Evaluation of Diabetic Rats Behavior after Treatment by *Artemisia herba-alba* Relative to Insulin

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**Abstract**

The aim of the study was to evaluate the behavioral signs of diabetic rats after treatment by *Artemisia herba-alba* (AHA) and insulin. **Method:** Based on the induction of diabetes in wistar rats by intraperitoneal (I/P) injection of 60 mg/kg streptozotocin (STZ), then treated by AHA as 20 mg (I/P) and insulin subcutaneously (S/C). The samples of rats were: 1) Diabetic control, 2) Injected with insulin, 3) Injected with AHA, 4) Non diabetic rats, which were fostered for 21 day; then weighted and the behavioral tests were conducted. **Results:** The board hole tests (BHT) showed that: the induced diabetes reduced the cognition of the rats in view of Latency of the First Head Dipping (LFHD) in seconds, number of head dipping (NHD) and duration of head dipping (DHD) by 49.3%, while it's improved in AHA and insulin treated rats by 52% and 69% in average respectively. The exploratory activity reduced in diabetic rats by 36%, while AHA and Insulin treated rats increased by 53% and 72% respectively. The rearing test showed an increase of anxiety among diabetic in form of duration of rearing, number of rearing and time spend in center by 51.7% in average respectively, while the anxiety reduced after treatment by AHA and Insulin by 39% and 47.3% in average respectively. Also the diabetes increased the depression state by 106%, while the treatment by AHA and Insulin reduced the depression state by 77% and 88% respectively. And VPT showed that: motor impairment occurred in diabetic cases and improved after AHA and insulin treatment.
Keywords
Diabetes, Artemisia, Behavioral, Herbal, Treatment, Insulin

1. Introduction
Diabetes mellitus is a serious metabolic disorder in humans. It is a chronic endemic endocrine disease, resulting from defective insulin secretion or resistance to insulin action, or both [1]. The disease is characterized by hyperglycemia leading increased fatigability, polydipsia, polyuria, polyphagia, weight fluctuation, blurry vision, irritability, infections, poor wound healing, microangiopathy, and it may ends with serious complications. The main two types of the diabetes mellitus were type 1 diabetes (insulin-dependent diabetes mellitus) which is characterized by an autoimmune-mediated destruction of pancreatic β-cells, leading to insulin deficiency and without treatment the case turns to ketoacidosis. Autoantibodies against insulin can be detected in 85% - 90% of patients at the time of diagnosis [2]. The incidence of type 1 diabetes is 8 - 20 per 100,000 patient-years in children up to 18 years of aged. After the age of 20, the incidence is lower, with little further effect of aged. Type 1 represents 15% - 20% of all diabetic patients [3]. Type 2 diabetes is characterized by insulin resistance and relative insulin deficiency [4]. Treatment aims to reduce insulin resistance (for example, with diet, exercise or drug therapy) and to increase endogenous insulin secretion. The incidence of type 2 diabetes (previously defined as non-insulin-dependent diabetes mellitus) is estimated to be about 5 per 100,000 patient-years in subjects up to 29 years of age, increasing sharply up to 500 per 100,000 patient/years in subjects over 70 [5]. The prevalence of diabetes ranges from about 2% in subjects aged 20 - 44 years to 18% in subjects aged 65 - 74 years, and over 20% in subjects of 85 and older [6]. Both forms of diabetes are associated with long-term complications that affect the eyes, kidneys, heart, blood vessels and nerves [7]. The development of these complications is dependent on the duration of diabetes and the quality of metabolic control [8] and can be only partially prevented by intensive insulin treatment. Both types of diabetes affect nervous system and showed neuropsychological changes [9]. Type 1 diabetic patients show different ranges of cognitive changes and impairments in learning and memory, problem solving, and mental and motor speed [10] [11]. As well type 2 diabetic patients showed chronic hyperglycemia, dyslipidemia peripheral neuropathy which were prone to moderate cognitive impairment particularly in tasks involving verbal memory or complex information processing, while attention processes, motor reaction time and short-term memory are relatively unaffected [9]. In addition, vascular dementias, Alzheimer’s disease [12], cortical and subcortical atrophy [13], and white-matter hyperintensities [14] have association with diabetic disease. The efforts to manage such drastic endemic disease have been carried out by many scholars, and the major components of the treatment are: Diet (combined with exercise if possible), Oral hypoglycemic therapy and
insulin treatment [15], and the Appropriate medication targeting glycemic con-
trol, hypertension, and lipid management for reducing morbidity and mortality, 
and improving long-term quality of life for patients diagnosed with type 2 di-
abetes mellitus (T2DM) [16], in addition to herbal medication efforts as gurmar 
leaf (Gymnema sylvestre), bitter gourd fruit (Momordica charantia), and fenugreek 
seeds (Trigonella foenumgraecum) may be among the best in terms of ef-
ficacy and safety [17] up on which 65% - 80% of world population depends on 
for their medication [18]. As the attempt has been continuing to beer out the ef-
iciency of herbal medication, the trend of the current work is to evaluate the 
behavioral signs of diabetic rats after treatment by Artemisia herba-alba (AHA) 
relative to Insulin. AHA or (Shih) exists in North Africa, the Mediterranean ba-
sin, parts of Asia and Middle East extending into northwestern Himalayas and in 
Spain [19]. Its extraction is popularly used in treatment of diabetes mellitus, due it 
is hypoglycemic activity [20], administration of 85 mg/kg body weight of AHA 
might be useful in preventing hyperglycemia and reduces blood glucose in both 
normoglycemic and hyperglycemic rabbits [21], and its phenolic constituent 
showed significant higher antioxidant effect, which is closer to that of the syn-
thetic antioxidants [22]. The full mode of action of the AHA in lowering blood 
glucose is under investigation, although some scientists propose, that the herbal 
drugs can show their effects by acting as; α-Glucisidase or α-Amylase Inhibitor 
[23] or Increases Insulin Secretion [24] [25] or stimulate β-cell regeneration by 
proliferation of its precursor or cells in the pancreatic duct [26], recently the 
AHA has been found to act as anti-diabetic derivative, by increasing insulin se-
cretion through regenerating new β-cells mass in STZ diabetic induced rats [27], 
so relieving of diabetes in rats will improve the symptoms accompanied the di-
isease including the behavioral ones.

2. Methodology

2.1. Tools & Equipments

- The Drugs

1) Insulin: long-acting insulin, ultralentehumulin U (lily), 0.5 ml/rat, S/C (sub-
cutaneous).

2) Herbs: AretemisiaHerba Alba was purchased from Khartoum medical 
herbs market. It was finely grinded, packed and subjected to 70% ethyl alcohol 
extraction. The extract was left to dry and dissolved as four grams of the herb in 
24 ml of 6% DSMO (dimethylsulphoxide-dissolver, used as vehicle transport to 
other material without affecting the tissue [28]) +376 distilled water = 400 ml of 
extract solution, each ml =10 mg of dissolved herb to be administered as 2 ml 
(20 mg)/Rat, I/P (intraperitoneal).

3) Glucometer: (i care TD-4279) Blood Glucose Monitoring System.

4) Streptozotocin (STZ): (STZ, has a chemical formula of C_{15}H_{15}N_{5}O_{7}, 265 
g/mol which implies nitrosourea moiety with a methyl group attached to one 
end and a glucose molecule at the other end. STZ was prepared by adding 750 
mg of STZ to 1.47 grams of sodium citrate (100 mM) to 50 ml of 9% sodium
chloride, the solution was injected I/P, 60 mg/kg.

- The Hole Board Test apparatus: (The hole board test was developed by Boissier and Simon, [29]. The arena comprised of a square Plexiglass box measuring 80 cm × 80 cm with equally spaced 16 holes in the floor 6 cm in diameter)
- Vertical pole Test apparatus: is cloth-tape covered vertical pole of an iron fixed on a piece of concrete. It is 150 cm long and 3 cm in diameter.
- Wistar Rats: rats of same ages, and sexes (males) were brought from the animal house of the faculty of medicine.

2.2. Methodology

Sixty four (64) Wister rats of same ages and sexes were brought from the animal house of the faculty of medicine. The rats were housed in a well prepared research room, encaged in sixteen cages, four rats per each, and left for two week before the experiment start to be adapted for the new housing conditions. Then the rats were labeled and weighed which were in range of 328.74 grams as average.

Four days later the effect of STZ (60 mg/kg I/P) was examined and showed an increase of blood glucose from 70 mg/dl to 320 mg/dl after 5 day flowing injection. Two weeks later the blood glucose of all rats was estimated; which was in the range of 70 - 110 mg/dl, then forty (40) rats were randomly selected and injected with STZ (60 mg/kg I/P). And after five days their blood glucose raised to more than 320 mg/dl (diabetic) as tested, from which twenty four (24) rats were randomly selected and divided into three (3) groups each implies eight rats and nominated as:

Group A: as diabetic treated with insulin, with S/C dose of 0.5 ml insulin.
Group B: as diabetic treated with AHA with (I/P) dose of 20 mg/Rat.
Group C: diabetic, injected with 2 ml of distilled water (I/P) daily (diabetic control).

The rest of the sample (24 normal rats) a group of eight (8) rats have been selected randomly and nominated as:

Group D: non-diabetic, injected with 2 ml of distilled water (I/P) daily (normal control).

The rats of all groups were weighed (Group A = 301.88 ± 118.9 Group B = 336.57 ± 42.9 Group C = 355.63 ± 68.1 Group D = 320.88 ± 647) and each group kept in a separate cage for 21 days.

During these 21 days, the experimental study has been carried out as general health observation; which showed no mortality among the sample. On twenty second (22nd) day; the rats were weighed in grams (Group A = 344.13 ± 53.4, Group B = 340.57 ± 40.0, Group C = 342.13 ± 56.1 and Group D = 325.13 ± 66.1) and their blood glucose was estimated (Group A = 93 mg/dl, Group B = 100 mg/dl, Group C = 355 mg/dl and Group D = 80 mg/dl) then all of the rats subjected to behavioral studies.

Behavioral Test

1) Vertical Pole Test (VPT):
VPT has been used to assess basal ganglia-related motor movement disorders in rodents [30] [31]. The rats are placed head-up on a cloth-tape covered vertical pole (150 cm long, 3 cm in diameter); when placed on the pole, animals orient themselves downward and descend the length of the pole. Animals with deficits in motor coordination and balance will fall off the pole [32] [33]. Animal time to orient downward (t-turn), reflect of cognitive abilities and the total time the animals stays on the pole (t-total), reflect of motor coordination ability [34] are determined from video recordings for a maximum of 180 seconds.

2) Hole Board Test (HBT):

The procedure of this experiment can be modified depending on the behavior being measured. Rats were individually subjected to the test. During the test, each rat was placed at the center of the platform and the activity in the arena was video recorded for a 5 min period. The video recordings were scored and the following exploratory behavior parameters were measured: the Latency of First Head Dipping (LFHD), Number and Total Duration of Episodes of Head Dipping through the holes (“nose-poking”, which represent a possible way to escape from the aversive environment and therefore reflect the escape response of the animal, which is a normal cognitive ability [35], the total distance travelled on the arena (ambulation score) rearing episodes, and the latency to the first grooming were recorded. The apparatus was cleaned with 95% alcohol after every trial.

1) Cognition: Estimated by counting the Latency of Time in seconds that taken by every rat for the first head dipping in a hole, by counting the head dipping numbers and by counting the head dipping duration in seconds.

2) Exploratory activity: done by measuring the distance traveled by a rat in the arena of the board/cm.

3) Anxiety: done by counting the numbers of rearing against wall of the box of the board, duration of rearing/s and the time spent in centre of the board/s by each rat.

4) Depression: estimated by the latency of time in seconds spent by a rat before commencing the first grooming episode.

3. Results

The following are the results of Rats behavior after induction of diabetes and treatment with AHA and Insulin, with specific attention to Latency of First Head Dipping per seconds (LFH/s), Number of Head Dipping (NHD) and the Head Dipping Duration per seconds (HDD/s) for the control group, diabetic group, group treated with AHA and group treated with Insulin.

4. Discussion

Based on the accumulated knowledge that: diabetes used to change the host behavior, which can be improved by the anti-diabetic medication, it is found that treatment of diabetic rats with AHA and insulin improve some behavioral signs compared to the non-treated ones out of behavioral tests (HBT and VPT).
In the following study; the board hole tests (BHT) showed that: the induced diabetes reduced the cognition of the rats in parameters of Latency of the First Head Dipping (LFHD) in seconds, number of head dipping (NHD) and duration of head dipping (DHD) from (100%) 35 s to (60%) 22.4 s, from (100%) 9.6 times to (58.3%) 5.6 times, and from (100%) 20 s to (50%) 10 s respectively; while its improved in AHA and insulin treated rats to (92.6%) 32.4 s, (108.3%) 10.4 times, (123%) 24.6 s, and ((114.3%) 40 s., (133.3%) 12.8 times, and (128%) 25.6 s) respectively as shown in Figure 1, same results have been highlighted by Rogacki et al., [36] in which they stated that: the healthy animals take longer time to begin the first head dipping. The exploratory activity was reduced in diabetic non treated rats from (100%) 1440 cm to (63.9%) 920 cm while AHA and Insulin treated rats increased to (88.9%) 1280 cm, and (108.3%) 1560 cm respectively as shown in Figure 2, in the same realm Deacon et al., [37] found the decrement of exploratory activity which indicates the impairment in motor

**Figure 1.** Shows latency of first head-dipping/seconds, head dipping number and head dipping duration/seconds after induction of diabetes by STZ relative to treatment with AHA, and insulin (100% = 35 s, 9.6 times & 20 s respectively).

**Figure 2.** Shows distance traveled on the arena after induction of diabetes by STZ and the relative distance traveled on the arena after treatment with AHA, and insulin (100% = 1440 cm).
function. Also rearing test showed an increase of anxiety among diabetic in form of duration of rearing, number of rearing and time spend in center as from (100%) 12 s to (33.3%) 4 s, from (100%) 4 times to (50%) 2 times, and from (100%) 370 s to (62.2%) 230 s respectively, while these parameters (duration of rearing, number of rearing and time spend in center) reduced in AHA and insulin to (58.3%) 7 s and (66.7%) 8 s, (90%) 3.6 times and (120%) 4.8 times, and (113.5%) 420 s and (100%) 370 s respectively as shown in Figure 3, such result has been mentioned in the introduction section by Moriera et al., [38] and Brown et al., [35]. While the BHT reveal the elevated depression state of diabetic rats in form of increasing the latency of first grooming episode (LFGE) from (205.9%) 350 s to (100%) 170 s; and the LFGE reduced to (76.5%) 130 s and (88.2%) 150 s in AHA and insulin respectively as shown in Figure 4, same result has been stated by Pires et al., [39] in the introduction section. The VPT revealed

Figure 3. Shows rearing episode number, rearing episode duration & time spent in the arena center after induction of diabetes by STZ relative to the treatment with AHA, and insulin (100% = 4 times, 12 s & 370 s respectively).

Figure 4. Shows latency of first grooming episode/second, after induction of diabetes by STZ and the relative grooming first latency after treatment with AHA, and insulin (100% = 170 s).
the motor impairment in diabetic rats in form of time taken on pole (TTP) and increase time to begin the first turn (TTFT) as decrement and increment by (39.1%) 36 s and (216.7%) 13 s relative to normal (100%) 92 s and (100%) 6 s respectively, while TTP and TTFT improved by increment and decrement as (82.6%) 76 s and (100%) 92 s and (50%) 3 s and (100%) 6 s respectively as shown in Figure 5 and Figure 6, ascertaining this fact the result obtained by Simmons et al., [32] and Tanriover et al., [32] [33]. The general improvement of the behavioral signs among diabetic rats after injection of AHA was ascribed to the new generation of \( \beta \)-cells in the islets of Langerhans of the pancreas as confirmed in histological section done by Yahia et al. [27].

5. Conclusion

The utilization of AHA as traditionally being used as antidiabetes by some universe population as well as classical herbal medicine for gastric disturbance, it showed a considerable obvious improvement in behavioral sign among diabetic rats; which encourage and open the trends for scholars and pharmacologists to

![Figure 5](image1.png)  
**Figure 5.** shows total time on the pole and the latency of the first turn in the vertical pole test (a) the healthy non diabetic rat; (b) STZ diabetic induced non treated rat.

![Figure 6](image2.png)  
**Figure 6.** Motor impairment in vertical pole test: Shows total time taken on the pole and time taken to begin the first turn/seconds after induction of diabetes by STZ and the relative treatment with AHA and insulin (100% = 92 S, 6 S).
search deeply to emphasize it is benefit and further the commercialization issues of AHA.

**References**


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