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Panax quinquefolium involves nitric oxide pathway in olfactory bulbectomy rat model

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HIGHLIGHTS

- Depression is associated with decline in mental and functional capacities.
- Olfactory bulbectomy (OBX) is a reliable model to mimic depression like behavior.
- Therapeutic effects of *Panax quinquefolium* (PQ) against OBX induced depression
- Nitric oxide (NO) is an intercellular messenger found in the brain.
- Possible involvement of nitric oxide pathway in the protective effects of PQ

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ABSTRACT

Olfactory bulbectomy (OBX) is a well known screening model for depression. *Panax quinquefolium* (PQ) is known for its therapeutic potential against several psychiatric disorders. Nitric oxide (NO), an intercellular messenger has been suggested to play a crucial role in the pathogenesis of depression. The present study was designed to explore the possible involvement of NO mechanism in the protective effect of PQ against olfactory bulbectomy induced depression. Wistar rats were bulbectomized surgically and kept for a rehabilitation period of two weeks. PQ (50, 100 and 200 mg/kg; p.o.) alone and in combination with NO modulators like L-NAME (10 mg/kg, i.p.) and L-arginine (100 mg/kg; i.p.) were then administered daily for another two weeks. Ablation of olfactory bulbs caused depression-like symptoms as evidenced by increased immobility time in forced swim test, hyperactivity in open field arena, and anhedonic like response in sucrose preference test. Further, OBX caused elevation in serum corticosterone levels and increased oxidative–nitrosative damage. These deficits were integrated with increased levels of neuroinflammatory cytokines (TNF-α), apoptotic factor (caspase-3) and a marked reduction in neurogenesis factor (BDNF) in both cerebral cortex and hippocampal regions of bulbectomized rats. Treatment with PQ significantly and dose-dependently restored these behavioral, biochemical and molecular alterations associated with OBX. Further, pretreatment of L-NAME with subeffective dose of PQ (100 mg/kg) significantly potentiated its protective effects; however L-arginine pretreatment reversed the beneficial effects. The present study suggests that protective effect of *P. quinquefolium* might involve nitric oxide modulatory pathway against olfactory bulbectomy-induced depression in rats.

1. Introduction

Olfactory bulbectomy (OBX) is a well known animal model of depression that leads to a variety of behavioral alterations, many of which are similar to those seen in patients with major depression [1,2]. OBX is a reliable model to evaluate anti-depressant activity since its behavioral changes are normalized following chronic but not acute administration of antidepressants [2]. OBX is associated with a variety of behavioral abnormalities such as hyperactivity in the open-field test [3], increased immobility time [2] and anhedonia like state in sucrose preference test [4]. These behavioral changes are independent of anosmia and result due to retrograde neuronal degeneration after bulbectomy [2]. Further, removal of olfactory bulbs has also been reported to reduce neurogenesis and promote neuronal cell death in different regions of the brain, a putative pathogenic mechanism in depression syndrome [5,6]. Although the current pharmacotherapy of depression includes a battery of drugs, clinicians are still looking for alternative therapies to exploit herbal medications for the treatment of psychiatric disorders. The approach towards development of dietary and medicinal phytochemicals as novel therapeutics may prove to be a useful tool in recognition of natural medicines globally.

Panax quinquefolium; PQ (American ginseng) belonging to the family *Araliaceae* is a native plant of North America and cultivated in many

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countries. The active constituents responsible for most of the central nervous system (CNS) related bioactivities of PQ are ginseng saponins, namely ginsenoside [7]. Ginsenosides are well-known for their antioxidant and free radical scavenging properties [8]. Studies have shown antidepressant effects of oral ginsenosides in both forced swimming test and chronic mild stress model of depression [9]. Further, ginsenosides are also well known for their neuroprotective effects in several animal models [10,11]. Earlier, studies from Sheikh et al. [12] showed that stress induced alteration in plasma corticosterone levels in rats was normalized on treatment with PQ. Saponins derived from PQ have shown a significant attenuation effect on apoptotic factor (caspase-12) [13] and are known to protect hippocampal neurons against ischemia [14]. These results clearly show the neuroprotective potential of ginsenosides; however, the exact cellular or molecular pathway in their protective effect has not been reported so far.

Nitric oxide (NO) is an intercellular messenger in the brain, synthesized from L-arginine by nitric oxide synthase (NOS) and plays an important role in synaptic plasticity, learning, memory, aggression and depression [15]. Nitric oxide has an unpaired electron, and therefore acts as reactive free radical species. The generation of reactive nitrogen species is associated with nitration of proteins [16] and lipid peroxidation [17] and promotes carbonylation [18]. Inhibition of NOS is known to produce anxiolytic and antidepressant-like effects in experimental animal models [19]. Inhibition of neuronal or inducible NOS (nNOS) in the rat hippocampus exerts antidepressant-like effects [20]. Moreover, reports have suggested that L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) is an important signaling pathway involved in depression [21]. With this background, the present study attempts to elucidate the behavioral, biochemical and molecular aspects of PQ with respect to its antidepressant activity against olfactory bulbectomy model and to further investigate the involvement of nitric oxide signaling pathway.

2. Materials and methods

2.1. Animals

Twelve week old male Wistar rats (200–240 g) were procured from Central Animal House, Panjab University, Chandigarh and from Animal House of Panacea Biotec Ltd., Lalru (Panjab). Male rats were chosen to avoid the influence of female estrogen hormone on depression like behavior. Animals were housed under standard (25 ± 2 °C, 60–70% humidity) laboratory conditions and maintained on a 12 hour natural day–night cycle, with free access to food and water. Animals were acclimatized to laboratory conditions before the experimental tests. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Panjab University (IAEC/282/UIPS/39 dated 30/8/12) and conducted according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines of the Government of India.

2.2. Surgical procedure (olfactory bulb ablation)

After the accommodation period, animals underwent either olfactory bulbectomy or sham surgery. Animals were anesthetized with ketamine (75 mg/kg, i.p) and xylazine (5 mg/kg, i.p) combination prior to surgery. Bilateral olfactory bulb ablation was performed as described by different investigators [22]. The animals were fixed in a stereotactic frame (Stoelting Co., USA), 1 cm rostral–caudal midline incision was made in the skin of the head, and two small burr holes (2 mm in diameter) were drilled into the skull 6 mm rostral of bregma and 1 mm lateral of the midline. Both olfactory bulbs were removed by suction and hemostatic sponge (AbGel, absorbable gelatin sponge USP, Srikrishna Laboratories, India) was inserted into the cavity to control bleeding. The incision was then closed with absorbable sutures (Ethicon 4-0, absorbable surgical sutures USP (Catgut), Johnson and Johnson, India) and animals were injected with sulprim injection® (each ml containing 200 and 40 mg of sulfadiazine and trimethoprim respectively), intramuscularly (0.2 ml/300 g) once a day for 3 days to prevent post surgical infection. Sham animals were given similar treatment as OBX animals except the removal of the olfactory bulbs. The success and validation of the OBX surgery were verified by using two methods: (a) dissection and direct observation of remaining olfactory bulb tissue and by (b) measurements of key behavioral variables altered by OBX, namely hyperactivity behavior during open field test. The OBX/Sham animals were housed singly in cages for two weeks (14 days) of surgical rehabilitation period and drug treatments were started after that. Pictogram of the entire protocol is represented in Fig. 1.

2.3. Drugs and treatment schedule

P. quinquefolium (American ginseng), L-NAME and L-Arginine were purchased from Sigma chemicals Co. (St. Louis, MO, USA). ELISA kit for TNF-α and caspase-3 was purchased from R&D Systems (USA). While ChemiKine™ Brain Derived Neurotrophic Factor (BDNF) kit was procured from Millipore (USA). All other chemicals used for biochemical estimations were of analytical grade. The animals were randomly divided into nine experimental groups with eight animals in each. Out of the total of 72 animals used in the study 6 animals died of surgery (similar to 5–10% mortality reported in olfactory bulbectomy experiments) and were replaced by fresh animals and surgery was performed. The first and second groups were named as sham and OBX (ablation of olfactory bulbs) control groups respectively. *P. quinquefolium* (PQ) (50, 100 and 200 mg/kg) was treated as groups 3–5 respectively. Pretreatment of L-NAME (10 mg/kg) and L-arginine (100 mg/kg) with PQ (100 mg/kg) served as groups 6–7. Treatment of L-NAME (10 mg/kg) and L-arginine (100 mg/kg) per se was categorized as groups 8 and 9 respectively. *P. quinquefolium* (PQ) was prepared in peanut oil where as L-NAME (10 mg/kg) and L-arginine (100 mg/kg) were dissolved in normal saline and administered orally on the basis of body weight (0.5 ml/100 g). The doses of *P. quinquefolium* were selected on the basis of literature [21]. Solutions were made freshly at the beginning of each day of the protocol. Drugs were administered daily once a day for a period of 14 days.

2.4. Behavioral assessment

2.4.1. Sucrose preference test

The rats were tested for sucrose consumption as described earlier [23]. Animals were housed individually throughout the test duration and presented two bottles simultaneously in the home cage, one containing a 1% w/v sucrose solution and the other containing standard drinking water during the 48 h training session. To prevent the preference to position, the location of the two bottles was varied during this period. After an 18 h period of food and water deprivation, an 8 h test session was conducted. The amount of liquid remaining in each bottle was measured at the end of the testing period. The sucrose preference score was expressed as percent of total liquid intake. Sucrose preference (SP) was calculated according to the following equation:

$$SP = \left(\frac{\text{sucrose intake(g)}}{\text{sucrose intake(g)} + \text{water intake(g)}}\right) \times 100.$$

2.4.2. Open field exploration

Open field behavior of rats was recorded in a circular arena of diameter 80 cm, surrounded by a 30 cm high wooden wall [24]. The arena painted white, was divided in to 25 small sections. Each rat was carefully placed in the center of circular arena and allowed to explore the open field for 5 min. During this period, the ambulatory activity, in terms of the number of sections crossed, and the frequency of rearing were