIOCHEMICAL PARAMETERS. A: ACTIVITY OF THE ALAT; B: ASAT ACTIVITY AND C: GLUCOSE. The results are expressed in Mean ± SEM. * significant difference at 0.05 significance level

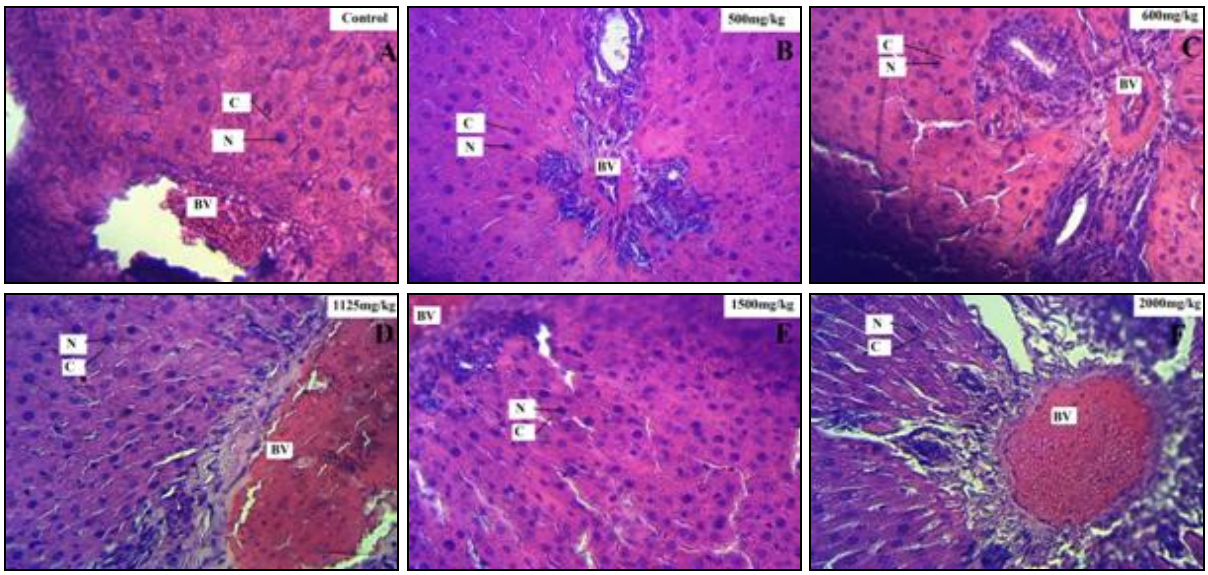

FIG. 6: HISTOLOGICAL SECTIONS OF HEPATIC TISSUE IN CONTROL (A) AND EXPERIMENTAL GROUPS (B, C, D, E, AND F); 40X10; STAINING: HEMATOXYLIN-EOSIN. C: HEPATOCYTE, N: CORE; VS: BLOOD VESSEL (CENTRILOBULAR VEIN)

Histological Examination:

Liver: The histological sections of the liver of control rats showed normal hepatocyte plates. The examination of liver tissue of treated rats, stained with hematoxylin and eosin, revealed centro-lobular necrosis inflammatory cells, and sinusoidal stasis in the liver parenchyma. The hepatocytes showed a clarified appearance with irregularly shaped and enlarged nuclei; the hepatic sinusoids were unevenly dilated and distributed in the hepatic lobule; the portal tructs were enlarged and filled with red blood cells **Fig. 6**.

Kidney: The kidneys of the control animals presented normal aspect. The histological analysis of kidneys of rats receiving different doses of *Chrysophyllum perpulchrum* ranging from 500 to 2000 mg/kg, revealed that these doses induced tubular degeneration without glomerular and the glomerular presented congestion and a slight inflammation and renal tubules dilation with eosinophilic deposition into the tubule lumen {presence of vascular fibrosis, glomerular fibrosis (or glomerulosclerosis)} **Fig. 7**.

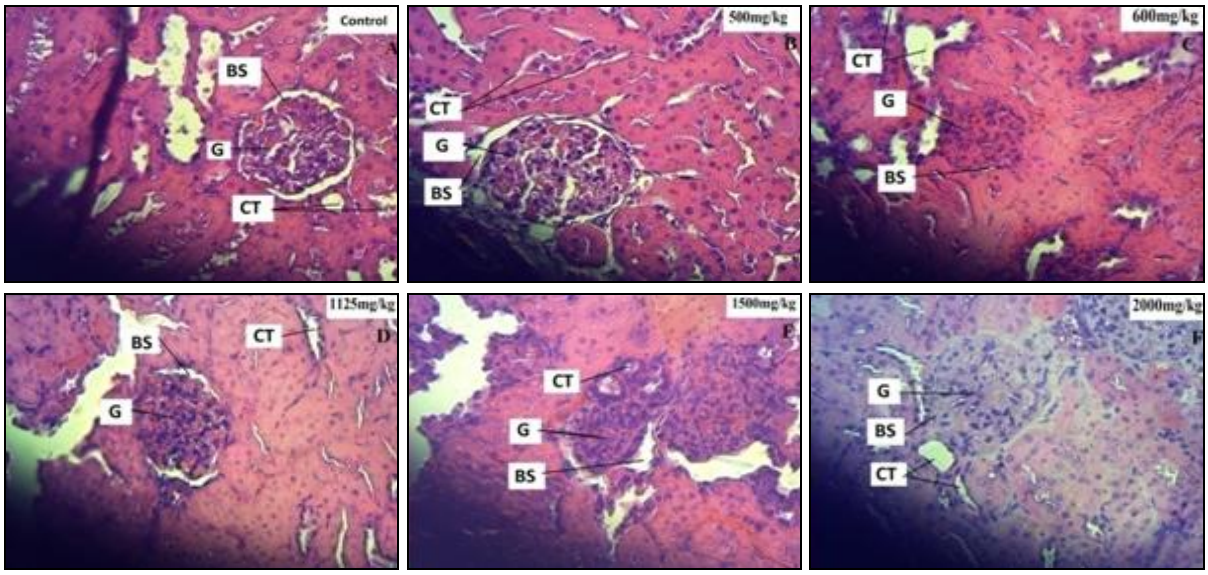

FIG. 7: HISTOLOGICAL SECTIONS OF RENAL TISSUE IN CONTROL (A) AND EXPERIMENTAL (B, C, D, E, AND F) RATS; 40X10; STAINING: HEMATOXYLIN-EOSIN. G: GLOMERULUS, EB: BOWMAN'S SPACE; TC: THE COLLECTING TUBES

DISCUSSION: In our study, the acute toxicity results showed that increased doses of methanolic extract of *C. perpulchrum* led to increased mortality in the tested rats. Therefore, there is a dose-response relationship between the dose administered and the rate of mortality recorded [11]. Also, the LD_{50} value is estimated to 1500 mg/kg of body weight (b.w); According to the Hodge and Sternercette scale, the LD_{50} value indicates that the methanolic extract of the *C. perpulchrum* plant, administered orally, is slightly toxic [12]. The clinical signs of toxicity presented in the rats after 5 min are: the acceleration of the respiratory rhythm, decrease of the locomotor activity, straightening of their hair, and sometimes nosebleed was observed (epistaxis). In accordance to our study; Philippe *et al.*, 2011 [5] showed that the administration of *C. perpulchrum* extract in mice also causes an acceleration of the respiratory rate, tremor, and immobilization of the animal.

Regarding to relative weight of organs, it has been observed that there is a significant increase in liver and stomach weight, and decrease testes weight; at macroscopic levels we noticed the presence of whitish nodular lesions in the liver; however all organs remain without apparent variations especially: the heart, the brain, the lung, the kidneys, and the spleen.

All these observations were recovered gradually in survived rats and can be explained in the fact that product accumulates in the body and its elimination is difficult, resulting in a longer recovery time [13]. The return to normal of the experimental rats after a few days, compared to the controls, is the proof of a plausible elimination by the urinary system of the degradation metabolites of this extract. The persistence of toxicity symptoms indicates that *C. perpulchrum* biotransformation is slow, resulting in damages to the organs and tissues.

This suggests that the variations noted in food intake, water and body may be the result of a modification of certain biochemical and tissue parameters due to their inflammation following the administration of the methanolic extract.

The acute oral treatment of male rats with methanolic extract of *Chrysophyllum perpulchrum*, in general, did not induce significant modifications of the biochemical profile when compared to the control group. However, a decrease in AST and ALT serum blood levels were observed in the animals that received the four doses (600, 1125, 1500 and 2000 mg/kg bw mg/kg) in comparison to the control group **Fig. 5A, B**. ALT is a more specific marker of liver cell damage, because it occurs more frequently in the liver while AST is also found in heart, skeletal muscle, kidneys, brain, pancreas and blood cells [14]. In the liver, ALT is confined to cytoplasm, while AST is found in both mitochondria (80% of total intracellular enzyme) and cytoplasm (20%) [15]. In patients with kidney failure, the AST and ALT are significantly lower than in healthy individuals, due to decreased free pyridoxal-5-phosphate, which reduces the enzymatic activity [16]. Changes in these enzymatic levels may suggest liver disease too, but the absence of these changes does not exclude the occurrence of a clinical disorder, as it is the case of the advanced stages of chronic hepatitis C with normal ALT levels [17]. In agreement with our study Philippe *et al*., 2011 [4] who showed that there is no significant variation in the level of enzymes (ASAT, ALAT, ALP, CPK, and LDH) in the blood serum of experimental rats compared to controls. This suggests that the administration of *C. perpulchrum* causes no change in the heart and liver in terms of activity.

Nevertheless, the same team observed an increase in urea, uric acid and creatinine in the blood serum. This suggests that the kidneys have been damaged by a decline in renal filtration [5]. However, the results obtained from the glycemic determination, our results showed significant increases in blood glucose level of treated rats compared to the control group; suggesting a hyperglycemic effect in these cases of the plant extract.

The histopathological evaluation indicated that the extract has some adverse effect onthe morphology of the tissues and these observations supported the biochemical results mentioned above. Therefore, it is concluded that *Chrysophyllum perpulchrum* can produce some toxic effect on male albinos rats.

The locomotor activity is considered as an index of alertness, and a decrease in it is indicative of sedative activity [18]. In our study the total number of squares crossed by different treated animals was different depending on the administered dose, we showed there was a significant decrease in the number of crossed squares observed in groups G2 (500 mg/kg) and significant increase of total visited squares in group G5 (1500 mg/kg) compared to control rats ($p<0.05$). However, none of the doses (600, 1125 and 2000 mg/kg) of *C. perpulchrum* extract were found to have any effect on the locomotor activity. Moreover, the lack of effect on locomotor activity works to the advantage of the plant demonstrating nootropic activity.

We used EPM to confirm deficits in emotional responsiveness evidenced in OF test. The elevated plus-maze (EPM) is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (fear of a novel open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans [18]. An anxiolytic agent increases the time spent in open arms and decreases the time spent in the enclosed arm of the EPM. In the present study, oral administration of *Chrysophyllum perpulchrum* extract demonstrated at the same time an anxiolytic-like effect, as it significantly increased the time spent in open arms and decreased the time spent in closed arms and also an anxiogenic-like effect in the rat (more entries into enclosed arms than open arms).

At our knowledge there is no data about the effect of this plant on anxiety, but we suggest the capacity of this plant to modulate the level of anxiety, depending on the molecule, dose and the method of administration, more studies are needed to prove the positive/negative effect of the plant on mood and anxiety (or vice versa).

Regarding learning and memory performances, no behavioral modifications were observed in treated rats compared to controls, suggesting the inability of methanolic extract of C. *perpulchrum* to impair

the cognitive functions after 14 days of exposure. Therefore, the absence of learning disturbances observed may be related to multiple factors including both variations in molecular structure, ways of metabolism, toxicokinetic characteristics, brain targets of interactions, way of administration and levels of exposure. Whereas the chemical composition of the plant is characterized by the presence of flavonoids with has similar activity to that of quercetin [2], it has been demonstrated to modify several functional endpoints in the brain including neuronal excitability, oxidative stress, and several neurotransmitters [20, 21, 22].

It has been established that flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in Alzheimer's disease model mice [23, 24, 25]. This suggests the use of the extract in improving the performance in competition and to promote poor learners. However, little is known about the brain cellular and molecular toxicity of *Chrysophyllum perpulchrum*, requiring the testing of the effects of this compound**.**

CONCLUSION: In this work, the LD_{50} value indicates that the methanolic extract of the *C. perpulchrum* plant, administered orally, is slightly toxic and can induce some slight behavioral disturbances related to anxiety whereas the learning and memory capacities remained unaffected. These effects were studied in several neuropharmacological models provided evidence that the methanolic extract of *Chrysophyllum perpulchrum* possesses a wide spectrum of CNS activity.

In the end, it will be necessary to carry out the specific binding studies on the pharmacological mechanisms that might account for the observed anxiogenic effects and neurotransmitters modulation in the brain and to verify the capacity of this plant to modulate the level of anxiety, depending on the molecule, dose and the method of administration.

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