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ORIGINAL ARTICLE

Thuja Occidentalis I. Expedites Functional Recovery after Sciatic Nerve Crush Injury in Mice

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ABSTRACT

This study was conducted to find the potential of Thuja occidentalis (crude powder) in promoting the rate of recovery following the sciatic nerve injury in a mouse model. Thuja occidentalis was administered orally at a dosage of 2g/kg body weight. The motor functional recovery was measured using muscle grip force, sciatic functional index (SFI), and muscle weight. While the hotplate test and formalin test were performed to measure the recovery of sensory functions. Other biochemical tests were performed to analyze oxidative stress. We noted an early recovery of motor functions in the treatment group as determined by SFI ($P<0.001$) and grip strength test ($P=0.01$). Additionally, the mass of Gastrocnemius ($P=0.04$) and the Tibialis anterior ($P=0.008$) muscle was also recovered in response to the treatment. Similarly, it significantly expedites the recovery of sensory function as checked by the hotplate test ($P=0.005$). Moreover, the reduced oxidant status ($P=0.03$) and elevated antioxidants capacity ($P=0.005$) were also found in the treatment group. Collectively, all of these findings highlight that the Thuja occidentalis possesses the potential to accelerate functional recovery. The enhanced antioxidant capacity and reduced oxidants status of the biological system appears the possible reason for this improved functional regain after the sciatic nerve crush.

Keywords: Peripheral nervous system, nerve injury, oxidative stress, sensorimotor functional regain, Thuja occidentalis.

INTRODUCTION

The peripheral nervous system comprises long delicate thread-like bundles of axons called ‘nerves’ responsible to connect the nervous system with the whole of the body. Despite having excellent anatomical protective tissue around long distant nerves, they are more likely to be harmed as a consequence of any stressful incident which eventually leads to loss of respective bodily functions or permanent physical disability due to sustained denervation of effector tissues (i.e., muscle) for an unlimited time. An injured peripheral nerve has an innate ability to regenerate itself but it has to go through a series of degenerative processes that further act as a precursor for the initiation of the process of regeneration which is a time-consuming process and its accomplishment depends on the severity of the injury. In-time evaluation of nerve injury’s type and selection of an optimal therapeutic option accordingly is the prerequisite to attaining in-time and precise nerve regeneration (Aziz et al., 2019; Rasul et al., 2019; Razzaq et al., 2020a). Over the last few years, peripheral nerve trauma has become a serious health concern due to the increasing rate of accidents particularly in overcrowded underdeveloped countries. Various treatment alternatives exist and are used to restore sensorimotor functions following such injuries, they do not guarantee the recovery of complete and exact functions. In such conditions of incomplete functional recovery, muscular atrophy irreversibly worsens the condition (Lopez et al., 2019; Razzaq et al., 2020b). The exploration of the therapeutic agents (either natural or synthetic), potent enough to accelerate injured nerve regeneration, is still being awaited to achieve this unmet objective of accelerated functional recovery to avoid everlasting disability (Kamran et al., 2020; Sajid et al., 2021).

Plants, being the richest source of potent compounds, have always been considered for discovering effective interventions against life-threatening ailments for the last few decades. Phytochemicals, being non-hazardous, have established their worth as promising candidates for drug development because of having tremendous pharmacological actions like anti-oxidant, anti-nociceptive, anti-inflammatory, anti-proliferative, anti-cancerous, anti-HIV, as well as several others. They are also reported to be effective against Alzheimer’s disease (AD), Huntington’s disease (HD), and Parkinson’s disease (PD) (Imran et al., 2019a; Maqbool et al., 2021; Zafar et al., 2020, 2021).

Thuja occidentalis (T. occidentalis), a native European tree, is generally utilized in phytotherapy and homeopathy. It is also known as eastern arborvitae or northern white-cedar. This plant exhibits anti-oxidant, anti-inflammatory, anti-proliferative, anti-convulsant, and analgesic actions (Lokesh et al., 2011; Naser et al., 2005). Being part of folk medicine, this plant has been considered a good candidate for the formulation of anxiolytic, nootropic, and anticonvulsant drugs (Lokesh et al., 2011). Moreover, it has also been reported that this plant can increase neuromuscular coordination and improve exploratory behavior in mice (Riaz et al., 2017). Based on this evidence, it can be assumed that this plant may possess positive effects on accelerating injured nerve regeneration or promoting functional recovery. Though the data about this aspect is not available yet. Therefore, this preliminary type study was conducted to evaluate whether T. occidentalis promote the regain of sensorimotor functions following induced sciatic nerve injury. For this, the already established model of induced sciatic nerve injury was used to study the neuro-regenerative capacity of T. occidentalis in mice.

MATERIALS AND METHODS

Animals: The albino mice (weight: 25-35 g: age: 8-10 weeks) were procured from the animal facility of the Department of Physiology, Government College University Faisalabad. They were split into two categories i.e., control (n=6) and treated (n=6) and kept in clean cages as one mouse per cage and had provided free access to food and drinking water. A standard control environment having a temperature of 25+2ºC, 12 hours of dark/light cycle, and optimal humidity in the housing facility was ensured throughout the experiment. All behavioral experiments were conducted in the light cycle at the same time.

Plant collection, material preparation, and supplementation: Thuja occidentalis’s leaves and stems were obtained from the local market of Faisalabad, Pakistan, and were recognized (Herbarium Number 271-bot-20) by Botany Department, Government College University Faisalabad. Following identification, plant parts were dried in shade and grounded into a powder. An equal quantity of stems and leaves were taken for processing. The powdered plant material (2g/kg) was uniformly mixed in the rodents’ diet (Sunila and Kuttan, 2006). The plant material was given to the treatment group from the day of nerve compression until the termination of the trial. The body weight and food usage were noted daily (Cheng, Xiong, and Xiang, 2013).

Sciatic Nerve Crush Injury Induction: Following the period of acclimatization, all experimental mice were induced sciatic nerve lesion surgically under anesthesia (Aziz et al., 2019; Hussain et al., 2013; Sajid et al., 2021). Ketamine and Xylazine, at the dose of 70mg/kg and 5mg/kg of body weight were injected intraperitoneally, respectively. An incision was given in the skin (clearly shaved off) on the right thigh of each mouse to expose the thigh muscles. Muscles were carefully separated with the pair of forceps and the sciatic nerve was disclosed and compressed by forceps for 12-15 sec. After that 2-4 stitches were applied to the skin and the wound was cleaned with absolute alcohol to prevent the infection. The mice were decapitated at the end of the experiment by giving deep anesthesia at the same dose as given before, and tissue samples were taken, as well as blood was taken.

$$\mathrm{SFI} = \left(-38.3 \times \frac{\mathrm{EPL} - \mathrm{NPL}}{\mathrm{NPL}}\right) + \left(109.5 \times \frac{\mathrm{ETS} - \mathrm{NTS}}{\mathrm{NTS}}\right) + \left(13.3 \times \frac{\mathrm{EIT} - \mathrm{NIT}}{\mathrm{NIT}}\right) - 8.8$$

Behavioral tests

Sciatic functional index (SFI): To assess motor functional retrieval, this test was calculated. The mouse's hind feet were dyed using blue ink and they were permitted to travel along the wooden trail. The paw prints were measured, and the SFI was calculated using the following formula.

intermediate toe spread (IT) refers to the distance between the 2nd and 4th toes. Toe spread (TS) is the distance between the 1st and 5th toes, whereas print length (PL) is the distance between the heel and the tip of the 3rd toe. Whereas NPL, NTS, and NIT correspond to the reading of the Normal group, and EPL, ETS, and EIT correspond to the reading of the Experimental group (Rasul et al., 2019).

Hot plate test: This was used to assess the mice's sensory function recovery in their hind limbs. Mice were permitted to stand with their experimental hind paw in direct contact with the hot plate surface at 56+2°C till they displayed any reaction. This duration was recorded as the hot plate latency (HPL). The mouse was separated from the instrument following any response (Imran et al., 2019; Yu et al., 2008). Three analyses were taken with a time gap of 2 minutes for every mouse.

Chemical withdrawal threshold: The threshold of paw withdrawal in reaction to a chemical stimulus was assessed twice throughout the study; pre and post to the sciatic nerve injury. In this test, formalin (10µl of 5%) was used to inject on the dorsal side of the experimental hind paw with a 50µl Hamilton syringe (Gong et al., 2014). The mice were monitored for five minutes to note their initial lick or jerk action.

Grip strength of muscle: Using the capacity of mice to grip the grid, this approach is used to assess mouse muscular strength in vivo. It is done with the use of a grip strength meter (Bioseb, Chaville, France). The mice are put on a metallic grid, which naturally stops the experimenter's inadvertent rearward movement. The mice continue to do so until the power of the drawing decreases their grip force. The peak of the animal pulling force was measured using a strength meter, and three values were recorded for the ipsilateral limb and contralateral to the injury (Hussain et al., 2013).

Organs weight: The mass of Gastrocnemius and anterior Tibialis muscle was measured by using a functional parameter of muscle weight. Both of these muscles were harvested from the normal and T. occidentalis chow group and used for the assessment of muscle mass (Li et al., 2013; Navarro, 2016; Tuffaha et al., 2016). At the end of the investigation, the variation between the muscle mass of control and treated mice was accounted for in the evaluation of muscle atrophy.

Random blood glucose: The glycemic level is used to understand the association between the development of metabolic-related pathogenic pathways in the damaged neuron. It was measured in both groups by using the glucometer (Accu-chek). In this test, a tiny drop of blood was extracted from the mice's tails (Asmat, Abad, and Ismail, 2016; Menon et al., 2004).

Analysis of Systemic Indexes

Total Antioxidant Capacity (TAC): The total antioxidant capacity (TAC) is a tool for assessing the biological system's antioxidant capability. The method given by Erel and Aziz was used to determine the TAC level (Aziz et al., 2019; Erel, 2005). This test is based on the principle of the formation of ABTS•+ following 2, 2´-azinobis 3 ethylbenzothiazoline-6-sulfonate (ABTS) and H_2O_2 incubation. A semi-automated chemistry analyzer (Biosystem, BTS-330) is used to measure TAC and its measurements are expressed in mmol Trolox Equiv/L (Rubio, 2016).

Total Oxidant Status (TOS): In this study, the TOS was determined by employing a known method (Erel, 2005) which is based upon the formation of ferrous ions into ferric ions in the presence of an oxidant. This ferric ion conversion was assessed by using xylenol orange to evaluate the oxidant status in the serum sample as previously described.

Ethical approval of the study: The Institutional Review Board of Government College University, Faisalabad, Pakistan, authorized the study plan and animal usage for this investigation (IRB No. 811). All rodent tests were conducted in compliance with the established rules, regulations, and recommendations.

RESULTS

Effects of T. occidentalis on Food Consumption and Body Mass: The body mass and diet intake were noted and compared during the whole study period (before and after inducing the sciatic nerve injury) in both groups as mentioned in Fig.1 a & b. A statistically non-significant difference suggests that the addition of T. occidentalis crude powder in the mice's diet did not affect the eating behavior of animals, which means that its supplementation does not affect the taste, granularity, and smell of the food. A comparatively constant increase in body mass of mice with non-significant differences between groups showed that neither sciatic nerve lesion induction nor the incorporation of T. occidentalis in diet alter the mice' metabolism pattern drastically. Thus, it can be speculated that comparatively improved observations in incorporated analyses in this study would be due to the addition of T. occidentalis.

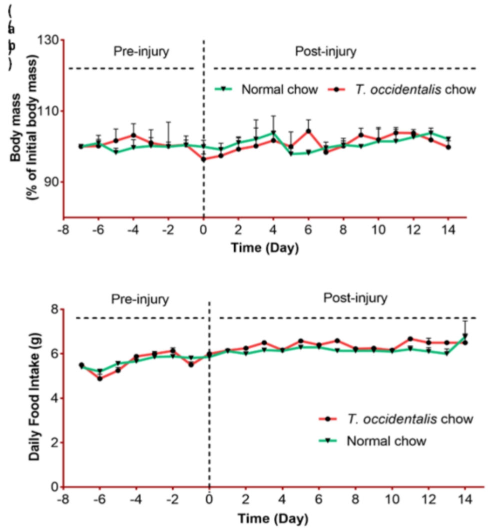

Fig 1: Impact of T. occidentalis on mouse's body mass and food intake pattern.

Readings are given as mean ± SEM (n=6) and are analyzed by two-way repeated measure ANOVA and Bonferroni's post hoc test (a) Time course of body mass (% of the average of initial body mass (g) measured on day 7 pre-injury) of mice served with normal chow throughout the study period (green line) or T. occidentalis chow following nerve injury (red line). Non-significant differences have been found between both groups at all-time points. (b) Duration of diet intake (g) in mice as in (a). Non-significant differences have been found between both groups at all-time points.

Effects of T. occidentalis on motor functions recuperation and muscle mass: Following sciatic nerve lesion, findings of grip strength analysis suggest that T. occidentalis promoted the retrieval of gripping force of ipsilateral hind paw significantly (P<0.01) on day 9 and 12 after injury induction, than the control group (Fig. 2a). Similarly, findings of SFI analysis showed a highly significantly improved SFI value (P<0.001) in T. occidentalis chow group on day 6 and day 9 post-injury than normal chow group, which further suggests the accelerating role of the plant in doing functional recovery following nerve injury. It was also found that the mass of Gastrocnemius (Fig. 3a) and anterior Tibialis (Fig. 3b) muscles were similar in both hind limbs of the T. occidentalis chow group (significantly improved mass ratio (P=0.013 for gastrocnemius and P=0.003 for Tibialis anterior)) than the normal chow group, suggesting the restoration of conduction of nerve impulse to the muscles. Collectively, these observations suggest that T. occidentalis not only helps to accelerate functional recovery but also restores muscle mass.

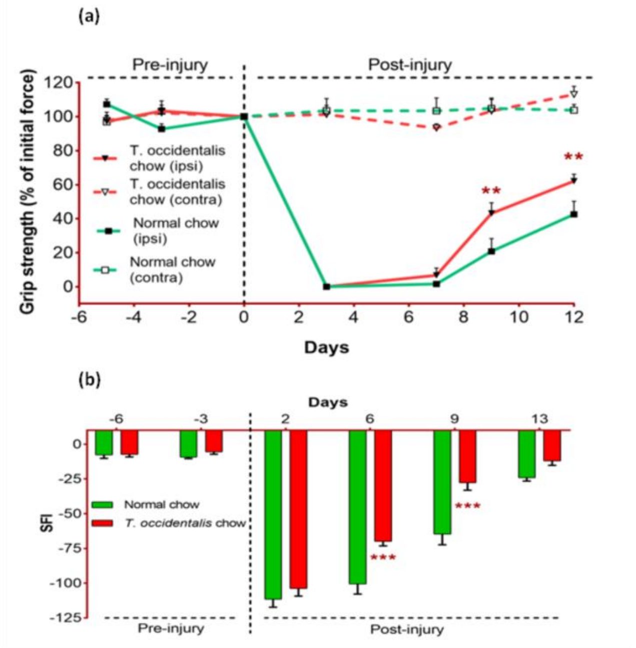

Fig 2: Impact of T. occidentalis on motor function regain following sciatic nerve lesion.

The features are given as mean ± SEM (n=6) and are analyzed by two-way repeated measurement ANOVA followed by Bonferroni's post hoc test. (a) Measurement of muscle grip strength of hind limbs (both ipsilateral (compact line) and contralateral (dotted lines) to the nerve lesion) of mice served with normal chow (green line) or T. occidentalis chow (red line) at various time points. A significant difference (**P<0.01) has been seen for the ipsilateral hind paw on day 9 and 12 following injury between both groups. (b) Estimation of the sciatic functional index in mice served with normal chow (green bar) or T. occidentalis chow (red bar) at different time points. A highly significant difference (***P<0.001) has been seen on day 6 and 9 after injury between both groups.

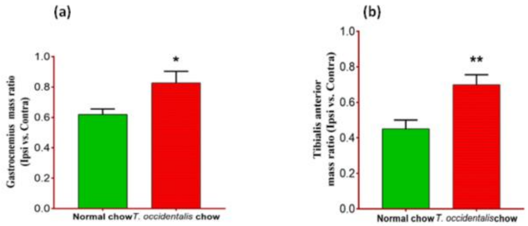

Fig 3: Assessment of skeletal muscle mass.

Details are given as mean ± SEM (n=6) and are analyzed by unpaired t-test. The green bar represents the normal chow group. The red bar represents T. occidentalis chow group. (a) represents Gastrocnemius mass ratio. A significant difference (*P=0.013) has been found between both groups. (b) represents Tibialis anterior mass ratio. A highly significant difference (**P= 0.003) has been found between both groups.

Effects of T. occidentalis on sensory functions recuperation: For the assessment of the extent of sensory function recovery in mice, a hotplate test and formalin test were conducted at various time points both prior to and after nerve injury introduction. Earlier paw withdrawal from the hot surface of mice, belonging to T. occidentalis chow group, on day 8 post-injury, revealed significant (P=0.017) restoration of sensory response (Fig 4a). The comparatively earlier response shown by licking the injection site on the paw following intradermal formalin injection, in mice of T. occidentalis chow group on day 14 post-injury, gave a clue about the restoration of sensory functions in them. However statistical comparison between both groups was found to be non-significant (Fig. 4b).

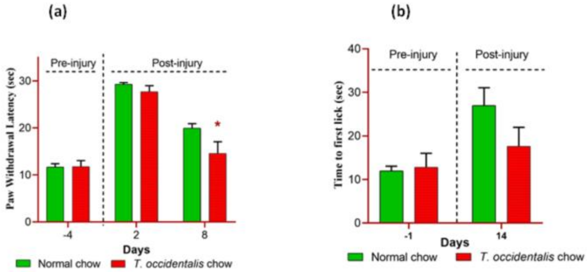

Fig 4: Impact of T. occidentalis on sensory function regain following sciatic nerve lesion.

Values are given as mean ± SEM (n=6) and are analyzed by two-way repeated measure ANOVA after Bonferroni's post hoc test. (a) Assessment of paw withdrawal response from the hot surface in mice served with normal chow (green bar) or T. occidentalis chow (red bar) at different time points. A significant difference (*P=0.02) has been seen between both groups on day 8 post-injury. (b) Assessment of onset of nociception following intradermal formalin injection in mice served with normal chow (green bar) or T. occidentalis chow (red bar) at different time points. A non-significant difference (P=0.1) between both groups, was observed on day 14 post-injury.

Effects of T. occidentalis on oxidative stress and blood glucose level: The measurement of oxidative stress markers including TAC and TOS suggests that the T. occidentalis chow group showed a high level of TAC (P<0.001) and low levels of TOS (P=0.017) (Fig. 5a and b). It is known that the balance between TAC and TOS is also sensitive to the endogenous glycemic level. It was observed that the T. occidentalis chow group has lower glycaemia than the normal chow group, however, the difference was non-significant statistically (Fig. 6).

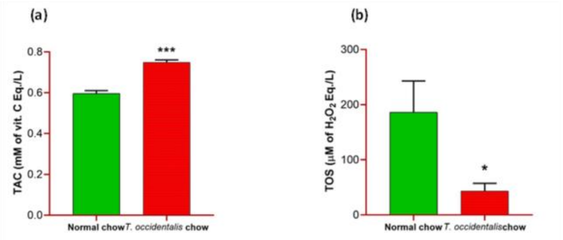

Fig5: Impact of T. occidentalis on systemic oxidative stress following sciatic nerve lesion.

Facts and figures are given as mean ± SEM (n=6) and are analyzed by unpaired t-test. The green bar represents the normal chow group. The red bar represents T. occidentalis chow group. (a) represents a measurement of TAC in both groups. A highly significant difference (***P<0.001) has been found between both groups. (b) represents a measurement of TOS in both groups. A significant difference (*P= 0.017) has been found between both groups.

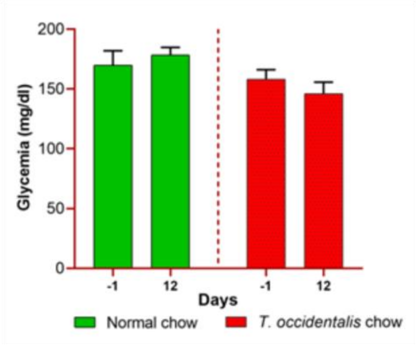

Fig 6: Impact of T. occidentalis on glycaemia.

Details are given as mean ± SEM (n=6) and are evaluated by ordinary one-way ANOVA followed by Bonferroni's post hoc test to compare the glycemic level 1 day before injury induction and 12th day after injury induction in the normal chow group (green bars) and T. occidentalis chow group (red bars). A non-significant difference has been observed between pre and post-injury glycaemia values for both groups.

DISCUSSION

Thuja occidentalis, Northern white-cedar, is extensively used in homeopathy and phytotherapy. This plant has anti-inflammatory, antioxidant, anti-proliferative, anti-convulsant, and analgesic characteristics, making it an excellent option for the creation of anxiolytic and nootropic medicines (Lokesh et al., 2011). Tannins, flavonoids, polysaccharides, oil, and a variety of other compounds are abundant in the leaves and branches of T. occidentalis. Diterpenes, monoterpenes thujone, fenchone, and sesquiterpene are significant bioactive chemicals found in the essential oils of the Thuja genus and are assumed to be responsible for the genus' various pharmacological and therapeutic benefits (Chang et al., 2000).

To date, no known study has evaluated the possible role of T. occidentalis in peripheral nerve lesions. The results suggest that T. occidentalis can induce a speedy regain of both sensory and motor functions. In the case of motor functions recovery in the T. occidentalis chow group, the results of the grip strength test were found significant (P=0.05). The level of significant difference appears more on day 9 and it becomes stronger on day 12. Similarly, the SFI was also significantly different (P=0.001) on days 6, 9, and 12 indicating the early functional retrieval in the treatment group. Two of the most important muscles; Gastrocnemius and Tibialis Anterior are involved in carrying the motor activities of the hind limbs. In the case of sciatic nerve injury, an interruption in basal electrical stimulus causes these muscles to lose their functions and if this interruption persists for a longer period they undergo atrophy. Therefore, the measurement of muscle mass is also an important factor. The restoration of muscle mass indirectly assesses the rate of nerve regeneration (Li et al., 2013; Navarro, 2016; Tuffaha et al., 2016). We found that T. occidentalis yielded a restoration of muscle mass of gastrocnemius muscle (P=0.04) and Tibialis anterior muscle (P=0.008). Collectively, all of these significant results suggest that T. occidentalis exhibits an early recovery of the motor functions in the mechanically injured sciatic nerve. Besides this, the T. occidentalis treatment group displayed an early paw withdrawal of ipsilateral hind limbs (P=0.005). Similarly, early time to first lick response was noted in the T. occidentalis treated group which further concomitantly signified an important role of this plant in the restoration of sensory functions.

Oxidative stress is considered the most potent mediator of the pathogenesis of peripheral nerve injuries. Under such situations, the reduced level of oxidative status and increased level of antioxidant capacity are taken as favorable conditions for healing an injured nerve. The anti-oxidative potential of T. occidentalis has already been reported in the literature (Yogesh and Ali, 2014). Accordingly, our results show a significant increase in TAC (P=0.005) and a decrease in TOS (P=0.03) levels in the treated group. Furthermore, the most important factor that inhibits functional recovery after peripheral nerve damage is a high glycemic level. Because it activates a slew of undesirable metabolic pathways, it adds to the recovery time (Asmat, Abad, and Ismail, 2016; Latini et al., 2019; Menon et al., 2004). Moreover, the antioxidant properties of T. occidentalis can be a potential therapeutic approach to treat diabetes (Alamdari et al., 2017). We observed a reduced glucose level in the treated group than in the normal chow group. However, the difference in the results of both of the groups was statistically non-significant. Overall, T. occidentalis has been shown to have beneficial benefits on the regenerated peripheral nerves following a severe lesion. Although this study presents a few pieces of evidence on rapid function recovery, more powerful component identification and mechanistic investigations will pave the road for clinical usage.

CONCLUSION

The results of our investigation imply that supplementing with T. occidentalis speeds up the functional healing of the damaged sciatic nerve. It improves the oxidant state in the biological system by increasing antioxidant capacity and decreasing oxidative conditions. Our findings point to the need for more research to identify and define the plant's real active constituent (s), which might be a useful target for medication development in the future.

Authors' contribution: T.F, G.H, and A.H conceived and designed the experiments. A.R, R.A, H.A, and S.A.M have equally contributed to conceptualization and data interpretation. R.A, F.S, S.N, and T.I helped in statistical analysis, data interpretation and wrote the manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Abbreviations: HPL: Hot plate latency; WRL: Withdrawal reflex; TAC: Total antioxidant capacity; TOS: Total oxidant status; TEAC: Trolox equivalent antioxidant capacity; ABTS: 2, 2´-azinobis 3 ethylbenzothiazoline-6-sulfonate.

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