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Antipsychotic Property of Methanol Crude Extract of *Peperomia pellucida* (L) Kunth. (Piperaceae) Whole Plant against Ketamine Induced Psychosis in Mice

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Abstract

Medicinal plants have been discovered and used in traditional medicine practice since prehistoric times. Numerous plants contain diverse phytochemicals and possess pharmacological actions, many of which remain unassessed by rigorous scientific research of defined efficacy and safety. This study evaluated the antipsychotic activity of *Peperomia pellucida* methanol crude extract against ketamine induced psychosis in Swiss albino mice. The preliminary phytochemical analysis was carried out following standard procedures, while stereotypy test, Y-maze test, object recognition and forced swim test (FST), were the behavioral models used for the antipsychotic study. The Histopathology of the mice brain was also carried out. Statistical analysis was done using one-way ANOVA followed by Dunnett's post hoc test and $p < 0.05$ was considered significant. Phytochemical analysis revealed the presence of saponins, tannins, flavonoids and alkaloids in the pulverized plant. The crude extract at 125 mg\kg reduced stereotype behavior in Ketamine-induced mice. The extract enhanced cognition in the animals by producing a significant dose-dependent decrease in the number of alternate arm entries and increases the time spent in recognizing a novel object compared to the negative control. In the FST model, 500 mg\kg was more effective in decreasing immobility as compared to negative control. The histopathology of the mice brain showed that the extract at the dose of 500 mg/kg produced a similar effect to the standard drug and at 250 mg/kg produced a regenerative effect on brain of the mice.

The results established that *Peperomia pellucida* possesses antipsychotic property against positive, negative and cognitive symptoms of psychosis.

Key words: *Peperomia pellucida*, Antipsychotic, Y-maze, Stereotypy, Forced swim test

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INTRODUCTION

Medicinal plants are defined as plants used in traditional or modern medicine for their therapeutic properties. They contain active chemical compounds that have pharmacological effects on the body and can be used to treat or prevent various diseases and health conditions [1].

Plants synthesize hundreds of these chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential established biological activity have been identified [2].

A single plant contains widely diverse phytochemical content and pharmacological actions, many of these plants with medicinal potential remain unassessed by rigorous scientific research of defined efficacy and safety [3]. Drug research sometimes makes use of ethnobotany to search for pharmacologically active substances and the approach has yielded hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes; alkaloids, glycosides, polyphenol and terpenes [4, 5]. Research into African plants use showed that traditional medicine is commonly used to treat neurological disorders in West African region [6].

Peperomia pellucida commonly known as shiny bush plant, pepper elder, silver bush is a west African plant, characterized by succulent stems, shiny, heart-shaped, fleshy leaves and tiny, dot-like seeds attached to several fruiting spikes. It has a mustard-like odor when crushed. The plant *Peperomia pellucida* was found to have variety of chemical constituents including alkaloids, cardenolides, saponins and tannins, while anthraquinones was observed to be absent [7]. The stem also contains alkaloid, tannins, flavonoids and steroids except saponins [8]. Additionally, the roots of *Peperomia pellucida* was reported to contain alkaloid, tannins, steroids and carbohydrates [8]. The essential oil of the plant was found to contain carotol, hydroxylated sesquiterpene. acacetin, apigenin, isovitexin, pellucidatin, campesterol stigmasterol, pellucidin A among others [9, 10].

Peperomia pellucida (PP) has been used for treating abdominal pain locally, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain [11]. It is used topically for skin disorders such as acne and boil. In the western parts of Nigeria, the water extract is used to treat high blood pressure, urinary tract infections and insomnia. The plant is use among other recipe to treat mental disorder in southwestern Nigeria (unpublished).

Although, Khan *et al*., [12] investigated the antidepressant activity of the petroleum ether and ethyl acetate fractions of *Peperomia pellucida*, to the best of our knowledge, there is no scientific investigation on the methanol extract of this plant against positive, negative as well as cognitive symptoms of psychosis. Hence, this study aim is to investigate the antipsychotic property of *Peperomia pellucida* leaves methanol extract using different behavioral models in mice.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh whole plants of *Peperomia pellucida* of the family Piperaceae were collected at the Faculty of Pharmacy, Olabisi Onabanjo University premises in the month of September, 2021. The plant was authenticated at Forest Herbarium Ibadan (FHI), Ibadan, Oyo State. Herbarium samples were

prepared and deposited with FHI Number; 113414.

Experimental animals

Swiss albino male mice weighing 15 – 20 g were used for the study. The mice were obtained from animal house Olabisi Onabanjo University. The animals were acclimatized to the environment of the study for one week in a cage lined with wood shavings at room temperature. The animals were fed on standard animal pellets and given access to water *ad libitum*, with a 12 h light/dark cycle. The protocols of the experiments were in accordance to the National Institutes of Health Guide for care and use of laboratory animals and the Ethics Committee of the Olabisi Onabanjo University, Sagamu, Nigeria.

Preparation and extraction of the leaves of *Peperomia pellucida*

The fresh whole plant of *Peperomia pellucida* were washed properly to remove earthy impurities, weeds that were not part of the plant were removed. The leaves were dried under the shade for a period of two weeks for complete drying. The dried leaves were pulverizeded into powder with the aid of mechanical grinder. About 100 g of the pulverized leaves of *Peperomia pellucida* were macerated in 100% methanol (analar grade) for 72 h. The extracts were then filtered and the filtrate obtained was concentrated to dryness using rotary evaporator and regulated water bath (40^{o}C). The residue was re-macerated to obtain a maximum yield. The percentage yield was calculated and recorded [13].

Phytochemical analysis

The preliminary phytochemical analysis of crude extract was carried out according to standard protocols [14, 15], to detect presence of metabolites of pharmacological importance.

Grouping of animals

The mice were divided into five groups

Group 1: Each mouse received 125 mg/kg of methanol extract + ketamine 30 mg/kg

Group 2: Each mouse received 250 mg/kg of methanol extract + ketamine 30 mg/kg

Group 3: Each mouse received 500 mg/kg of methanol extract + ketamine 30 mg/kg

Group 4: Each mouse received ketamine 30 mg/kg (negative control)

Group 5: Each mouse received ketamine 30 mg/kg + haloperidol 0.5 mg/kg (positive control)

Behavioral assessment

Animals were assessed for behavioral effects using stereotype behavioral tests in a transparent observation chamber [13, 16], Y-maze tests, object recognition and forced swim test [17, 18, 19, 20, 21].

Stereotype behavior test

The effect of the crude extract of *Peperomia pellucida* was tested on ketamine-induced stereotype behavior in a transparent observation

chamber (L X B X H: 16 cm x 10 cm x 6 cm). Animals were pretreated orally with crude extracts at different doses (125 – 500 mg/kg), Haloperidol or distilled water for one hour, thereafter, ketamine (30 mg/kg i.p.) was administered to induce psychotic symptoms in the mice. Five minutes after ketamine injection, each mouse was placed in a transparent observation chamber to observe for repetitive behavior at every 2 min in 5 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min of the experiment. The stereotype behavior observed were scored as; 0= no stereotypy behavior; 1= head movement; 2= intermittent sniffing; 3= chewing; 4= intense licking. The transparent chamber was cleaned with 70% ethanol after each session, to remove residual odour [22, 23].

Y-maze Test

Spontaneous alternation is a measure of spatial working memory. The Y-maze can be used as a measure of short-term memory, general locomotor activity and stereotypy behavior [24]. Hence, spontaneous alternation was assessed using a Y-maze composed of three equally spaced arms (120˚, 41cm long and 15 cm high), the floor of each arm is made of ply-wood, 5 cm wide. The arms were labeled A, B, or C, arm A was used as the start arm and each mouse placed in arm A was allowed to move freely into the other arms (B or C). A complete arm entry is when the mouse with its tail completely enters the arm. The sequence of arm entries is manually recorded. An alternation is defined as entry into all three arms consecutively, for instance if the animal makes the following arm entries ACBBCABACABC, in this example, the animal made 12 arm entries, 6 of which are correct alternations. The percentage correct alternation was calculated as the total number of arms entered minus two multiplied by 100. The Y – maze test was carried out for 5 minutes. The apparatus was cleaned with 70 % ethanol and allowed to dry before next test session [21, 25].

Object recognition test

The mice were presented with two similar cubes (green colour) during the training session (familiarization session), after which one of the two cubes was replaced by a new object (red colour) during the second session (test session). Two identical cubes were placed in an open field for the mice to play for 5 min, and then there was a second 5 min session when one cube was replaced with an unfamiliar object. The exploration time for both objects during the test phase was recorded. The weights of the objects were heavy enough that the mice could not move them. All the objects used for this test have the same odour [17, 26].

Forced Swim Test (FST)

The forced swim test is a behavioral assessment of despair [27]. Animals were pre-treated orally with different doses of methanol extracts of *Peperomia pellucida* (125 – 500 mg/kg) for 1 h, the mice were then induced with ketamine (20 mg/kg) for 10 days before the test was carried out

24 h post-treatment. During the test session, mice were placed in a glass cylinder containing water and forced to swim. Swimming activity was recorded for 5 min after 1 min of acclimatization. Duration of immobility was observed and recorded; mice were considered immobile when they were floating motionless in water. The duration of immobility was taken as time of hopelessness or behavioral despair in the animal [18, 21].

Harvest of mice brain

The animals were euthanized by cervical dislocation 24 h after the end of the FST experiment. The mice were decapitated and the brains were excised for the histopathology study.

Method of histopathology of the mice brain

After the brain was excised, homogenization was carried out. The brain tissue was dehydrated using alcohol of various percentage (70%, 80%, 90%, 95% and 100%). The alcohol was then removed using xylene for 1 h. thereafter, molten paraffin wax at 58 – 60ºC was used to create an internal support for the tissue, thereby filling the interstitial space or matrixes in-between the cells. The embedded tissue was sliced using a microtome, mounted on a slide and stained with hematoxylin and eosin stains (H-E stains). The slides were viewed under the microscope to check for any cell damage.

RESULTS AND DISCUSSIONS

Peperomia pellucida is used ethnobotanically as medicine, food and flavoring agent in various parts of the world. The leaves and stems are frequently consumed as spicy leafy vegetable, cooked or in salads and condiment in many parts of the tropics. The ethnomedicinal uses of the plant vary depending on the region. Aerial parts, young shoots, leaves and whole plant are used in the form of decoctions, juice, paste, to treat several diseases such as fever, cold, cough, viral diseases, rheumatic pain, asthma, vaginal infections and kidney infections [27]. It has been used in traditional medicine in the treatment of abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, rheumatic joint pain and adjuvant in the treatment of psychosis.

The percentage yield calculated from 100 g of leaves sample (macerated twice) was 3.9%. This plant tested positive in the test for flavonoids, saponin, alkaloids and tannins but tested negative for anthraquinone, cyanogenetic glycosides, amino acid and steroids. Flavonoids are an important class of polyphenolics and a neuroprotective metabolite. Dietary flavonoid has been reported to involve a number of effects within the brain, including potential to protect against injury induced by neurotoxins, an ability to suppress neuro-inflammation, and the potential to promote memory, learning and cognitive function [28, 29, 30]. Therefore, their presence in plant is expected to promote memory function. Similarly, saponins are biological emulsifiers. Accumulated evidence suggests that saponins have significant neuroprotective effects on attenuation of central nervous system disorders, such as stroke, Alzhemier's disease, Parkinson's

disease, and Huntington's disease [31, 32]. Tannins (condensed tannins) are also a class of polyphenolics and have been established to have anticholinesterase activity and portray their neuroprotective effects in Alzheimer disease [32]. Alkaloids exert neuro-protective activities in numerous diseases such as epilepsy psychological disorders, cerebral ischemia and memory impairment, depression, anxiety among others [32].

Ketamine modulates neurotransmission at postsynaptic receptors such as N-methyl-D-aspartate (NMDA) glutamate receptors and gamma-aminobutyric acid (GABA) receptors. As an uncompetitive antagonist, it also blocks NMDA receptor and induces a dissociative anesthesia [33]. In rodents, acute sub-anesthetic doses of ketamine produce a schizophrenia-like symptomatology, including enhanced stereotype behaviors, cognitive and sensorimotor gating deficits, and impaired social interactions [34]. The antipsychotic activity of the methanol extract of the whole plant of *Peperomia pellucida* were evaluated using the stereotype behavioral model, Y – maze test, object recognition test and forced swim test. The mice were pretreated orally with 125 mg/kg, 250 mg/kg and 500 mg/kg of the extract, which was followed by induction of psychosis using ketamine 30 mg/kg after 1hour.

The effect of methanol extract of *Peperomia pellucida* on graded doses of 125, 250 and 500 mg/kg was assessed using the stereotypy model. In this test, haloperidol, a standard antipsychotic drug gave time-dependent calming effect right from 5 min of experiment as compared with ketamine (negative control) group ($p = 0.0026$). In mice administered with 125 mg/kg, stereotype behavior was observed in the first 15 min of the experiment at different doses of the extract. However, a tranquilizing effect was observed at 20 min, up till 60 min of the experiment. Out of all the doses, 125 mg/kg gave a better tranquilizing effect (Figure 1). In support of this findings, extracts of *Synedrella nodiflora*, *Palisota hirsuta* and *Costus afar* have been reported to antagonize stereotype behavior in mice [20, 35].

Y-maze and object recognition tests are models use in detecting cognition in mice [18, 21]. The reverse of ketamine-induce cognitive impairment by the methanol extract of *Peperomia pellucida* at graded doses of 125 - 500 mg/kg was investigated by recording the sequence of arm entry and number of arm entries using the Y-maze test. Mice pretreated with methanol extract of *Peperomia pellucida* at graded doses of 125 - 500 mg/kg produced a significant ($P < 0.0001$) dose-dependent increase in correct alternation compared to the negative control (Figure 2). This implies that as the dose of the extract increases, there was enhanced cognition in the memory of the animals. This study is in agreement with Saxena *et al.*, [36] and Sonibare *et al*., [21], which revealed that hexane extract of *Ficus carica*, and dichloromethane and ethyl acetate fractions of *Philenoptera cyanescens* restored memory dysfunction in the Y-maze model, respectively.

Furthermore, the methanol extract of *Peperomia pellucida* at graded doses of (125 mg/kg – 500 mg/kg) was assessed using object recognition test on time spent exploring familiar and novel object. The result showed significant ($p < 0.05$, $p < 0.01$, $p < 0.001$) dose-dependent increase in time spent exploring the novel object (Figure 3). The significance produced by 500 mg/kg is comparable with the standard drug. *Willughbeia cochinchinensis* was reported to prevent memory loss using the object recognition task in previous study [37].

Forced swim test has been used to detect negative symptoms of psychosis in rodents [16, 18]. Intra-peritoneal administration of ketamine (30 mg/kg) for 10 days repeatedly, increases the duration of immobility in experimental mice in the forced swim test. However, the protective effect of the administration of methanol extract of *Peperomia pellucida* at doses of 125 – 500 mg/kg consistently for ten days produced significant dose dependent decrease in the duration of immobility, when compared with the negative control (Figure 4). This implies that the mice administered with the different doses of extract were active and never feel depressed. This result support previous studies, Khan *et al*., [12] revealed the antidepressant activity of the petroleum ether and ethyl acetate fractions of *Peperomia pellucida*. Furthermore, Ashok Kumar *et al.*, [38], showed that *Amaranthus spinosus* possesses anti-depressant activity in rats using the Forced Swim Test.

From the histopathology result of the mice brain (Figure 5), it showed that mice induced with 30 mg/kg of ketamine and treated with 500 mg/kg of extract gave a complete regeneration of neuronal and neuraglia cells with no loss of function, this complete regeneration was similar to haloperidol. Mice induced and treated with 250 mg/kg of extract showed complete regeneration of the neuronal and neuroglia cells at the molecular layer and granular layer without loss of function. The mice that were induced for psychosis with 30 mg/kg of ketamine and treated with 125 mg/kg of the extract showed no significant effect of the extract on regeneration of the brain compared to the positive control (Haloperidol) group with complete regeneration.

CONCLUSION

Findings from this study provide scientific justification for the traditional use of *Peperomia pellucida* in the treatment of mental disorder. The plant was found to restore positive, negative and cognitive symptoms of psychosis in mice. This restoration maybe attributed to the phyto-constituents present in the plant such as tannins, alkaloids and flavonoids.

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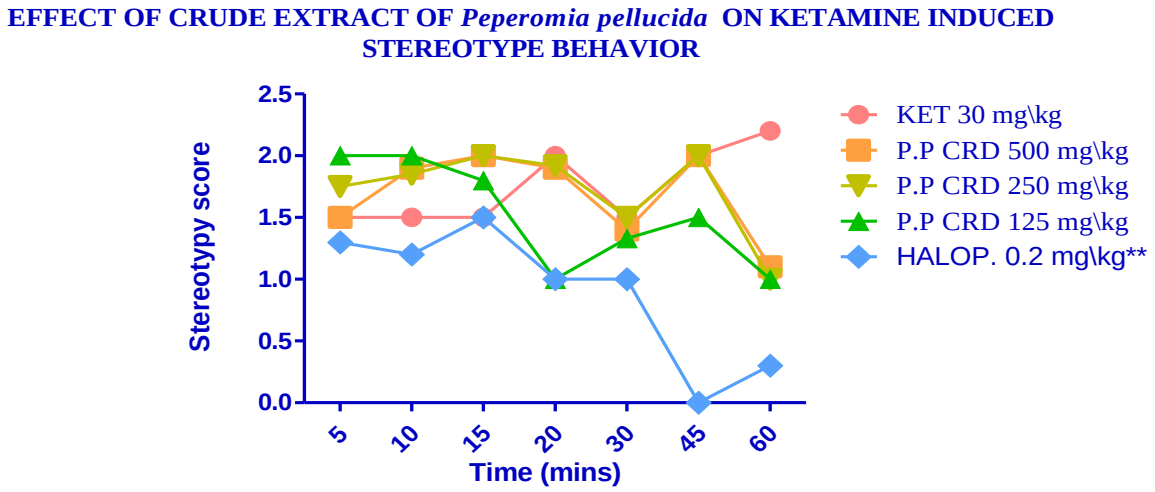

Figure 1: Effect of crude extract of *Peperomia pellucida*, Haloperidol and Ketamine on ketamine induce stereotype behaviour in a transparent observation chamber. Data were mean ± SEM (n = 6). **p = 0.0026, compared with negative control group (one-way ANOVA followed by a Dunnett's multiple comparison post hoc.

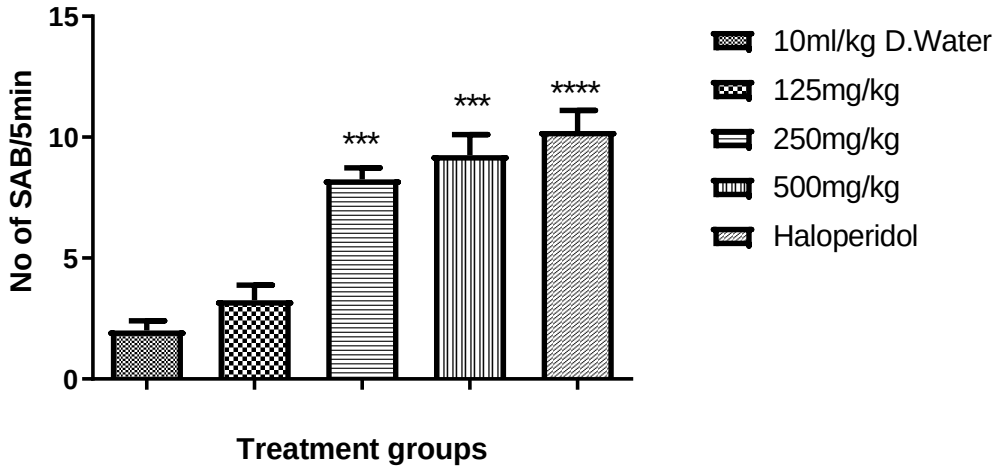

Figure 2: Effect of acute oral administration of *Peperomia pellucida* on Spontaneous Alternation Behavior (SAB) in mice in Y – maze Task. Data represent Mean ± SEM of six mice **** $p < 0.0001$, analysis was by one – way ANOVA followed by Dunette's Multiple comparison test compared to the vehicle group.

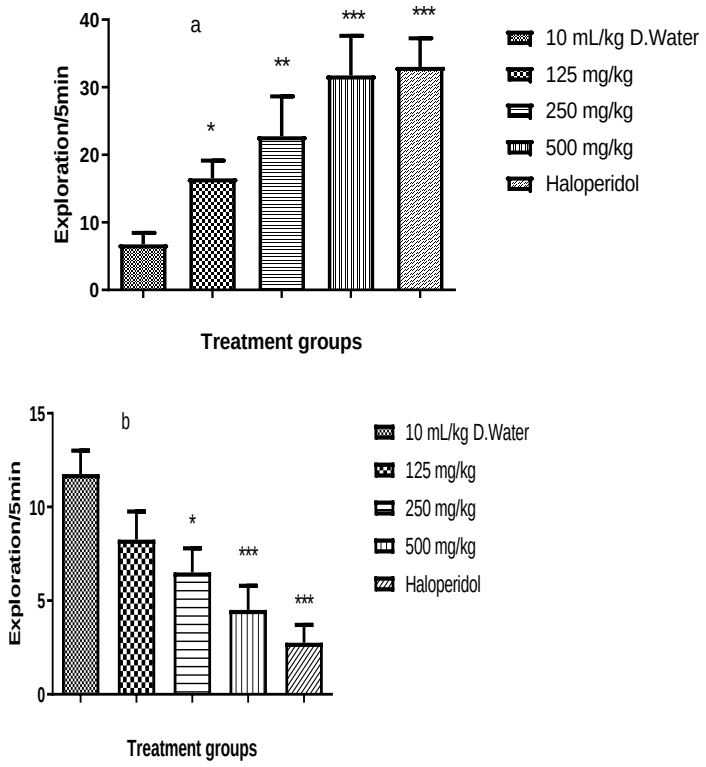

Figure 3: Effect of graded oral administration of *Peperomia pellucida* on Time spent exploring novel (a) and familiar (b) objects in object recognition test in mice. Data represent Mean ± SEM of six mice per group. *p<0.05, **p<0.01, ***p< 0.001, analysis was by one-way ANOVA followed by Dunette's multiple comparison test compared to the vehicle group.

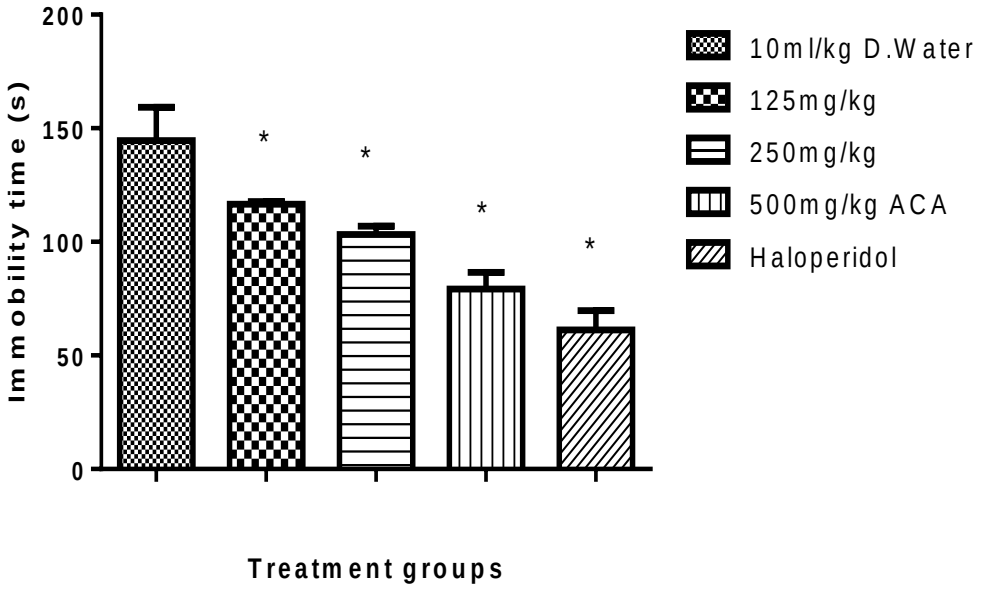

Figure 4: Effect of graded doses of crude extract of *Peperomia pellucida* on Immobility time in Force Swim Test in mice. Data represent Mean ± SEM of six mice. *p< 0.05, analysis was by one-way ANOVA followed by Dunett's Multiple comparison test compared to the vehicle group.

PHOTOMICROGRAPH OF MICE BRAIN TISSUES

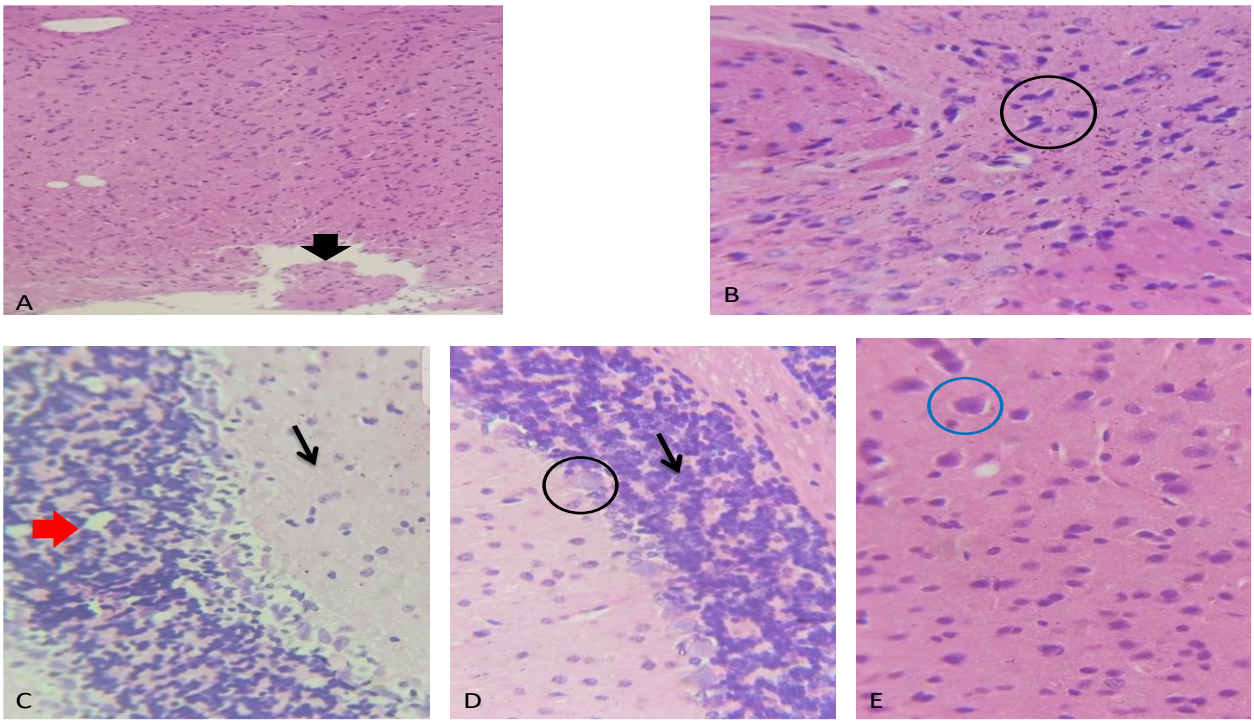

Figure 5: Photomicrograph of mice brain tissues

A. Negative control slide induced with ketamine alone shows severe degeneration of the tissue (black thick arrow) with loss of neuronal and neuroglial cells

B. Positive control slides induced and treated with the standard drug (haloperidol) shows complete regeneration of neuronal and neuroglia cells (black circle) with active functionality.

C. Induced and treated with 125 mg/kg of *Peperomia pellucida* extract shows no significant effect of the extract on regeneration of the brain granular layer (red thick arrow) with slight distortion, loss of neuronal cells at the molecular layer (black thin arrow).

D. Induced and treated with 250 mg/kg of extract shows complete regeneration of the neuronal and neuroglia cells at the molecular layer, purkinje cells (black circle), and granular layer (black thin arrow) without loss of functions.

E. Induced and treated with 500 mg/kg extract shows complete regeneration of the neuronal and neuroglia cells (blue circle) without any loss of functions H/E X400.