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Original Research Article

SPECTROSCOPIC, ANAESTHETIC AND HISTOLOGICAL ASSESSMENTS OF *VITEX DONIANA* SWEET ON RETICULAR FORMATION

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ABSTRACT

The study investigated spectroscopic property, anaesthetic and histological effects of *Vitex donianna* sweet. Spectroanalysis was conducted using atomic absorption spectroscopy (AAS) and Fourier Transform Infra-Red (FTIR) analysis; anaesthetic and histological assessments were carried out on four groups (A, B, C and D) of four rats each. Group A received 0.1ml of physiological saline, group B; 50mg/kg of ketamine alone (i.m), while groups C and D were administered 200mg/kg and 400mg/kg body weight (i.p) of the leave extract respectively 30 minutes later, 50mg/kg of ketamine was given intramuscularly. Rectal temperature, heart rate and respiratory rate (abdominal beats) were measured. The periods of onset and recovery from anaesthesia were recorded. Reticular formation of the brainstem was harvested, manually processed and stained for morphological changes and Nissl granules using H& E and Cresyl fast violet. The AAS and FTIR analysis revealed presence of zinc and functional groups such as ethyl, carboxyl, ether, acetyl and hydroxyl groups which have pharmacological relevance to the extract property. The results showed that administration *Vitex doniann* produced a dose dependent anesthetics effect. The light microscopic study reveal deeply stained nissls granules and few prominently stained pyramidal cells. In conclusion, this could translate into an increased synthesis of serotonin and glutamates neurotransmitters in the reticular formation, which accounts for the anaesthetic and reversal of ketamine effects observed in this study.

KEYWORDS: RAS, Vitex Donianna, Anaesthetic, Histology, Glutamate, Serotonin.

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INTRODUCTION

Interests in herbal medicaments have grown exponentially in recent years resulting in gradual shift from the synthetic drugs to plants products of medicinal value. This is ignited by the inefficacy of the existing orthodox therapies to provide cure for diseases while acting as a monotherapy or combined to achieve a synergistic pharmacologic activity. Majority of the Africa populace hold high opinion that herbal plants exhibit various activities on different parts of the body; this belief has constituted part of the traditional primary health care system. Some of the herbs have demonstrated central nervous system (CNS) excitatory and/or inhibitory effects, the later causing depression of the CNS [1]. The inhaled decoction of the leaf of Lippie Alba has been shown promotes sleep [2]. The roots and leaves are also used in treatment of some disease associated symptoms such as nausea, colic, epilepsy, rheumatic pains and inflammatory disorders [3].

Vitex doniana sweet also called black plum is of the family verbanaceae and the commonest of the vitex species grown in the open woodland and savannah regions of Northern, Western and Eastern Nigeria [4]. Its local names in Nigeria include; dinya (Hausa and Igala), Ucha-koro (Igbo) and Orin-ola (Yoruba).The stem barks and leaf decoctions of *Vitex donianna* are consumed locally in the North- Eastern part of Nigeria for the treatment of sleep disorders. *Vitex donianna* is used to treat mental diseases among the kanuri's of North Eastern Nigeria [5]. The aqueous extract of the stem bark of V. doniana sweet has been reported to cause depression and muscle weakness in experimental rats [6]. The administration of *V. doniana* sweet alone has been shown to maintain vital parameters such as temperature, respiratory and heart rates as well as prolonged the sleeping time in rabbits [6]. In a study, the stem back induced graded contraction of the uterine musculature and potentiated the contractile activity of prostaglandin, ergometrine and oxytocin [7].

The above herbal effect suggests a clear CNS depressant activity as it is experienced using anaesthetic drugs such as ketamine [8]. These anaesthetic drugs exert depressant effects on the reticular formation, a set of midline connected nuclei of the brainstem that are responsible for regulating the biological cycles of wakefulness and sleep transitions. Particularly, the ascending reticular activating system (ARAS) is in a constant cross talk with other relevant parts of the brain such as the ventral lateral posterior nucleus of the hypothalamus to maintain a state of on and off switch between sleep and wakefulness [9]. Hence, most anesthetic agents influence the state of unconsciousness by exerting inhibitory effect on the RAS via the serotonin neurotransmitter modulation.

In addition to the above property, anesthetics also elicit physiologic states such as analgesia, inhibition of sensory and autonomic reflexes, and skeletal muscle relaxation [8]. Consequently, an ideal anesthetic agent is expected to produce smooth and rapid loss of consciousness, wide margin of safety, devoid of side effect while promoting a swift recovery phase [8]. Rarely does any single anaesthesia effectively satisfy these criteria, thus classically; in modern practice of anaesthesiology the combina-tion of anesthetics is advocated for induction and maintenance of anaesthesia to achieve balanced anaesthesia techniques.

Ketamine, an intravenous anaesthesia is often used for induction of anesthesia while isoflurane and nitrous oxide inhalation anesthetics are mostly used for the maintenance of the anesthetic state. Inhalation anesthetics are associated with respiratory depression, a decreased arterial blood pressure and cerebral metabolic demand while increasing cerebral blood flow [8]. We Identified gaps within these existing anesthetics including: (i) unavailability of a single anaesthesia that meets all criteria for a balanced procedure (ii) Lack of available oral anesthetic agent for maintenance when ketamine is used for induction instead of an inhaled anesthetic (iii) pivotal is the need for a natural (herbal) agent that will possess the above properties and yet have little or no adverse physiologic or anatomical effects, particularly, on the respiratory rate, heartbeat, temperature and reticular activating system (RAS) of the brain stem. Notably, not much has been done on the central nervous effects of the various extracts of the *Vitex doniana* sweet particularly the leaf. Therefore, this study investigated the effect of the leaf extract of *Vitex doniana* (sweet) on the histology of the reticular activating system of wistar rats and accessed the anesthetic property of the effect of varying dose of the extract on the sleep-time, recovery time and other physiologic parameters. This is to further justify the folklore use and an attempt to bridge the gaps aforementioned.

MATERIALS AND METHODS

Authentication and preparation of the plant material: The fresh leaves of *V. doniana* sweet were collected from Ezza community in Ebonyi State (06° 152 183 N; 8° 052 553 E) during the month of August, 2015, and authenticated at the Department of Biotechnology, Ebonyi State University, Nigeria by Dr Nnamani, C.V. The fresh leaves of *V. doniana* sweet were washed in distilled water; it was cut into smaller sizes and room-dried for seven days to prevent the ultra-violet rays from inactivating the chemical

constituents. The dried leaves were pulverized into fine powder in a mortar using pestle and weighted using the electronic weighing scale (Binatone) with sensitivity 0.0001g

Eugenia uniflora

Spectroanalysis:

Atomic Absorption Spectroscopy (AAS)/ Fourier Transform Infra-Red (FTIR) Analysis: Portion of the pulverized leaves was used for Fourier Transform Infra-Red (FTIR) and Atomic Absorption Spectroscopy (AAS) analyses. Fourier Transform Infra-Red (FTIR) Analysis of *V. Doniana* was carried out using the Schimadzu FTIR- 8400S Spectrophotometer Europe. The sample was prepared using KBr and the analysis was done by scanning the sample through a wave length rage of 400 to 4000cm^{-1}. The sample was first digested using nitric acid (HNO3) and hydrochloric acid (HCl) with the addition of deionized distilled water. Then, the Atomic absorption spectroscopy which is the measurement of the radiation absorbed by the unexcited atoms of the chemical substance that has been aspirated into a flame or in the absence of a flame directly into the path of radiation was also performed. Concentrations of nickel, copper, iron, lead, cadmium and zinc were determined using a UNICAMM 969 atomic absorption spectrophotometer. Calibration curves for each element were prepared and the concentrations of the elements were extrapolated from their respective plots Tables 1 and 2.

Extraction of leave of V. Doniana: The pulverized *V. Doniana* sweet (500g) was macerated in 1.5 litre of analytical graded 95% ethanol in extraction bottle and allowed to stand with continuous stirring for 48hrs. The mixture was filtrated with the whatman filter paper No 1; thereafter the filtrate was allowed to stand on the bench to allow for total evaporation of the residual solvent. The pulverized *V. doniana* yielded 37.4% of extract, this crude extract was subsequently referred to as extract in this study. It was kept in a refrigerator at 4°C until required for use. Dissolving 1g of extract in 10ml of distilled and administered per body weight of each animal.

Drugs and chemicals: Ketamine and Thiopental Sodium (Rotex Medica-Trittau-Germany) were purchased from a local pharmacy store. All other chemicals were of laboratory graded and freshly prepared as at when required.

Animals: A total of sixteen wistar rats of both sexes age 8 to 10th week old average weight 150 g were obtained from animal laboratory of the Department of Anatomy, Ebonyi State University Abakaliki. The rats were housed in a temperature-controlled (20 - 22°C) room under 12 h : h light/dark cycles with free access to standard rodent chow food pellets and tap water for two weeks habituation period. After this habituation period, at the age of 10 to 12 weeks, the rats were randomized into three and weight-matched groups, n = 4): group A (controls) and B and C experimental groups by generating random numbers using standard.

Experimental grouping

Group A: Control received normal saline 0.1ml

Group B: Rats given ketamine 50mg/kg

Group C: Rats given 200mg/kg extract + ketamine 50mg/kg

Group D: Rats given and 400mg/kg of extract + ketamine 50mg /kg

Protocol for anesthetic evaluation: The effect of *Vitex doniana* (sweet) leaves extract on ketamine anaesthesia, the method of Sanni (6) was adapted with slight modification. Four groups of four rats each were used, group A received 0.1 normal saline, group B was given 50mg/kg of ketamine alone while groups C and D were administered 200mg/kg and 400 mg/kg body weight of the leave extract respectively 30 Minutes prior to the administration of ketamine. All extract treatments were through intra-peritoneal route, while ketamine was given

intramuscularly. The vital parameters such as rectal temperature, heart rate (measured by the use of stethoscope) and respiratory rate (abdominal beats) were measured prior to, and after the administration of the agents. The periods of onset and recovery from anaesthesia were recorded.

Histological Evaluation: All the experimental animals were anaesthetized using thiopental sodium. In the unconscious state, the brain was carefully dissected out of the skull of each of the rat using a surgical blade and fixed in 10% neutral formal saline for 48hr. Thereafter, a sagittal section was made across the brain stems dividing it into two asymmetrical sections to expose the reticular activating system in the midline. Each specimen was dehydrated ascending grades of alcohol (50%, 70%, 90% & 95%) and embedded in a paraffin block thin sections of 5µm. The sections were stained with hematoxylin and eosin for general morphological observation and Cresyl fast violet for examination of Nissl granules as described by Elizabeth [10].

RESULTS

Elemental analysis

Table 1: Mean concentration of some heavy metals in the samples.

V. Doniana	(mg/kg) ±SD
Cu	19.20±0.0003
Pb	53.90 ± 0.0004
Ni	8.20 ± 0.0012
Zn	48.60 ± 0.0006
Fe	290.90 ± 0.0046
Cd	1.10 ± 0.0006

Fig.1: FTIR Spectrum of *V. Doniana.*

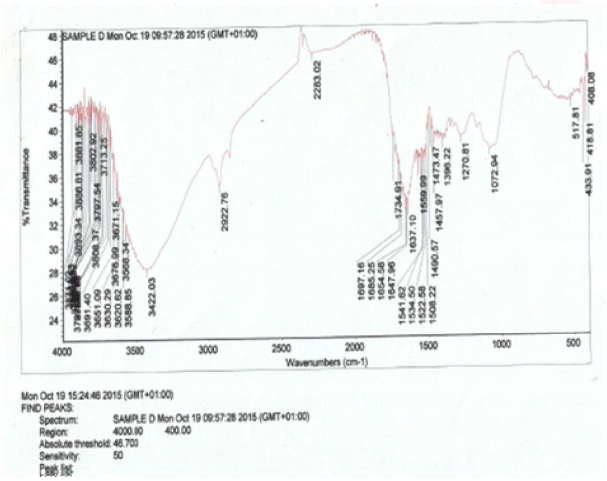

Table 2: Peak, Frequency and Assignment of FTIR Absorption Bands of V. Doniana.

S/N	Peak	Intensity	Assignment/functional group
1	408.08	44.769	C-C bending
2	517.81	41.844	CO2 bending
3	1072.94	37.924	C-O stretch
4	1396.22	38.611	O-H bending
5	1457.97	38.048	O-H stretch
6	1508.22	36.797	N-O asymmetric stretch
7	1647.96	32.575	C-O stretch
8	1734.91	38.729	C=Ostretch due to acetyl group
9	2922.76	35.136	C-H stretch
10	3322.03	28.056	O-H stretch

Anaesthetic Evaluation: In group A, the animals carried on a normal life style; slept when they wanted to and stayed awake and alert anytime. In group B, it was observed that the animals were calm at first on extract administration; they maintained that state but became alert after some time. In group C, on ketamine induction, the rats exhibited unsteady gait, they staggered for few minutes and then became unconscious with moderate muscle relaxation, speedy heart rate and respiratory rate, and there was very minimal change in temperature. In group D, A higher dosage of extract was administered and after 45 minutes ketamine was administered. In this group, there was no total anaesthesia rather they made staggering movements from time of administration till time of full recovery. Still under the influence of the anesthetic agents, after some minutes of administrations, they became hyper active, running round the cage frantically. Between 45 – 60 minutes, they became calm and soon fell asleep which lasted for about 30 minutes. There was slight increase in temperature. A greater increase in heart rate and respiratory rate were recorded. The results of physiological parameters: temperature, heart rate and respiratory rate were analyzed and represented in figures 2, 3, and 4 below.

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Fig. 2: Shows heart rate changes (beats/mins) within and between groups before and after the experimental. There was significant ($p<0.05$, ANOVA) increase in heart rate in all the treatment groups compared to control.

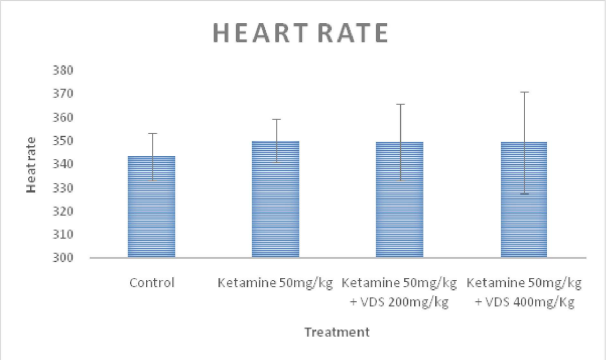


Fig. 3: Shows temperature changes (in Degree Celsius) within and between groups before and after the experimental.

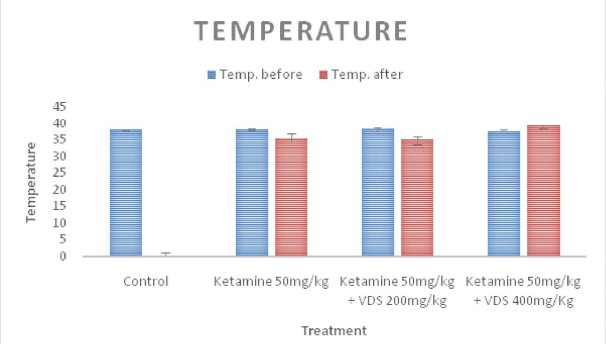


Fig. 4: Shows durations of anaesthesia (minutes) within and between groups before and after the experimental.

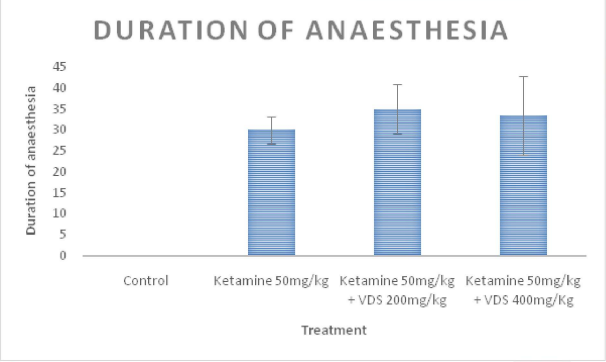
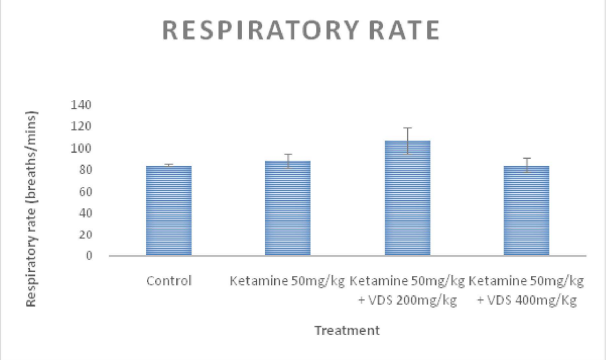
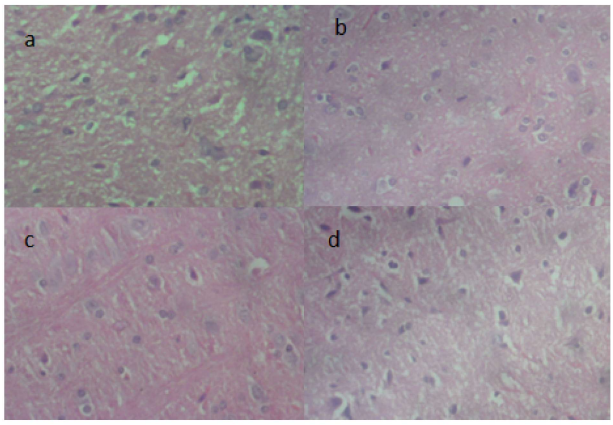


Fig. 5: Shows respiration rate changes (Breaths/mins) within and between groups before and after the experimental. There was significant ($p<0.05$, ANOVA) increase in respiration rat in ketamine with VDS 200mg/kg group compared to control.



Histological findings

Fig. 7: Sections of RAS of rat (a) Control (b) Ketamine (c) Low dose extract (d) High dose extract. Cresyl Fast Violet. X200



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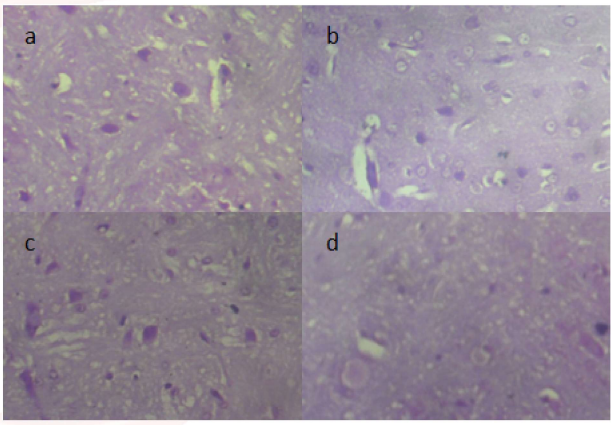

DISCUSSION

The extract of the Vitex doniana leaf contains alkaloids, flavonoids, cardiac glycosides, terpenes and steroids and resins but tannins, saponins, balsam and phenols were absent [3]. The result of AAS shown in Table 1 reveals high mean concentrations of Iron and lead. Iron is an essential element needed by humans in protein synthesis and in the formation of some blood cells. Next to iron in terms of importance is zinc. Zinc is an essential mineral that stimulates the activity of about 100 enzymes in the body. It also supports healthy immune system and it is necessary in the synthesis of DNA, essential for wound healing and supports the healthy growth and development of the body during adolescence, childhood and pregnancy.

The concentration of zinc in the studied sample was far below that of iron. On the other hand, concentration of copper in sample was relatively low compared to other essential metals.

However, copper is a trace element needed by the body in minute concentration. Copper is required in the formation of haemoglobin, red blood cells as well as bones. Copper helps in the formation of elastin as well as collagen and is therefore necessary for wound healing. Deficiency of copper may lead to increased blood fat levels. Copper is also necessary for the manufacture of the neurotransmitter noradrenaline and for the pigmentation of human's hair and from the analysis copper was 19.20 mg/kg. The concentrations of some heavy metals such as nickel, lead and cadmium were higher in the studied sample hence the heavy metals in the studied samples were above permissible limit set by World Health Organization and Food and Agricultural Organization of the United Nations.

The *vitex doniana* sweet leaf FTIR spectrum showed specific functional groups such as C-C, C-O, O-H and N-O. The presence of these carbon chained compounds signifies that the leaf extract contains organic compounds. The analysis of the spectrum presented in table 2. Shows that the functional groups C-C bending has the highest level of intensity (44.769) at the smallest peak (408.08) while O-H stretch has the least intensity (28.056) of all the functional groups at the highest peak (3322.03). These revealed the following functional groups: ethyl, carboxyl, ether, acetyl and hydroxyl groups. The presences of these functional groups will determine the chemistry of the vitex doniana. These will also impact specific pharmacological, toxicological and physiological properties on the extract. Similarly, anaesthetic agents also contain the ether, ethyl and carboxyl groups that constitute their pharmacological properties. The detection of the above functional groups in the leaf extract of vitex doniana suggests it possess anaesthetic property.

Anaesthetic agents are known to depress the CNS in order to induce the state of unconsciousness. They exert various effects on the heart rate, respiratory rate, temperature, cerebral blood flow and pressure. Thus, these vital parameters are monitoring of in patients under anaesthetic influence and considered in the choice of suitable anesthetic. In this study, the control group A maintained normal sleep cycle and vital parameters. Group B (50mg/kg) calmed the animals but in group C (200mg/kg) the duration of anaesthesia was short compared to recovery. In group D, the administration of 400mg/kg of the extract prolonged the onset of anaesthesia and recovery period. This was similar with the findings of [6] which showed that the extract of stem bark of vitex doniana enhances anesthetic tie of ketamine. Besides the anaesthetic effect, the vital parameters; temperature, heart rate and respiratory rate remained fairly normal. Thus the ethanol extract of the leaf of vitex doniana altered ketamine anesthetic properties in dose dependent manner.

Neuronal cells have centric nuclei within the cytoplasm, Result of the light microscopic study reveal normal neuronal histoachitecture in the control group. This was expected since the group didn't receive any drug hence the normal integrity of the neurons were preserved. However, the ketamine administration alone in group B altered the neuronal histoarchtecture, several neurons showed eccentric or pyknotic nuclei. This is typical of cellular adaptive changes in pathologic sites, which can also be attributed to the adverse effect of ketamine. Ketamine neurotoxic effects have been consistently reported in previous studies [11].

The co-administration of the extract in various dosages (200mg/kg and 400mg/kg) with ketamine showed relatively normal neuronal histoarchtecture depicting the reversal of ketamine neurotoxic effect with the effective dose of 400mg/kg which also imply a clear dose dependent effect. Several agents have been known to reverse the effects of ketamine including benzodiazepine and antipsychotics: clozapine, chlorpromazine, neostigmine and herbal agents such as memantine have exhibited/demonstrated anti-ketamine activities [10-13]. Ketamine is an NMDA receptor anta-gonist that depletes glutamergic activity where as this agent causes reversal of ketamine effect which suggests possible increased in the glutamergic activity particularly in pyramidal neurons.

The Nissls substance is a granule in neurons; its presence in neurons has been linked to synthetic activity of neurohormones and neurotransmitters. Thus increase Nissls granules reflect in tissue section of Cresyl fast violet

stained by increase intensity of stain, likewise the decrease staining as decrease intensity. In the present study, normal Nissls activity was observed in the control while the ketamine group showed decrease Nissls stain thus confirmed the earlier H and E result of the same group where cytoplasmic vacoulation was seen. This implies actual depletion of neurons and consequently decreased synthetic activity in the RAS. Functionally decrease Nissls is translated as decrease neurotransmitter synthesis in the region of particular the serotonin transmitter is known to modulate the sleep-wake transition that characterised the biological clock. On the contrary, the extract revealed deeply stained Nissls granules with few prominently stained pyramidal cells. This signifies an increased pyramidal cells synthetic activity in the RAS. Thus, serotonin and glutamates neurotransmitters increase secretion this accounts for the anesthetic and reversal of ketamine effects observed in the groups earlier.

CONCLUSION

The vitex doniana leaf contains functional groups which impacts anesthetic effect. It also enhances ketamine duration of anaesthesia and histologically, reversed ketamine neurotoxicity and enhanced Nissls granules activity in the RAS neurons.

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Conflicts of Interests: None

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Ethical Consideration: Ethical issues including plagiarism, misconduct, data fabrication, falsification, double submission and publication have been carefully checked. All conditions and handling of the animals during the experiments wereapprovedby the Ethics Committee of the Faculty of basic Medical sciences and were carried out according to the ethics of the institution and national institute of health guide for care, handling and use of laboratory animals.

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