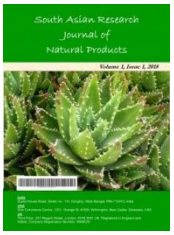

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Study on the Anti-inflammatory and Anti-nociceptive Activities of Methanol Extracts of *Terminalia ivorensis* A. Chev

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAA collected the plant sample and isolated the crude extracts. Authors IAO and ONA designed the study, performed the statistical analysis and wrote the protocol. Author ONA supervised author MHZ in the anti-inflamamtory and anti-nocicpetive studies. Author OAL managed the literature searches. Author IAO wrote the first and final drafts of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study was aimed at the determination of the anti-inflammatory and anti-nociceptive potentials of methanolic extracts of the stem and root bark of *Terminalia ivorensis* A. Chev (Combretaceae).
Study Design: The study design involved isolation of crude extracts from *T. ivorensis* and evaluation of the anti-inflammatory and anti-nociceptive potentials.
Place and Duration of Study: *T. Ivoriensis* Stem Bark (TISB) and Root Bark (TIRB) were collected from a location in Ore, Ondo State, Nigeria, in January 2017.
Methodology: Plant samples were extracted with 90% methanol using Soxhlet extractor. Egg-albumin induced inflammation and hot plate tests were used to evaluate the anti-inflammatory and anti-nociceptive activity respectively at a dose of 100, 200 and 400 mg/kg.

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Results: Extract yield of the TIRB and TISB were 20% and 24% respectively. Both extracts displayed high anti-nociceptive activities (at the peak of $p<0.001$) relative to time and concentration except the 400 mg/kg of TISB with reduced activity ($p<0.01$ at the 4th h). The egg-albumin induced inflammation was inhibited by the extracts in different doses. TISB displayed non-significant inhibition properties for all the doses. However, the 100 mg/kg of root bark showed a $p<0.05$ activity minimal at the 30th and 60th min but declined to non-significance as the reaction proceeds. The dose of 400 mg/kg showed a steep rise in activity from $p<0.05$ to $p<0.01$ at the 3rd and 4th h.
Conclusion: This study has shown that TISB and TIRB possess both anti-nociceptive and anti-inflammatory properties and thus the plant can be exploited for such.

Keywords: Terminalia ivorensis; anti-inflammatory activity; anti-nociceptive activity.

1. INTRODUCTION

Terminalia ivorensis A. Chev (Combretaceae) is a deciduous tree growing to 30 m by 30 m (98ft) at a fast rate [1]. Mature trees are very flat topped with a wide horizontal canopy of evenly distributed foliage arising from the apex of the straight bole. In young trees, the branches are whorled; deciduous, young shoots and foliage falling a few years after initial growth, leaving sockets to mark their original position on the bole [2]. When young, the bark is smooth and of light gray colour,but as it matures, it becomes blackish with lengthy fissures which breaks off after intervals. *T. ivoriensis* flowers between April and June in native regions but may vary in other regions, while fruiting begins in December and completely blossom by end of March [3].

Aqueous and ethanolic extracts of *T. ivorensis* showed significant antibacterial activity against tested organisms [4]. The hydroalcoholic extract showed best antifungal activity towards *Candida albicans* and *Aspergillus fumigatus* [5] and *Trichophyton mentagrophytes var. interdigitale* [6]. A recent finding suggest that extracts of *T. ivorensis* exert its antipsychotic-like activity, via a neuroprotective compensatory mechanism of action, and as such, could be relevant in the management of schizophrenia [7]. Ethanol extracts of the plant inhibited apomorphine or ketamine-induced stereotypy, and ketamine-induced hyperactivity, which suggest that *T. ivorensis* contain biologically active constituents that possess antipsychotic activity [8]. The ethanol bark extract of *T. ivorensis* possess sedative and analgesic effect, thus supporting its folkloric use in pain management and as a tranquilizer in psychosis [9]. The ethyl acetate and ethanolic fractions of *T. ivorensis* exhibited ED_{50} values of 11.11 and 12.32 µg/mL respectively against against *T. brucei rhodesiense* [10] indicating prospect for the development of potential trypanocidal agent from the plant. In an earlier work on the screening of some medicinal Nigerian plants, biological activities associated with the plant include anti-inflammatory and anti-arthritis [11].

Two oleanane-type triterpenes named ivorengenin A (3-oxo-2*α*,19*α*,24-trihydroxyolean-12-en-28-oic acid) and ivorengenin B (4-oxo-19*α*-hydroxy-3,24-dinor-2,4-secoolean-12-ene-2,28-dioic acid), together with arjungenin, arjunic acid, betulinic acid, sericic acid, and oleanolic acid, were isolated from the barks of *T ivorensis* [12]. The compounds exhibited significant antioxidant and antiproliferative activities against MDA-MB-231, PC3, HCT116, and T98G human cancer cell lines [12]. The isolation and characterization of sericic acid and lonchoterpene (28-hydroxy-18α-glycirrhetinic acid) have been reported [11]. In addition, ivorenosides A, B and C were isolated from the bark of *T. ivorensis* [13]. Ivorenoside B and C exhibited scavenging activity against DPPH and ABTS(+) radicals while ivorenoside A showed antiproliferative activity against MDA-MB-231 and HCT116 human cancer cell lines with IC_{50} values of 3.96 and 3.43 µM, respectively [13].

In continuation of our extensive study on the biological activities of Nigerian medicinal plants [14-16], the present study was undertaken to evaluate the *in-vivo* anti-inflammatory and hot plate induced anti-nociceptive potentials of methanol extracts of the stem bark and root bark of *T. ivorensis* A. Chev (Combretaceae) on Wista rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All the

chemicals used including the solvents, were of analytical grade. Acetylsalicylic acid (ASA) and Diclofenac injection (RX, Nigeria Ltd) were purchased from Lagos State University Pharmacy manufactured by May and Baker, Nigeria.

2.2 Animals

Eight weeks wistar rats of average weight of 150 to 200 g of either sex were bought and kept in the animal house of the Department of Biochemistry, Lagos State University, Nigeria. Standard conditions of temperature (23 ± 2°C), light accessibility (12 h light and darkness cycle) with free access to standard pellet feed, tidy environment and water *ad libitum*. All experimental procedures were approved under the Lagos State University Research Ethical Clearance Committee (RECC) of the University (Approval no: 012/2017/LASU/BCH).

2.3 Preparation of Plant Sample

The stem bark and root bark of *T. ivoriensis* were collected by Mr. M.A. Adebayo from a location in Ore, Ondo State, Nigeria, in January 2017. Botanical identification was carried out by Mr. G.A. Ademoriyo of the Herbarium, Obafemi Awolowo University, Ile-ife, Nigeria, where a voucher specimen, Ife 17708, was deposited. The stem bark and root bark of *T. ivoriensis* were properly separated from the stock and kept in a black polythene bag. The samples were air-dried for 12 days and pulverised with a laboratory blender.

2.3.1 Extraction of crude extracts

500 g of each of the pulverised samples was extracted with 90% methanol using Soxhlet extractor to produce a dense mass of methanolic extracts after extraction and kept in a refrigerator at 4°C until further analysis as previously described [14,15].

2.4 Study Design

The rats were randomly assigned to five groups of five animals each for the two different experimental models. The first group served as negative control receiving normal saline (1 mL/kg). The second were served 100 mg/kg standard drug, aspirin [17]. The methanolic extracts of *T. ivoriensis* were given at a dose of 100, 200 and 400 mg/kg. All extracts were dissolved in 90% DMSO and animals were treated with 1 mL of 100, 200 and 400 mg/kg of methanolic extracts respectively. All treatments were administered orally using the canula syringe.

2.5 Hot Place Induced Anti-nociceptive Test

The experiment was carried out using the modified method earlier described [18]. Twenty-five (25) mature wistar rats of both sexes were randomly divided into 5 groups of 5 rats per group. The grouping was used as above. The animals were fasted for 12 h with provision of clean water *ad libitum*. Each rat was placed upon the heated metal plate (Hot plate) maintained at the temperature of about 50-55^{0}C within the restraining plastic cylinder. Group 1 rat received 1 mL of distilled water and served as control. Group 2 rat received acetylsalicylic acid 100 mg/kg (ASA) (standard control) and groups 3, 4 and 5 received 100, 200 and 400 mg/kg of *T. ivoriensis* extract respectively per 0 s. Animal response to the heat varies and such changes includes; kicking of hind foot and jumping about, licking of foot, raising the foot, holding the foot tightly to its body or shaking of the foot. Readings were taken 0th, 30th and 60th minutes after administration. Wistar rats that presented baseline reaction of more than 45 s were taken as maxima analgesia of 100% [18].

2.6 Anti-inflammatory Test

A modified method for rat paw edema as earlier reported was used [14,15]. Wistar rats were assigned to one of 5 groups consisting of 5 animals as described previously [14-16] and were administered with the extracts as discussed in Section 2.4. However, the standard group was treated with Diclofenac Sodium injection 100 mg/kg, orally. Prior to experimentation, animals were starved overnight to allow for proper sample absorption into the blood stream through the stomach cavity and to empty part of gastrointestinal tract [19]. Thirty minutes later, 1.0 mL of 50% (v/v) of fresh egg albumin was injected subcutaneously into the subplantar surface of the right hind paw. Rat paw oedema was assessed by change in paw sizes measured with a vernier caliper before and after egg-albumin injection at 30 min, 1, 2, 3, and 4 h.

2.7 Statistical Analysis

Data were presented as mean ± standard error mean (SEM); n=5. The results were analyzed

using Graph Pad Prism software 6.0. Statistical significance was determined by Boornfontein test and *P* value less than 0.05 was considered as significant.

3. RESULTS AND DISCUSSION

3.1 Yields of the Extracts

The Soxhlet extracted methanolic extract of the stem and root bark of *T. ivoriensis* yielded a yellowish-greenish viscous extract of 24.0 and 20.0% extract respectively. Methanol was used due to its high polarity and can absorb most polar phytochemicals such as phenolic compounds, terpenoids and flavonoids.

3.2 Anti-nociceptive Activity

The results of the analgesic effect of the methanol extract of the galls of *T. ivorensis* using hot plate method are presented in Figs. 1 and 2 for the stem (*TISB*) and root (*TIRB*) bark extracts respectively. The analysis was conducted based on dose dependent of 100, 200 and 400 mg/kg of rat body weight. Statistical significant of $p<$ 0.05 was used to test the level of confidence on the extracts.

As shown in Fig. 1, the methanolic extract of the stem bark showed a activity dependent on dose and time. All the extracts exceeded the maximum analgesia period of 45s. In addition, the extracts showed a significant level of maximum possible analgesia for all the doses from the zero minute to the 60 minutes (*p* value varies between $p<0.01$ and 0.001), indication of its high level of peripheral cavity pain inhibition.

The anti-nociceptive activity of the root bark is shown in Fig. 2. Activities of extract were also dose dependent and were significant when compared to the control (saline solution). The 200 mg/kg dose however showed an interesting activity; at the baseline values (0 min), the activity was significant ($p<0.05$) but declined considerably at the 30^{th} min. The activity and reached a high peak of $p<0.001$ at the 60th min. The 400 mg roots bark extract declined in activity at the 60^{th} min after reaching a high peak at the 30^{th} min.

Results of the present study clearly demonstrate the analgesic (anti-nociceptive) properties of the stem and root bark of *T. ivoriensis.* The anti-nociceptive property shows the response of animals to heat which is exhibited by their jumping and limb biting. In this model, a rise in pain latency indicates the level of analgesia induced by the drug or extract. This test is preferred as it examines central analgesic activity caused by sensitivity to strong analgesics and partial tissue damage [20]. The advantage of this method over others is because human pain is supraspinal mediated (Central nervous system) which the hot plate model easily measures [21]. The stem and root bark extracts of the *T. ivoriensis* shows a considerable activity in suppressing the peripheral pain in the animal model studied. Adeoluwa et al. [9] examined the analgesic property of the ethanolic extract of *T. ivoriensis* in mice and obtained a high activity in the locomotor activity but less activity of thermal stimulus using tail flick immersion model. However, the present study had demonstrated the effectiveness in the methanolic solvent. Phytochemicals such as saponins, terpenes, tannins and flavonoids have been quantified in *T. ivorensis* and may be responsible for the observed activity [11-13].

A recent review had extensively showed the action and mechanism of phytochemicals inhibition of pain with emphasis on the binding activity and suppression of pain mediator's e.g the prostaglandins and the bradykinins [22].

3.3 Anti-inflammatory Activity

The egg-albumin induced oedema activity of the methanolic extracts of *T. ivoriensis* stem and root bark is shown above in Figs. 3 and 4 respectively. As expected, the standard drug, Diclofenac was effective throughout the reaction time. However, level of inhibition by *TISB* reduced considerably and were insignificant as the reaction proceeds on comparison to the control sample (Fig. 3).

The TIRB activity as shown in Fig. 4 were dose and time dependent. The 100 mg/kg dose (*TIRB*) showed a $p< 0.05$ activity at the 30^{th} and 60^{th} minute only but declined considerably as the reaction proceeds as shown in Fig. 4. The dose of 400 mg/kg showed a steep rise in activity from $p<0.05$ to $p<0.01$ at the 3^{rd} and 4^{th} h.

This study shows that the methanol extract of *T. ivoriensis* exhibits a relatively low anti-oedematogenic effect on egg albumin-induced oedema of the paw of wistar rats. Egg albumin-induced inflammation model is a significant predictive test for anti-inflammatory activity [23]. Several white blood cells and anti-inflammation mediators (biomarkers) are released by the cells

which help to regulate the progress of healing and activity of such mediator. Acute inflammation, such as egg albumin-induced oedema, involves the synthesis of these mediators at the injured site which prolongs over a few periods of days. These processes are influenced by the release of some mediators in three different phases [24]. Histamine and serotonin are synthesised in the first phase during the first 1.5 h [14-15]. The second phase involves the release of bradykinin from 1.5 h to 2.5 h, and prostaglandins between 2.5 h to 6.0 h phlogistic administration [25].

The present study shows that the methanolic extract on *T. ivoriensis* root bark phytochemicals at 100 and 400 mg/kg exhibits anti-inflammatory potential. Histamine and serotonin were inhibited

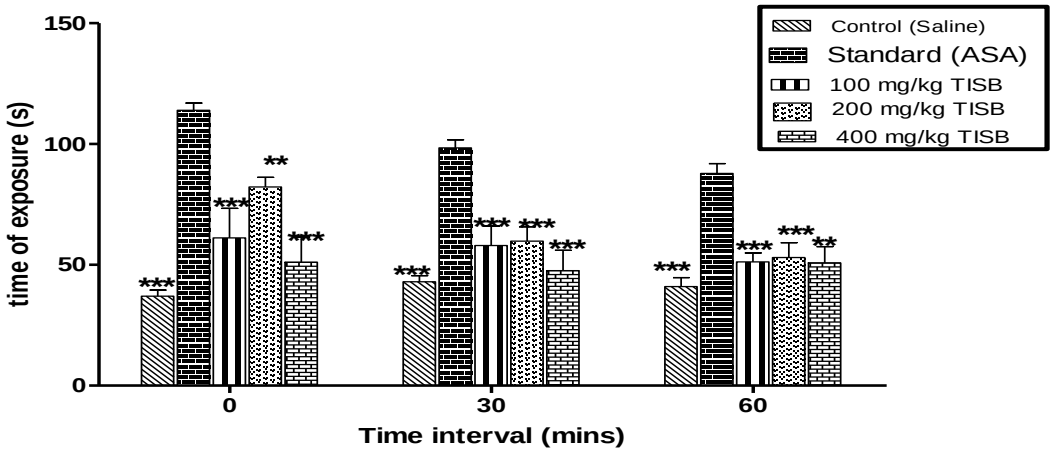

Fig. 1. Effect of methanolic extract of *T. ivoriensis* stem bark (TISB) on heat induced pain. Control, Standard, and TISB represent 1 mL saline solution, 100 mg/kg of ASA and 1 mL of 100, 200 and 400 mg of TISB respectively

**p<0.05, **p<0.01, *** p<0.001 statistically compared to control. (Non- asterisk represents non-significant)*

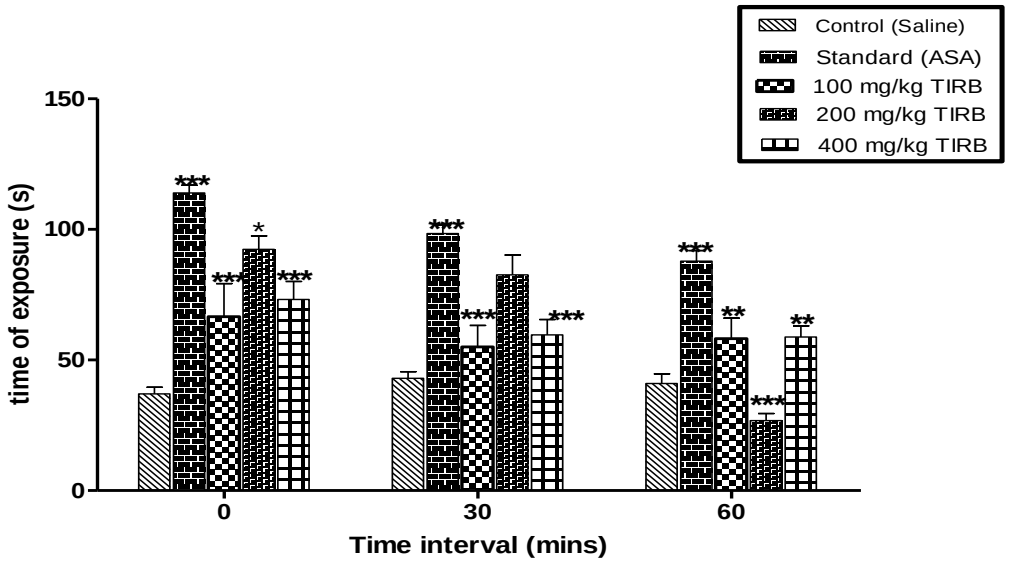

Fig. 2. Effect of methanolic extract of *T. ivoriensis* root bark (TIRB) on heat induced pain. Control, Standard, and TIRB represent 1 mL saline solution, 100 mg/kg of ASA injection and 1 mL of 100, 200 and 400 mg/kg of TIRB respectively

**p<0.05, **p<0.01, *** p<0.001 statistically compared to control. (Non- asterisk represents non-significant)*

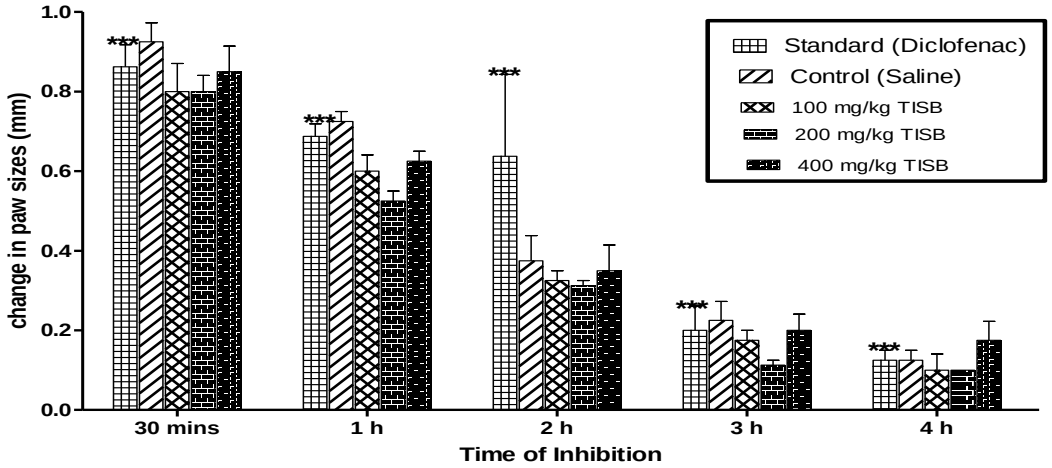

Fig. 3. Effect of TISB on egg-albumin induced inflammation. Control, Standard, 100 mg/kg of Diclofenac injection and 100, 200 and 400 mg/kg p.o. of TISB respectively

p<0.05, **p<0.01,* p<0.001 statistically compared to control. (Non- asterisk represents non-significant)*

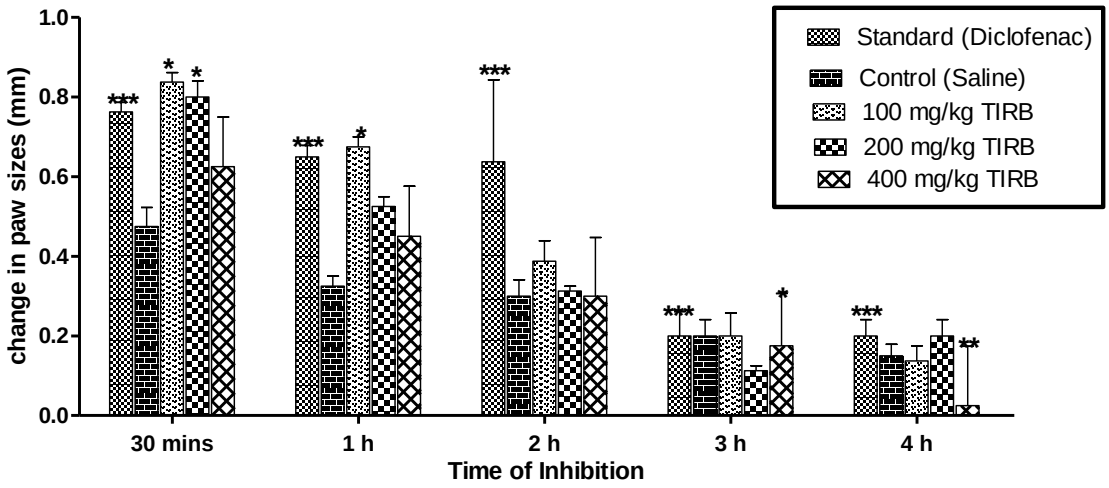

Fig. 4. Effect of TIRB on egg-albumin induced inflammation. Control, Standard, 100 mg/kg of Diclofenac injection and 100, 200 and 400 mg/kg p.o. of TIRB respectively

p<0.05, **p<0.01,* p<0.001 statistically compared to control. (Non- asterisk represents non-significant)*

by the 100 and 200 mg/kg extract within the 1st h but diminish in activity as the time of reaction prolongs. This type of activity could be due to the nature of the phytochemicals and their rate of absorption at the inflammation site. Furthermore, the 400 mg/kg extract only showed increased activities at the 3rd and 4th h of activity suggesting the release of bradykinins and prostaglandins. Earlier study has revealed the anti-inflammatory activities of extracts and isolate of *Terminalia* species. *In vivo* application of the ethanolic extract of *T. citrina* at a concentration of 2000 µg/mL showed 67.85% protection of Human Red Blood Cells in hypotonic solution compared with standard naproxen which showed 73.21% protection [26], in which the activity was as a result of the presence of saponins and flavonoids. Carragenan induced acute inflammation of the ethanolic extract of *T. arjuna* revealed a high inhibition caused by release of histamine [27]. It has been reported that the high anti-inflammatory activity of *T. chebula* fruits prolonged to the 5th h [28], which agrees with our present study as shown at the 4th h. Future study will reveal the mechanism of this activity and isolation of components responsible for such activity. Triterpenoids such as betulinic acid, ivorengenin A&B, sericic acid and oleanolic acid

had been previously isolated from the barks of *T ivorensis* [12] are known anti-inflammatory and anti-nociceptive agent.Their mechanistic pain pathway involves the inhibition PGE2, IL-6 production, COX-2 [29], TNF-α and NO production which are pain modulators [30].

4. CONCLUSION

The present study has shown the ability of the methanolic extract of *T. ivoriensis* stem and root bark to inhibit inflammation and as analgesic therapy. The activity shows that the extract is both a CNS stimulant and the other way applicable as a non-steroidal anti-inflammatory drug (NSAID) due to its ability to hinder at both phases (anti-noniceptive and anti-inflammatory). In addition, the study confirms the traditional use of the plant and the best plant parts for each activity.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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