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Biochemical, Haematological and Histopathological Changes caused by Senna obtusifolia (L) in Albino Rats

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Abstract: **This study held to determine the appetizing property of aqueous extract of fermented leaves of Senna obtusifolia when eaten by albino Rats, and also to evaluate their effects on liver, renal and hematological functions and the possible pathological effects on the Rats organs. Materials and methods: Plant leaves were powdered. 20 albino male rats weighed 90-125g were chosen and split to four groups: three test. Which fed on the plant in different doses, and fourth was a control group. Blood and tissue samples were taken to measure the effects of Senna obtusifolia. Internal organs were weighed between test and control groups for comparison. Results: There was an obvious significant gain in weight at ($p>0.05$) compared to control. The total protein in range in tested Rats was in normal range, WBCs were increased significantly, while there was an insignificant reduction on RBCs and haemoglobin. The renal and liver functions and gross histology appeared normal under the microscope. Conclusion: *Senna obtusifolia* succeded in increasing the weight of alino Rats, and caused no significant undesired effects in renal and liver functions.**

Keywords: **Senna obtusifolia, weight gain, albino Rats, Liver function, renal function.**

1. INTRODUCTION

Senna obtusifolia (L) (Chinese Senna, American Senna Sicklepod or Sicklepod) is a legume in the genus Senna, store line separated in the monotypic genus. The plant taxonomy of this genus is as follows: Kingdom Plantae; Order Fabales; Family Fabacae; Subfamily Caesalpiniodeae; Tribe Cassieae; Genus Senna; Species Obtusifolia, hence the binomial name is *Senna obtusifolia* L H .S –Irwin & Barneby [1], [2]. The green leaves of the plant are fermented to produce a high protein food product called locally in Sudan: (Alkawal) which is eaten by many people in Sudan as meat substitute [3]. It is leaves, seed, and root are also used in folk medicine.

Fermented Leaves of *Senna obtusifolia* (L) (Alkawal) are collected and grinded and buried in wetted place in apices of cloth underground for week to be fermented and then dried in sun light to become black color and bitter taste and unpleasant odor. They are saved in small ball shapes of approximately 5×5cm and powdered when needed. It's mainly used locally as a medicine for GIT upset, and also as a food appetizer.

This study objective is to determine the appetizing property of aqueous extract of fermented leaves of *Senna obtusifolia* (L) Alkawal when eaten by albino Rats (effects on food and water consumption and body weight on albino rats), also to evaluate the effect of *Senna Obtusifolia* (L) (Alkawal) on liver, renal and hematological functions and finally the histopathological effects on Liver and kidneys of albino Rats.

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Figure 1: *Senna obtusifolia* leaves.

2. MATRIALS AND METHODS

Plant collection and preparation of fermented leaves extracts:

Senna obtusifolia(L) (Alkawal) leaves were harvested from the plant that growing widely in Gezira scheme, then authenticated by Yahya .M .Adam at Medical and Aromatic plants institute where herbarium sample was deposited Leaves were kept in house and washed by water and they were kept away from direct sunlight underground for a week at least and put in ball shape and dried by direct sun light and then powdered.

Determination of body weights and food and water consumption:

The animals used in this experiment were 20 albino male rats (90-125g) the animals were kept at the pharmacology research lab at faculty of pharmacy, Omdurman Islamic university at room temperature. They were kept in rat cages and fed on commercial rat's food (meat, wheat flour, salt, and water) and water ad lipidium. The animals then allotted to four groups of five animals each. Group 1recieved oral dose of 1g/B.W of fermented Kawal leaves was re suspended in 5ml distilled water, Group 2 administered with 1.5g/B.W, Group 3 received 2g/B.W, while Group 4 was administered 5ml of water and served as control . The dosing of animal was continued for 14 days and body weight, and food and water consumption were recorded on daily bases until the end of experiment

Collection of blood samples and tissues:

At day 14 all animals were sacrificed and adequate amount of blood samples were collected by cervical decapitation from diethyl ether anesthesized rats into heparinized containers for hematological tests, other Blood samples was kept in clean Non- heparinized bottles for serum separation for liver and renal function tests, in addition the liver, kidneys, lungs, spleen, and heart were taken, weighted and kept in formalin 14% for histopathology. The tissues were fixed to preserve from degradation and to maintained the structure of organelles. Tissues were processed routine and water removed from tissues after dehydrated and cleared, tissues were in filtered with embedding in paraffin wax. Section of 5 nanometer thickness were cut with microtone, and stained with Hematoxylin and Eosin and examined under microscope and photomicrographs were taken.

Hematology: This experimental was carried following the method adopted by Lewis in 1991[4].

- *Hemoglobin determination (Hb %):* Determination of Hb% was adopted by SAHI method. The method depends upon the conversion of hemoglobin to acid ham tin which is brown in color. A volume of (0.02ml) of blood was added to 0.2ml of HCL O/N in haematometer calibrated tube. After 5µ minutes, distilled water added gradually until the color matched with Sahli apparatus. The concentration of Hb was obtained by multiplying the reading by 14.6g/dL.
- *Packed cell volume (PCV %):*Fresh blood sample was centrifuged in a micro-haematocrit tube for 5 minutes at 3000 r.p.m and the sample was read on the scale board [5].
- *Red blood cells count (RBCs):* The RBCs were counted with improved Neubauer haemocytometer slide (H.S.TM., CAT, No 500, England). A volume of 0.05ml formal citrate was used as a diluents added to 0.01 ml of blood measured by bulled haematometer pipette. Red blood cells count was expressed as $x10^3$Iitre.

- *White blood cells count (WBCs):* The WBCs were counted by using improved Neubauer haemocytometer slide (H.S, T.M., CAT.No.500, England) a volume of 0.05 ml Turkish fluid (1% glacial acetic acid, tinged gentian violet), was used as a diluent added to 0.1 ml of blood measured by bullied haematometer pipette. White blood cells count was expressed as $x10^{-1}$Iitre.
- *Mean corpuscular volume (MCV):* The mean corpuscular volume was obtained by using the following formula. *MCV (fI) = PCV (%) x10/RBC ($x10^6$/ml)*
- *Mean corpuscular hemoglobin concentration (MCHC):* The mean corpuscular hemoglobin concentration was calculated using the formula: *MCHC (%) = Hb (g/dL) x100/PCV (%)*
- *Mean corpuscular hemoglobin (MCH):* The mean corpuscular hemoglobin was calculated using the following formula: *MCH = Hb (g/dL) x10 RBC ($x10^6$/mI)* [6].
- *Red cell distribution (RDW): calculated as follows: RDW=SD/MCV × 100%*

  Where (SD) is the standard deviation in fl (femtolitres)

Sero- biochemical analysis: Sero-biochemical analysis is method used for determination of plasma constitutes. The method involves: total protein, albumin urea, acid, creatinine and liver function test.

- *Total protein:* Total protein was determined using spectrophotometer (Jenway, 6105 UV/vis, Germany) at a wavelength 540 nm, and calculated as follows: *Protein concentration (g/100ml) = T-Bx7.5*

  Where T: Test; B: Blank; S: standard (7.5 g/dl).
- *Total urea and Creatinine:* Total urea and Creatinine in was determined using even, method. In this method the absorbance was recorded at wavelength nm, using spectrophotometer (Jenway-U.V/vis-Germany), and calculated as follows: Urea concentration and Creatinine (mg/dL) = Test – blank x11—mg/dl [7].
- *Albumin:* Albumin was determined using bromcresol green method [8]. In this method the absorbance of blank and the sample was determined at lambda max 637 nm, and calculated as follows:

  Albumin concentration (g/100ml) = sample absorbance/standard absorbance x standard concentration.

  Where T: test; B: Blank; S: standard (5g/dL)

Preparation of the extract: -

10 g of each powdered plant sample was refluxed with 100 ml of 80% of ethanol four 4 hours. The cooled solution was filtered and enough 80% ethanol was passed through the volume of the filtrate to 100 ml. This prepared extract (PE) was used for the various tests.

Statistical analysis:

Result was expressed as mean ± standard error mean (SEM). The data were subjected to one –way analysis of variance (ANOVA) test and differences between samples were determined by Dunnetts multiple comparison test, using the Graph pad prism (statistical) software. Results were considered to be significant at $P<0.05$.

3. RESULTS

Administration of fermented aqueous *Senna obtusifolia* leaves 100mg/kg/BW to rats for 14 days caused an obvious significant gain in weight at ($p>0.05$) compared to control. The Administration of aqueous *Senna obtusifolia* showed that all experimental groups gained weight, than the control rats, and the food consumption increased with when the dose increased (See figure 2).

The total protein in range in Rats was in normal range; above 6g/dl and less than 8g. There was some reduction on RBCs and hemoglobin but not statistically significant, but the total white blood cells (WBCs) were increased significantly. The renal function of treated and control groups were normal, as the Creatinine ranged between 0.7mg/dl to 1.4mg/dl and Urea level was between 15mg/dl and 50mg/dl

Administration of aqueous *Senna obtusifolia* 100mg/kg/bw to rats for 14days showed no changes in renal nor hepatic histology (see figure 3).

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When tissues were weighted and compared, there was no variation between tested and controlled rats heart in range. The weight range of the hearts was 0.6 mg to 0.3 mg, and mean weight of the spleen was 0.3 mg. Lungs ranged in weight between 1.3 mg to 0.8 mg, Kidney o.5 mg to 0.3mg and Liver in range 4.6 mg to 3.3 mg (See figure 4).

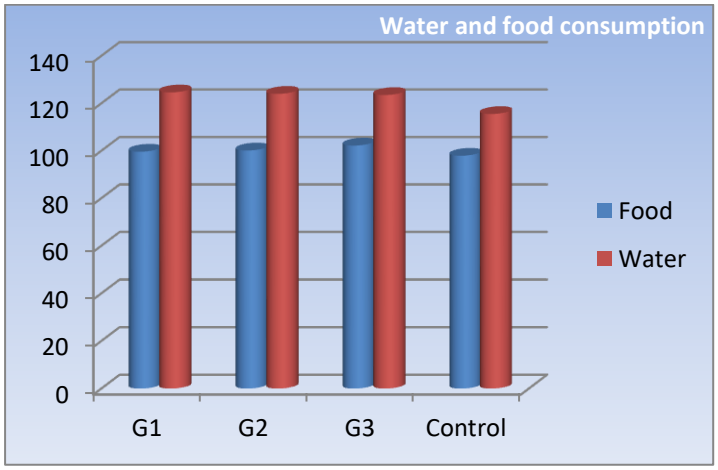

Figure (2): A histogram showing the effect of Senna obtusifolia (L) on water and food consumption; P-value < (0.05)

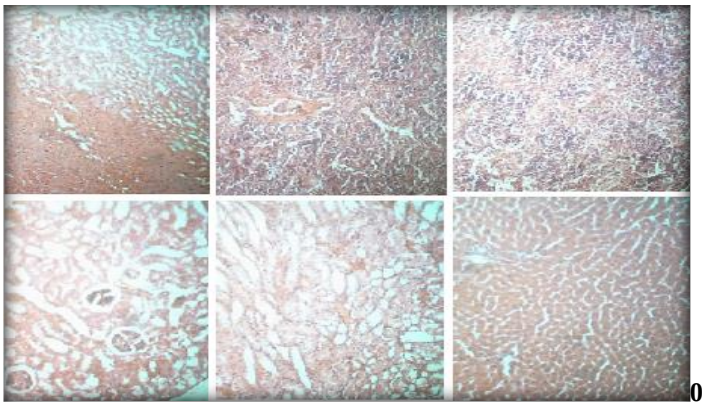

Figure (3): Photomicrograph section through the liver of an albino Rat (Up) showing normality of liver, and (Down) showing normal tissue of the kidneys in test albino Rats.

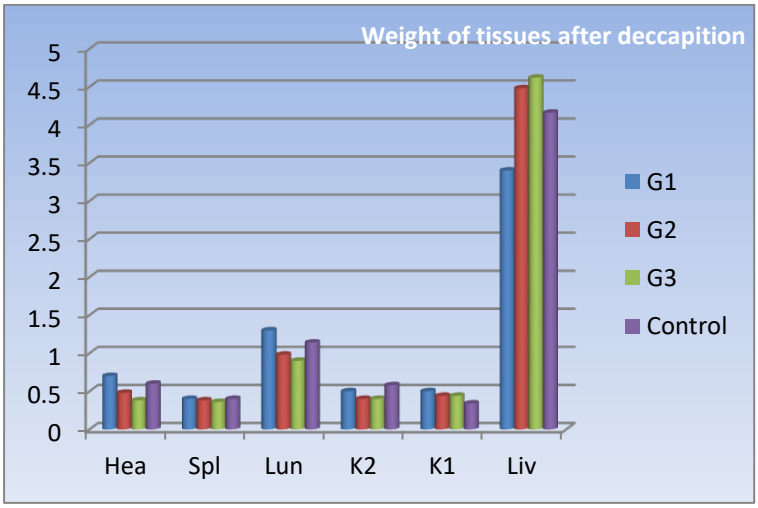

Figure (4): A histogram comparing the effect of *Senna obtusifolia* (L) on weight of tissues in different test groups after decapitation

4. DISCUSSION

Administration of fermented aqueous *Senna obtusifolia* leaves 100mg/kg/BW to albino rats for 14 days caused an obvious significant gain in weight at ($p>0.05$) compared to control. The Administration of aqueous *Senna obtusifolia* showed that all experimental groups gained weight than the controlled rats. Water and food consumption where tabled and observed increase in experimental.

Water and food consumption increasing gradually with increasing the dose, in group (1) day 1 food consumption 90 (g) ,till day 14 the total food consumption was108 (g) as the percentage of 20%, while, in Group (2) day 1 food consumption was 89 (g) till the day 14 in a total of food consumption was113 (g) as the total percentage of 26.9% , for Group (3) food consumption in day 1 was 91 (g) increasing gradually till day 14 in consumption of 114(g) with total percentage of 25.3%, therefore, the food consumption increased with when the dose increase , these results confirm that the *Senna obtusifolia* (L) Alkawal contained minerals and vitamins supplement by certain tribes in Kenya and Senegal and also these results in agreement with Becker, 1986 [9] who reported that the *Senna obtusifolia* (L) having a highly protein (14.4%) and highly palatable to poultry [10], therefore, this fermented plant contained highly percentage of vitamins and protein and act as appetizer.

Also the liver functions of the treated groups were compared with control group.

Despite of the normal total protein 6g/dl to 8g/dl, also Albumin 3g/dl to 4.5 and Globulin equals total protein minus albumin, therefore, the obtained results when compared, having a total protein in range above 6g/dl and less than 8g/dl and also the Globulin in normal range, this mean that it is normally ratio and there were no effects in liver functions. Also the pharmacological studies related with blood showed the hematological results when administration of aqueous *Senna obtusifolia* (L) on rats for 14days were caused reduction on RBCs and hemoglobin but not significant. Senna obtusifolia may affect bone marrow synthesis of enough erythrocytes. Plasma trapping is increased in macrocytic anemia [11], therefore it might be *Senna obtusifolia* has some compounds which lead to prevent plasma trapping and also are very rich with iron for this reason RDW was normal [12]. WBCs were increased significantly. WBCs play an important role in defense line and operate as phagocytes against invading microorganism. Change in liver histology may have caused elevation in WBCs or secondary compounds in *Senna obtusifolia* was increased represented as foreign bodies may be the reason for elevation of WBCs. And also renal function of treated and control groups, Creatinine normal rang 0.7mg/dl to 1.4mg/dl and Urea in a normal level of 15mg/dl to 50mg/dl [13], therefore, there were no change appeared, this was indicated that a safety of Alkawal.

Administration of fermented aqueous Senna obtusifolia (L) to rats for 14days100mg/kg/bw showed no alteration in Creatinine level and blood urea compared to controls which is in contrast with other studies [14], also administration of aqueous fermented leaves of *Senna obtusifolia* (L) 100mg/kg/bw for14days showed hepatic sinusoidal dilation with tiny cytoplasmic vacillations. Tissues, however, appeared normal with tiny liver parenchymal necroses; these necrotic areas may be due to secondary metabolites such as isothiocyanates which produce glycosides that might lead to necrosis. Tissues appear generally normal, probably due to the anti-oxidant compounds in the plant.

5. CONCLUSION

This study was performed to assess the leaves of *Senna obtusifolia* safety, appetizing potentials, and possible toxic effect on hematology and histological change in alino Rats. The aqueous leave extracts of *Senna obtusifolia* (L) Seclopode (Alkawal) had been used and they exhibited an appetizing appeal through the increased water and food consumptions and weight gain in Rats. It is relatively safe for human consumption. This plant may have no negative impact on tissue histopathology, since no significant lesions were observed in this study. This points to the fact that the plant is relatively safe for use nutritionally and medicinally. It is expected that the findings from this work may add to the overall value of medicinal and nutritional and potential of the plant.

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