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Neurobehavioural Study on the Effect of Aqueous Extract of *Citrus medica* Leaf on Prefrontal Cortex of Hyperglycemia Wistar Rats

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Abstract

The Prefrontal cortex is the anterior part of the frontal lobes of the cerebral cortex, lying in front of the primary motor and premotor areas of the frontal lobe. High blood sugar (hyperglycemia) happens when the body has too low insulin or when the body cannot use it properly and in train. It causes adverse effects resulting to brain tissue acidosis, ischemic, cerebral edema and hemorrhagic stroke. Plant materials have been used for medicinal purposes from time immemorial. *Citrus medica* is a plant whose extracts are being used to treat various ailments in folk medicine till present day. The ability of *Citrus medica* extracts to treat these ailments was traced to its constituents, which are flavonoids, vitamins C and E, lectins, phenols, alkaloids, steroids and glycosides. As a step in this direction, this study was carried out on effects of aqueous leaf extract of *Citrus medica* on Neurobehavioral study of the Prefrontal cortex of hyperglycemic Wistar rats.

Forty adult male Wistar rats weighing between 160-200 g were randomly grouped into four consisting of ten rats each *viz*: A. normal control, B. hyperglycemic only, C. hyperglycemic treated with *Citrus medica* and D. *Citrus medica* only. Hyperglycemia was induced by a single intraperitoneal injection of streptozotocin (70 mg/kg/body weight), freshly dissolved in 0.1 M citrate buffer at pH 4.5. After three days of uninterrupted hyperglycemia (blood glucose ≥250 mg/dl), aqueous leaf extracts of *Citrus medica* were administered orally at 400 mg/kg body weight daily for six weeks. Neurobehavioral parameters and blood glucose level were recorded weekly. After the sixth week extract of administration, animals were sacrificed by cervical dislocation. Organ weight was also taken. Data were analysed using excel and Student t-test. $p<0.05$ was considered significant.

The findings of this study showed that the blood glucose level of hyperglycemic+*Citrus medica* and *Citrus medica* only group significantly lowered, increase in body weight and relative brain weight were observed relative to the hyperglycemic group. $P<0.05$. The anxiety level in hyperglycemic+*Citrus medica* and *Citrus medica* only were not significantly different to control. $P>0.05$. Aqueous leaf extract of *Citrus medica* showed ameliorative potentials on the Prefrontal cortex of adult male Wistar rats against the damage initiated by hyperglycemia.

Keywords: Prefrontal cortex; Streptozotocin; *Citrus medica*; Hyperglycemia; Wistar rat

Introduction

Background of the study

The nervous system is the most complex system in the human body formed by network of more than a billion of neurons (nerve cells) assisted by many more glial cells (neuroglia). The nervous system includes both central nervous system and peripheral nervous system [1]. The central nervous system is made up of the brain and the spinal cord while the peripheral nervous system is made up of the somatic and the autonomic nervous system. One of the organs of the human central nervous system is the human brain [2]. It is located in the cranial cavity. It has the same general structure as the brains of other mammals but with a more developed cerebral cortex [3,4].

The adult human brain weighs on average about 1.2-1.4 kg or about 2% of total body weight [1,5] with a volume of about 1260 cm^3 in men and 1130 cm^3 in women, although there is substantial individual variation. [6]. Neurological differences between the sexes have not been shown to correlate in any simple way with intelligence quotient (IQ) or other measures of cognitive performance reported by Gur et al. [7] cited by Budhachandra et al.

The human brain is composed of neurons, glial cells, and blood vessels. The number of neurons is estimated at 100 billion [7]. Out of these, 16 billion (or 19% of all brain neurons) are located in the cerebral cortex (including subcortical white matter), 84 billion (or 80% of all brain neurons) are in the cerebellum [8]. The human brain is made up of three major parts: the cerebrum, cerebellum and brain stem [9,10].

The Prefrontal cortex is the anterior part of the frontal lobes of the cerebral cortex, lying in front of the primary motor and premotor areas of the frontal lobe [11]. It is made up of the dorsolateral and ventrolateral areas that receive their major afferents from the mediodorsal nucleus and there are additional inputs from the medial pulvinar, the ventral anterior nucleus and the paracentral nucleus of the anterior intra laminar group of nuclei in the thalamus [12]. Countless authors have

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illustrated an essential link between a person's personality and the functions of the prefrontal cortex reported by Miller et al. [13] cited by Anna [14]. Prefrontal cortex has been implicated in planning complex cognitive behaviour, personality expression, decision making, and moderating social behaviour [15]. The basic activity of this brain part is considered to be orchestration of thoughts and actions in accordance with internal goals reported by Liston [16] and cited by Makanjuola et al. [17]. Destruction of the anterior two-thirds results in deficits in concentration, orientation, abstracting ability, judgment, and problem solving ability; destruction of the frontal lobe results in inappropriate social behavior. Each of the different cortical layers contains a characteristic distribution of neuronal cell types and connections with other cortical and subcortical regions [18]. There are exact associations between different cortical areas and indirect connections via the thalamus; the neurons of the cerebral cortex are grouped into six main layers, from superficial layer (pia surface) to the deep layer (white matter).

Hyperglycemia is the technical term for high blood glucose or blood sugar. High blood glucose happens when the body has too little insulin or when the body cannot use insulin properly [19], other conditions that can cause hperglycemia are pancreatitis, Cushing's syndrome, unusual hormone-screating tumors, pancreatic cancer, certain medications and severe illnesses [20]. Temporary hyperglycemia is often benign and asymptomatic in nature. Blood glucose levels can rise well above the normal values for significant periods without producing any permanent effects or symptoms [20]. However, chronic hyperglycemia at levels more than slightly above the normal values can produce a very wide variety of serious complications over a period of years; chronic hyperglycemia that persists even in fasting states is most commonly caused by Diabetes mellitus [20].

Diabetes mellitus is a disorder of glucose metabolism whereby the body is not properly making use of glucose in the blood stream, therefore compromising a necessary function for cell nutrition and function [21]. Type 1 diabetes is also called insulin-dependent diabetes [22]. It used to be called juvenile-onset diabetes, because it often begins in childhood and accounts for about 10% of all diabetic cases, affecting approximately 20 million people Worldwide [22]. Type 2 diabetes is also known as adult-onset diabetes, accounting for about 95% of the cases of Diabetes mellitus in adults [23]. Gestational diabetes is known to be Diabetes triggered by pregnancy which account for about 2% to 10% of Diabetes mellitus pregnancies [23].

Diabetes mellitus had been connected with disabling and life threatening complications such as retinopathy, neuropathy, nephropathy, dermopathy, cardiomyopathy and hepatopathy [21].

After the classic work of Banting and Best [24] on insulin, indefinite number of findings had accumulated over the years. Currently, botanicals are being screened for their efficacy and safety in the management of Diabetes mellitus and its complications [25,26]. In this regard, there is laboratory-based evidence that the fruit juice of *Momordica charantia* reverses hyperglycemia in rats by decreasing gluconeogenesis and increasing insulin secretion [25,26]. Additional potential herbal sources of new chemical entities for the management of Diabetes mellitus include *Coccina indica* [27], *Gymnema sylvestre* [28] and *Panax quinquefolius* (ginseng) [29]. Others include *Annona muricata* [30], *Hypoxis hemerocallidi* [31], *Berberis lyceum* [32], *Aloe vera* [33], *Trichosanthes cucumerina* [34] and *Allium cepa* [35].

Chronic hyperglycemic oxidative stress is implicated in the pathogenesis of these complications [36]. Some reports have shown that antioxidants may protect people from the disabling effects of Diabetes mellitus by mopping up free oxygen and superoxide radicals [36]. It has also been shown by Opara [37] that depletion of antioxidant appears to be a major risk factor for developing complications, and that antioxidant supplements lowered the risk. Besides, impaired insulin levels or action in Diabetes mellitus predisposes to dyslipidemia and increased risk of atherosclerosis [38]. In view of the fact that emphasis is now on herbal therapy, searchlight is on herbs that are edible of which Citron is qualified. Moreso, Citron leaf has abundance of antioxidants [39].

The study is therefore aimed at investigating neurobehavioral study on the effects of *Citrus medica* leaf extracts on prefrontal cortex of STZ-induced hyperglycemia in wistar rats.

Materials and Methods

Mature fresh leaves of *Citrus medica* were taken to a botanist in Forestry Research Institute of Nigeria (FRIN), Jericho hill, Ibadan for authentication and voucher number (No. FHI. 110913) was assigned. *Citrus medica* aqueous leaf extracts were prepared as reported by Taha et al. [39]. Fresh leaves of *Citrus medica* were air-dried (under shade). The leaves were ground to coarse powder using an electric blender. The powdered sample of 400 g was soaked in 4000 mls of distilled water for 24 hours in a measuring cylinder. The mixture was homogenised in Explosion Proof Blender for 120 seconds, then filtered using a whatman filter (Grade 1circles, diameter 15 mm, Z274844). The filterate in the round bottom flask was put on a heating mantle (Barnstead/eletrothermal) at 100°C for 7 hours. The concentrate formed was taken to an oven at 50°C for 1hour; the final residue of about 80 g was a dark green mass which was stored at room temperature of 25°C until reconstituted for uses.

Animals and animal management

Forty adult presumably healthy male Wistar rats (*Rattus norvegicus*) were used for this study. The animals were between 8 to 10 weeks old; body weight (160-200 g). Animals were kept in four cages (10 rats per cage) and housed in the animal holdings of the Department of Anatomy, Faculty of Basic Medical Sciences, Animal House, Olabisi Onabanjo University Ikenne. They were maintained on standard animal feeds (Wealth-gate pelletized feeds) and allowed access to clean water and feeds freely.

Induction of hyperglycemia in rats

The blood glucose level was monitored weekly from four weeks (acclimatisation period) before the induction of hyperglycaemia. Hyperglycaemia was induced in twenty overnight-fasted randomly selected rats by a single intraperitoneal administration of Streptozotocin at 70 mg/kgbw [40]. Streptozotocin (STZ) was dissolved in citrate buffer (0.1 m, pH 4.5) just prior to injection. Hyperglycemia was allowed to develop for 72hours [41]. Rats with Fasting Blood Glucose ≥ 250 mg/dl were considered hyperglycemic [42]. Control rats (n=10) received a single intraperitoneal injection of 0.1 M citrate buffer (1 ml/kgbw; pH 4.5).

Experimental design

Forty male Wistar rats were divided into four groups of ten animals each. Control Group A was normoglycemic animals that received neither STZ nor *Citrus medica* leaf extract, Group B was hyperglycemic rats that received distilled water only, Group C was hyperglycemic rats that received *Citrus medica* extract only and Group D was

normoglycemic group that received only *Citrus medica* extracts.

Citrus medica mode of administration

The dose of the aqueous extracts of *Citrus medica* used in these studies was adopted from the report of Taha et al. [39]. *Citrus medica* was dissolved in physiological saline daily and was administered orally with use of oro-gastric cannula to Group C rats (n=10) at 400 mg/kgbw (at 9.00-10.00 a.m. each day) for a maximum period of six weeks, Group D rats (n=10) were administered 400 mg/kgbw of *Citrus medica* extracts. Group A rats (n=10) received neither STZ nor *Citrus medica* extract. These rats however received an intraperitoneal dose of 0.1 M citrate buffer (pH 4.5). Group B rats (n=10) received oral physiological saline only following STZ treatment.

Measurement of blood glucose

The blood glucose was evaluated in overnight fasted rats at 9:00-10:00 hours using Glucose oxidase method of one touch ultra 2 glucometer (Accu-Chek Compact Plus). Blood was obtained from the median caudal vein of the tail by snipping the tip of the tail. The blood glucose level was monitored weekly from four weeks (acclimatisation period) before the induction of hyperglycaemia and for six weeks of treatment [30].

Measurement of the body weight (g)

Body weight (g) of the rats were record for four weeks (acclimatisation period) prior to induction of hyperglycaemia and on a weekly basis during the experimental treatment for a period of six weeks. Body weight was taken with a weighing scale (Venus VT 30 SL) [34].

The relative brain weight (%)

The relative brain weight of the rat was evaluated as the ratio of respective weight of the brain and the terminal body weight of the same rat, the unit was recorded as percentage (%) using sensitive weighing balance (SonyF3G brand); [34].

Neurobehavioural studies

Anxiety, exploration and locomotor activities: The Anxiety, Exploration and Locomotor activities were studied using open field maze method [1,43]. The open field maze used was the large size (72 × 72 cm). The large open field used for measuring anxiety and exploration as well as locomotion as it has a large center arena.

The open field apparatus was constructed of plywood painted with white colour and measured 72 × 72 cm with 36 cm walls.

In Figure 1 red lines were drawn on the floor with a marker and were visible on the floor. The lines divided the floor into sixteen 18 × 18 cm squares. A central square (18 cm × 18 cm) was drawn in the middle of the open field [1,43]. The central square was used because some rat strains have high locomotor activity and could cross the lines of the test chambers many times during a test session. The central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations [43].

Procedures

Rats were carried to the test room in their home cages and were handled by the base of their tails at all times. Rats were placed into the center, or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minute test, rats were returned in their home cages and the open field maze was disinfected with 70% ethyl alcohol and permitted to dry between tests [1,43].

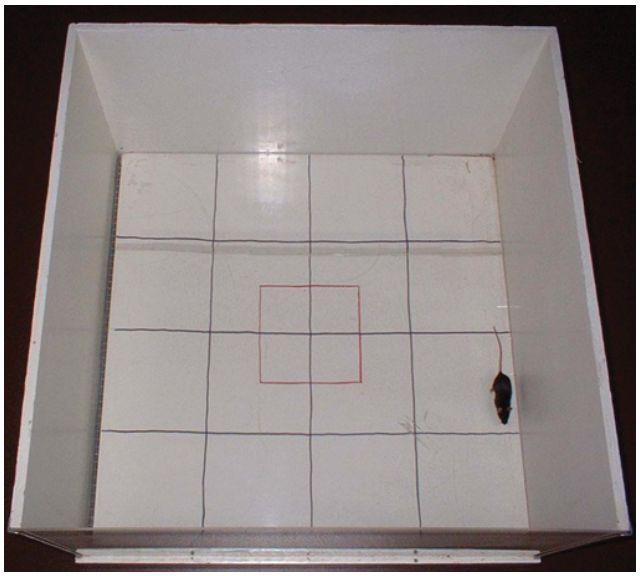


Figure 1: Open field maze.

Behavioural parameters

The behaviours scored [1,43] included:

1. **Line crossing:** Frequency with which each of the rats crossed one of the grid lines with all four paws in 5 minutes.

2. **Center square entries:** Frequency with which each rat crossed one of the red lines with all four paws into the central square (Number of times each rat entered the center square).

3. **Hinding:** Frequency with which the rat stood on its hind limbs in the maze.

4. **Walling:** Number of times the rat touched the maze wall.

5. **Urination:** Number of puddles or streaks of urine in 5 minutes.

6. **Defecation:** Number of fecal boli produced in 5 minutes.

Statistical analysis

Data were analysed using Excel 2016 (Microsoft Corporation, U.S.A). Data were expressed as mean ± standard error of the mean (mean ± SEM). Mean values were compared using student t-test. P values less than 0.05 ($P<0.05$) were taken to be statistically significant. All graphs were drawn with Excel 2016 (Microsoft Corporation U.S.A).

Results

Average body weights of the rats (g)

Figure 2 showed the weekly changes in body weight of rats in various groups. The body weight of rats in various groups during four weeks of acclimatisation were normal with no significant difference when compared to Group A (control) ($P>0.05$). At the second week, there was an increase of 8.3% in the average body weight of the rats in Group B of hyperglycemic only (174.16 ± 3.21 g), but after the second week of treatment, their average body weight started declining and by the sixth week (166.07 ± 3.28 g) there was a decrease of 3.7% compared

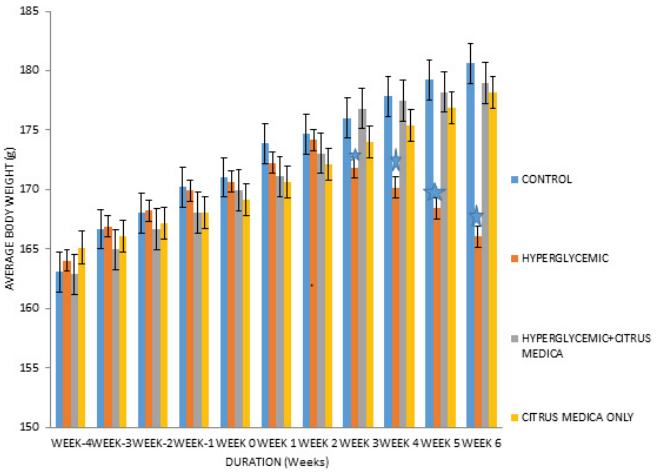


Figure 2: Histogram chart of average body weight on weekly basis.

to the initial average body weight at week 0 (170.64 ± 3.14 g) with significant difference when compared to Group A (P<0.05). In Group C of hyperglycemic+*Citrus medica*, there was an increase of 7.9% at second week of treatment (173.06 ± 3.79 g), but at sixth week (178.95 ± 3.37g) the increase was 12.1% when compared with their weight at week 0 (169.92 ± 3.88 g) with no significant difference when compared to Group A (P>0.05).

Relative brain weight (%)

The relative weight of the brain in various groups were shown in Figure 3. There was a significant decrease in brain weight in Group B (1.36 ± 0.056 g) compared to Group A (1.83 ± 0.091 g) (P<0.05). In Group C (1.62 ± 0.034 g) the relative brain weight was not significantly different from Group A (P>0.05).

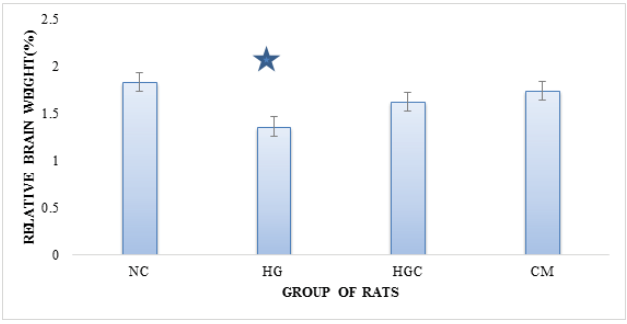
Blood glucose levels on weekly basis (mg/dl)

Figure 4 showed the blood levels glucose of different groups of rats on weekly basis. The blood glucose level of rats in various groups during 4 weeks of acclimatisation were normoglycemic in nature with no significant difference when compared with rats in Group A (P>0.05). The Group B rats were hyperglycaemic at week 0 (253.16 ± 5.77 mg/dl) and remained so until the end of the sixth week of treatment (367.53 ± 7.11 mg/dl). The value was significantly different from Group A (74.65 ± 3.38 mg/dl) (P<0.05). Whereas the Group C (265.15 ± 6.05 mg/dl) rats had high blood glucose level at week 0 up to week 3 which was significantly different from Group A rats at P<0.05. From the fourth week the blood glucose levels of Group C (98.17 ± 3.98 mg/dl) were comparable to Group A (74.65 ± 3.38 mg/dl) which were not significantly different at P>0.05.

Neurobehavioural study

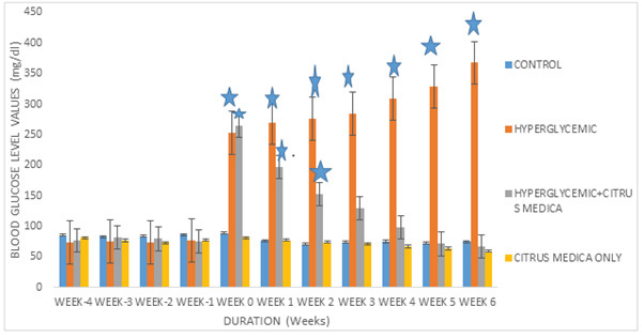
Anxiety, exploration and locomotor activities:

The frequency of lines crossed by rats: Figure 5 showed the weekly changes in the frequency of line crossing of rats in various groups of this study. The line crossing of rats in different groups (B, C and D) during a period of four weeks of acclimatisation were presumably normal with no significance different when compared to the control group of rats (Group A) (P>0.05). At the first week, there was an increase (54.4 ± 1.75), in the frequency of line crossing of the animals in Group B of hyperglycemic rats but after the first week their line crossing abilities



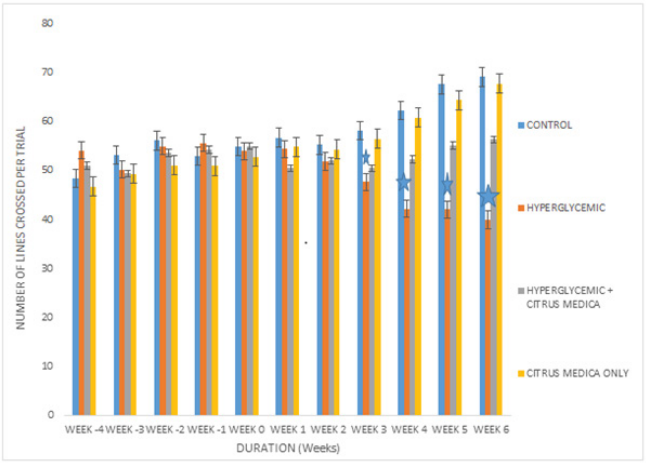
Note: NC: Normal Control, HG: Hyperglycemic, HGC: Hyperglycemic+Citrus medica, CM: Citrus medica only, Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05.

Figure 3: Bar chart of relative brain weight in percentage (%).



Note: Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

Figure 4: Bar chart of relative brain weight in percentage (%).



Note: Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

Figure 5: Histogram chart of lines crossed by rats per trial.

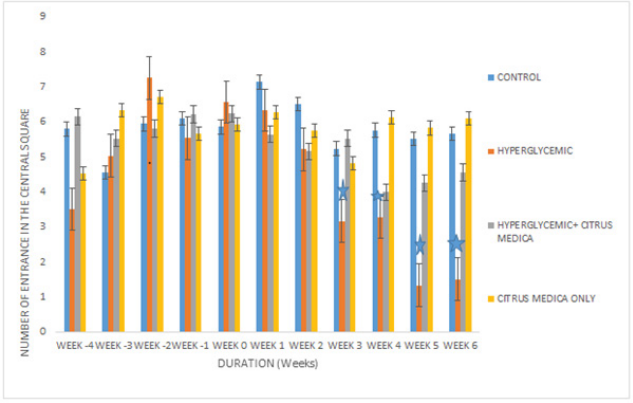
started declining and by the sixth week there was decrease (39.8 ± 0.95) locomotor activities when compared to their initial line crossing (54.0 ± 1.74) at week 0. In Group C of hyperglycemic+*Citrus medica*, there was a decrease (50.4 ± 1.67) in locomotor activity at the first week of the study but at the sixth week there was an increase (56.4 ± 1.77) when

compared with their line crossing at week 0 (55.00 ± 1.76) and also with control group rats there was no significant difference at P>0.05. There was significant difference (P<0.05) when Group B of hyperglycemic rats were compared to control group rats.

The frequency of the central square entrance: Figure 6 showed the weekly changes in the frequency of number of times a rat entered center square in the various groups of this study. The frequencies in groups (B, C and D) during a period of four weeks of acclimatisation were asumably normal with no significant difference when compared to rats in Group A(control) at P>0.05. At the first week of treatment, there was an increase in the frequency of the rats entered the center square in Group B of hyperglycemic rats (6.4 ± 0.24), but after the first week, the frequency of central square entrance started declining and by the sixth week (1.5 ± 0.096) there was a decrease when compared to the initial frequency of the rats entered center square at week 0 (6.6 ± 0.26). In Group C of hyperglycemic+*Citrus medica*, there was a decrease in the first week (5.8 ± 0.18) also at the sixth week (4.6 ± 0.18) there was a decrease and when compared with their center square entrance at week 0 (6.2 ± 0.24) no significant difference was observed when compared to the control group (P>0.05) however, there was a significant difference (P<0.05) when Group B of hyperglycemic rats was compared to Group A (control).

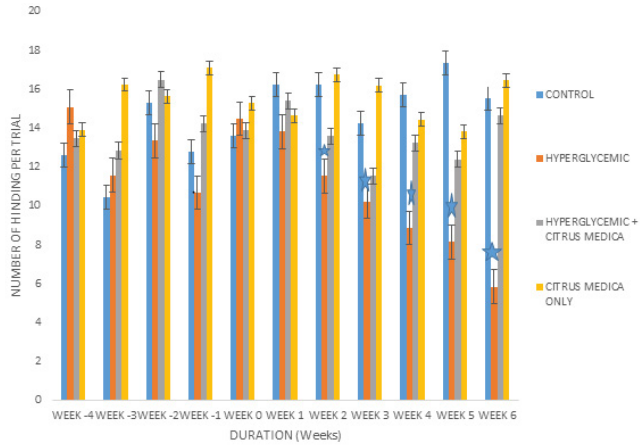
The frequency of hinding by rats: Figure 7 showed the weekly changes in the frequency of hinding of animals in various groups of this study. The frequencies of hinding of rats in different groups (B, C and D) during a period of four weeks of acclimatisation were presumably normal with no significant difference when compared to Group A of Control rats (P>0.05). At the first week of treatment, there was a decrease (13.8 ± 0.47) in the frequency of hinding in the rats in Group B of hyperglycemic after the first week their hinding still declined and by the sixth week there was a decrease (5.8 ± 0.18) when compared to the initial hinding at week 0 (14.6 ± 0.49). In Group C of hyperglycemic+*Citrus medica*, there was an increase (15.4 ± 0.50) in the first week of treatment also at the sixth week there was increase (14.6 ± 0.49) and when compared with their hinding at week 0 (13.8 ± 0.47) with no significant difference when compared to Group A (P>0.05). There was a significant difference (P<0.05) when Group B of hyperglycemic rats were compared to Group A (Control) rats.

The frequency of walling by rats: Figure 8 showed the weekly changes in frequency of walling of rats in various groups of this study.



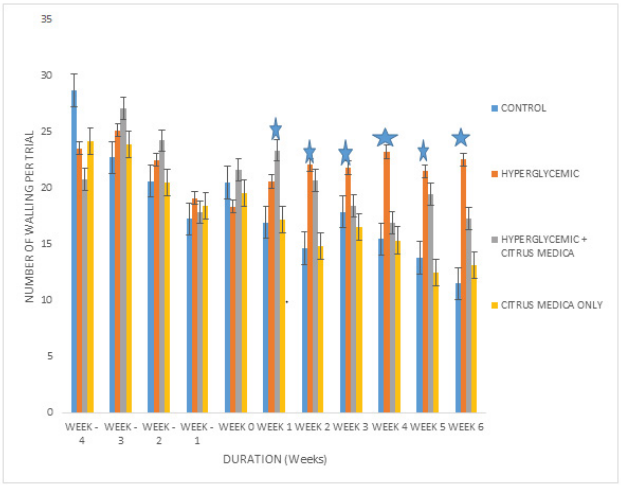
Note: Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

Figure 6: Histogram chart of the central square entrance in rats.



Note: Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

Figure 7: Histogram chart of hinding by rats.

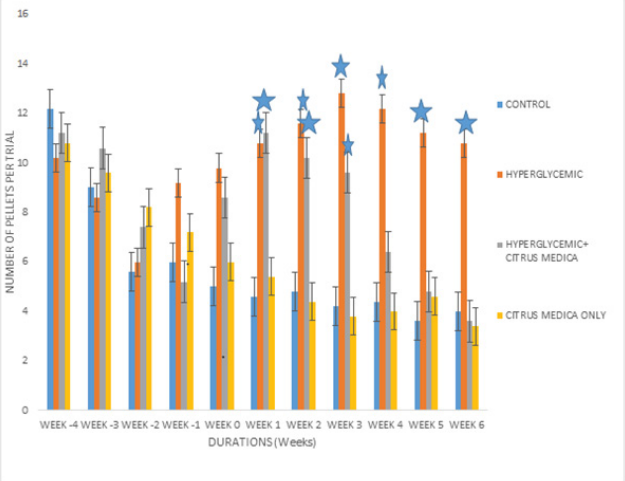


Note: Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

Figure 8: Histogram chart of walling by rats.

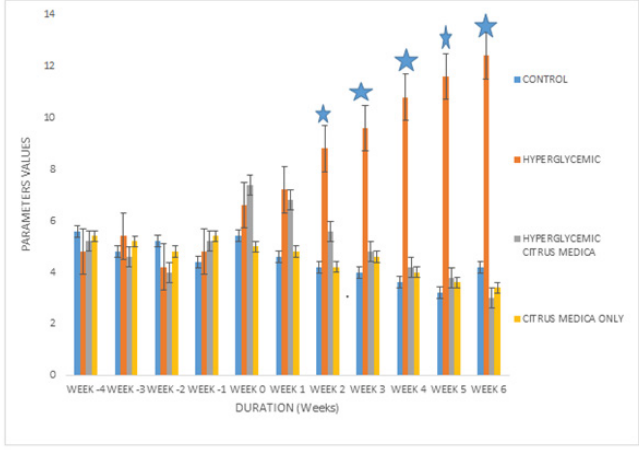
The frequencies of walling of animals in different groups (B, C and D) during a period of four weeks of acclimatisation were asumably normal with no significant difference when compared to the rats in Group A (Control) (P>0.05). At the first week of treatment there was an increase in the frequency of walling of the rats in Group B of hyperglycemic (20.6 ± 0.57), also after the first week their walling still increase and by the sixth week there was an increase (22.6 ± 0.61) when compared to the initial walling at week 0 (18.4 ± 0.52). In group C of hyperglycemic+*Citrus medica*, there was an increase (23.4 ± 0.60) in first week but at sixth week there was a decrease (17.4 ± 0.54) and when compared with their walling at week 0 (21.8 ± 0.57) with significant difference when compared to Group A (P<0.05). There was also a significant difference (P<0.05) when Group B of hyperglycemic was compared to Group A (Control).

The frequency of defecation by rats: Figure 9 showed the weekly changes in the frequency of defecation of rats in various groups. The frequencies of defecation of rats in different groups (B, C and D) during a period of four weeks of acclimatisation were presumably normal



Note: Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

Figure 9: Histogram chart of defecation by rats.



Note: Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

Figure 10: Histogram chart of micturition by rats.

with no significant difference when compared to the rats in Group A (Control) P>0.05. At the second week of the treatment, there was an increase (11.6 ± 0.40) in the frequency of defecation of the rats in Group B of hyperglycemic, after the second week their frequency of defecation still increase and by the sixth week there was a decrease (10.8 ± 0.36) when compared to the initial defecation at week 0 (9.8 ± 0.33). In Group C of hyperglycemic+*Citrus medica*, there was an increase (10.2 ± 0.36) in the second week of the treatment but at sixth week there was a decrease (3.6 ± 0.12) and when compared with their defecation at week 0 (8.6 ± 0.38) with no significant difference when compared to Group A (P>0.05). There was significant difference (P<0.05) when Group B of hyperglycemic rats were compared to Group A of control rats.

The frequency of micturition by rats: Figure 10 showed the weekly changes in the frequency of micturition of rats in various groups in this study. The frequency of micturition of rats in different groups (B, C and D) during a period of four weeks of acclimatisation were presumably normal with no significant difference when compared to Group A of control rats P>0.05. At the second week of treatment there was an increase (8.8 ± 0.41) in the frequency of micturition of the rats in Group B of hyperglycemic, after the second week their micturition still increased and by the sixth week there was an increase (12.4 ± 0.51) when compared to the initial micturition at week 0 (6.6 ± 0.32). In Group C of hyperglycemic+*Citrus medica*, there was a decrease (5.6 ± 0.21) in second week and also at sixth week there was a decrease (3.4 ± 0.05) and when compared with their micturition at week 0 (7.4 ± 0.31) with no significant difference when compared to Group A (Control) P>0.05. There was significant difference (P<0.05) when Group B of hyperglycemic rats were compared to Group A of control rats.

Discussion

The use of plants with medicinal properties for the treatment, cure and prevention of diseases is one of the oldest medicinal methods known in history. At the beginning of the 1990s, the World Health Organization stated that 65-80% of the population of developing countries depended on medicinal plants as their only form of basic health care [44].

The present study elucidates the possible ameliorative effects of citron on the hyperglycemic effect of STZ on the prefrontal cortex, the declining of the body weight of the rats in hyperglycemic group after second week might due to the low level of insulin which had been reported by Raheleh et al. [45] that low level of insulin leads to inability of the body to breakdown glycogen for energy and the body begins to breakdown its own stored fat foe energy, this causes rapid weight loss. In hyperglycemic+*Citrus medica* group there was an increase in the body weight from the second week of the treatment this might due to the presence of antioxidant in the *Citrus medica* which had been reported by Raheleh et al. [45] that is capable of reducing blood glucose level which was an agreement with this study.

The relative weight of the brain seen in the hyperglycemic group was low compared to other groups this might due to the neurodegeneration and it has been reported by Madhavan et al. [46] that hyperglycemia causes neurodegeneration. In hyperglycemic+*Citrus medica* group the relative weight of the brain is not significant to control this might due to the aversion of neurodegeneration by the chemical composition of *Citrus medica* [47].

The present study evaluated the possible ameliorative effects of citron on the hyperglycemia effects of STZ on the prefrontal cortex, treatment of STZ induced hyperglycaemia rats with aqueous leaf extract of *Citrus medica*, at a dose of 400 mg/kg/d, produced normoglycaemia in 83.2% of rats by the end of the third week of treatment and all the animals had become normoglycaemic by the end of the forth week. Normoglycemia was maintained in these rats from fourth week to the end of experiment. Antihyperglycemic activities of *Citrus medica* leaf extract had been reported earlier by Taha et al. [39], which was an agreement with the results of these study.

Antihyperglycemic activities are usually achieved through accentuation of release of insulin from B cells of islets of langerhans of pancreas, prevention of uptake of glucose from gastrointestinal tract as seen in alpha-gluconidase or pancreatic amylase enzyme inhibitors, prevention of gluconeogenesis and glucogenolysis [48,49]. *Citrus medica* antihyperglycemic activities had been reported to be due to the presence of alpha gluconidase [50] and pancreatic amylase enzyme inhibitors [51]. These enzymes inhibit the digestion of glucose into an absorbable product, hence the inability of blood glucose to increase after glucose intake. The presence of these inhibitors was reported in plants

like *Morus alba*, which was able to exhibit antihyperglycaemic activity [52]. Also the antihyperglyceamic activity of *Citrus medica* leaf extract was due to the presence of antioxidants like flavonoids, phenol in it [39]. Duong et al. [53] reported that flavonoids and phenols were powerful hydrosoluble antioxidants in biological fluids. The antioxidants were able to prevent further destruction of beta cells in the pancreatic islets.

The present study also evaluated the possible ameliorative effects of citron on the hyperglycemic effects of STZ on the prefrontal cortex appraised by locomotor activities, exploration and anxiety using the open field maze test. The behaviours scored in this study were line crossing, hinding, center square, walling, urination and defecation.

The line crossing is usually used as measuring of locomotor activity, exploration and anxiety [54], high frequency of the line crossing indicates increased locomotion, high exploration and lower anxiety [54,55]. The declining of line crossing frequency in the hyperglycemic group shows that there was low locomotion, low exploration and high anxiety this might be due to degeneration of neurons present in the frontal cortex. Hyperglycemia is associated with neuronal degeneration in the frontal cortex rats already reported by Madhavan et al. [46] that degeneration of the neurons in the frontal cortex could affect voluntary movements.

In the hyperglycemic+*Citrus medica* group there was a declined of line crossing frequency at the first week of treatment which later started increasing at the second week till sixth week this might due to presence of chemical composition in the *Citrus medica* such as flavonoids, phenols, lecitins, polypeptides and glycosides which they had been reported capable of preventing neurodegeneration [47]. Also, reversal of hyperglycemia from the third week could have contributed to this.

The decreasing of centre square frequency in the hyperglycemic group showed that there was low locomotion, low exploration and high anxiety this might be neurodegeneration of frontal cortex which was supported by Madhavan et al. [46] that degeneration of the neurons present in the frontal cortex can affect voluntary movements. In the hyperglycemic+*Citrus medica* group there was a decrease of centre square frequency at the first week of the treatment which keep on flunctuating till the sixth week. The rate of decrease of centre square frequency seen in Hyperglycemic+*Citrus medica* group was not significant to control compared to Hyperglycemic group that was significant to control. In this group there have been aversion of neurodegeneration due to the presence of chemical composition in the *Citrus medica* such as flavonoids, phenols, lecitins, polypeptides and glycosides which they had been reported capable of averting neurodegeneration [47]. In the hyperglycemic group, hyperglycemia was maintained till the end of experimental period and hyperglycemia had been reported as said earlier to be associated with neurodegeneration [46].

The declining of hinding frequency in the hyperglycemic group showed that there was low locomotion, low exploration and high anxiety this might be due to neurodegeneration of the frontal cortex which was supported by Madhavan et al. [46] that degeneration of the neurons present in the frontal cortex can affect voluntary movements. In the hyperglycemic+*Citrus medica* group there was increase in the frequency of hinding throughout the week of the treatment this might due to aversion of neurodegeneration both by chemical composition of Citrus media [47] and reversal of hyperglycemia from the third week by this plant extract.

The increase of walling frequency in the hyperglycemic group throughout the weeks showed that there is high anxiety this might be due to presence of neurodegeneration in the frontal cortex which is usually associated with hyperglycemia [46]. Robert et al. [56] reported that neurodegeneration diseases can cause high anxiety.

In the hyperglycemic+*Citrus medica* group there was an increase of walling frequency in the first week of treatment but at the sixth week there was a decrease. Hyperglycemia is associated with neurodegeneration [46] and also *Citrus medica* contains some chemical that avert neurodegeneration [47]. Since normoglycemic was achieved in this group in the third week and maintained throughout experimental period neurodegeneration would have been averted hence increase walling frequency.

The decreasing and flunctuating of grooming frequency in the hyperglycemic group showed that there was low exploration and high anxiety this might also be link to neurodegeneration diseases associated with hyperglycemia [46]. Findings of Robert et al. already reported that neurodegeneration of frontal cortex caused high anxiety.

In the hyperglycemic+*Citrus medica* group there was an increase in the frequency of defecation at the second week of the treatment which later started decreasing at the third week and maintained throughout the experimental period. The decrease in the rate of defecation was not significant to control. Increase rate of defecation was associated with anxiety which is usually due to neurodegeneration [57]. Also hyperglycemia is associated with neurodegeneration [46]. In this group, neurodegeneration was averted by attainment of normoglycemic from the third week and flavonoids, vitamins, phenols, lecitins, polypeptides and glycosides present in *Citrus medica* [47].

The high rate of the frequency of urine seen in hyperglycemic group shows that there was high anxiety this might be neurodegeneration [57] or ketoacidosis seen in chronic hyperglycemia [58-60]. In the hyperglycemic+*Citrus medica* there was an increase at the second week of the treatment which started decreasing from the third week till sixth week and when compared to control group there was no significant difference. Anxiety and hyperglycemia are associated with polyuria [61-63], in the hyperglycemic+*Citrus medica* group, the improvement might be due to reversal of hyperglycemia from the third week in this group and reduction of anxiety due to aversion of neurodegeneration as a result of chemical composition of *Citrus medica* and attainment of normoglycemia.

Conclusion

Aqueous leaf extract of *Citrus medica* possess antihyperglycemic effect and be an effective therapy in the management of hyperglycemia that leads to anxiety base on these research findings.

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