Zinc oxide nanoparticles synthesised from the *Vernonia amygdalina* shows the anti-inflammatory and antinociceptive activities in the mice model

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**ABSTRACT**

In this study, we synthesised the zinc oxide nanoparticles from *Vernonia amygdalina* and evaluated its anti-inflammatory and antinociceptive potentials against the different inflammation and pain induced mice model. The synthesised zinc oxide nanoparticles were characterised by UV, SEM, XRD and FTIR techniques. The anti-nociceptive effects of *V. amygdalina* were examined by different stimuli e.g. acetic acid, glutamate, capsaicin, and formalin-induced nociception in mice. The anti-inflammatory effects of synthesised zinc oxide nanoparticles were assessed by air sack assessment and the level of inflammatory cytokines were studied. The muscle tension of animals were studied through open field assessment. The present study exhibited proficient antinociceptive and anti-inflammatory actions of the synthesised Zinc oxide nanoparticles from *V. amygdalina*. The formulated zinc oxide nanoparticles were appreciably reduced the acetic acid, glutamate, capsaiacin, and formional-induced nociceptive responses in mice. Further the zinc nanoparticles were exhibited the potent anti-inflammatory actions via reducing the inflammatory response and pro-inflammatory cytokines level in the mice. In conclusion, the findings of this study proved the beneficial effects of zinc oxide nanoparticles from *V. amygdalina* against the different pain and inflammation-induced mice. Hence, it was clear that the zinc nanoparticles from *V. amygdalina* could be promising antinociceptive and anti-inflammatory agent in the future.

**Introduction**

The evolving of nanotechnology in an ecofriendly manner has become a part of greenery biotechnology [1] with crucial study of dimension and their shape and structure [2]. The ultimate intention of the nano medicine domain is to develop drugs with precise targets [3] and these nanoparticles can be manufactured by means of physical, chemical and natural process. Among these, the opting of biosynthesis is an ideal way in the terms of ecological friendly [4], which possess outstanding properties of antiseptic and antimicrobial and further are supposed to reveal substantial phototoxicity on budding flora [5].

Nanoparticles can be used as nanofertilizer for emergent of plants but although a number of researches utter nanoparticles prevent the enlargement of some plants, few others stated the development of some other plants [3,6]. The techniques comprise in the fusion of ZnO nanoparticles (ZnO NPs) are chemical steam toppling, gas stage method, drenched decomposition, hydrothermal mixture, micro suspension, electrochemical technique, thumped laser deposit, microwave blend, and the gel processing [7]. The significant of the produced ZnO has possible characteristics such as antimicrobial, gas detector, photoreactions, and deprivation of organic dyes [8–11]. *Vernonia amygdalina* is generally known as sour leaf by its bitter flavour, which usually grow in the tropical Africa as a small bush with 22 cm to 5 m long and belongs to the family of Asteraceae kin [12]. These leaves can be consumed as a cocktail snack and its water as a digestive stimulant [13]. The foliage are extensively worn for agitation and moreover as an alternate for quinine in Nigeria and other few African countries [14]. This herbal medicine can widely used as antiparasitic antimalarial, purgative, expectorant, enema and an inducer of fertility in women (subfertile) [15,16]. And it found to be a notorious plant to treat diabetes, fever, and possess many undocumented pharmacological properties for body pain and joints pain [17].

Inflammation or irritation is a multifactorial biological phenomenon with various processes, which formed by stimulus.
such as microorganisms injured cells in vascular tissues [18,19]. Preceding results demonstrated that the biosynthesis of zinc oxide nanoparticles from the V. amygdalina leaf extracts would be used as nano level fertilisers. It also possesses therapeutic principles by holding bio molecules with reduction capability. When in combination, it is used to treat inflammation, annoyance, pneumonia, swollen veins, common colds and infant tremor [20,21]. With all these supportive evidences, the present study evaluated the diverse anti-inflammation effect of V. amygdalina in dissimilar nociceptive mice models.

Materials and methods

Chemicals and reagents

Chemicals such as dexamethasone, diclofenac sodium, carrageenan, capsaicin, formalin, and dexamethasone were obtained from Sigma Aldrich, USA. All other analytical grade reagents used in this study were purchased from Himedia, USA.

Experimental animals

Swiss Albino male mice weighing about 25–30 g were selected for this study. The animals segregated in disinfected cages (plastic) at warmth of 20–25 °C by regular light and dark sequence for 12 h. The humidity of the mice domicile was maintained for about 50–60%. The mice were allowed to take feed and water. In the beginning, the animals were adapted for 10 days at regular laboratory circumstances. Then the animals were fasted in the night prior to the behavioural examination and the analysis was executed in the morning sessions (8.00 am to 12.00 am). All the experiments were approved by the institutional ethical committee, The Second Affiliated Hospital of Soochow University, Jiangsu, 215004, China.

Collection and preparation of V. amygdalina

The fresh and matured leaves of V. amygdalina was collected from the Classical Gardens of Suzhou (31°19′ N, 120°27′ E), Jiangsu province, China, and washed with the distilled water to remove the dust particles. Then the leaves were shade dried and powdered by using mechanical grinder. The resulted leaf powder was used to prepare the aqueous extract. The 15 g of the leaf powder was soaked in 100 ml of double distilled water and then heat macerated for 15 min at 75 °C. After that, the suspension was filtered and then resulted extract was used to synthesis the zinc oxide nanoparticles.

Nanoparticles synthesis and preparation

Illustration of zinc oxide nanoparticles was biosynthesized using 20% leaf extracts of V. amygdalina with zinc acetate dihydrate and sodium hydroxide as predecessor resources [22]. Then the amalgamation was kept in dark condition at room temperature and the mixture was centrifuged for 15 min at 10,000 rpm. Then the centrifugation pellet was allowed to dry and it was placed in a water bath at 45 °C for 10 h subsequent to centrifugation and dehydrated in a hot air oven for 6 h. The samples of V. amygdalina ZnO nanoparticles were synthesised and characterised.

Assessment by UV-Visible spectroscopy

The refuse of uncontaminated metal ions was recognised via shaping the amalgamation of retorting assortment by UV-Visible Spectrophotometer (1700 series Shimadzu model) as of 400 to 800 nm [23].

Investigation of Fourier transform infrared (FTIR)

FTIR is an important device to categorise the purposeful cluster to metal constituent part and organic molecules. The spectrums were established at 1 cm-1 movement by FTIR spectrophotometer (FTIR-8400S Shimadzu Corporation) by means of Potassium Bromide shot process [24]. It displayed the incidence in the range between 3500 and 500 cm\(^{-1}\).

Scanning electron microscopy

The formed nanoparticles were dotted in water and the suspension was detected by ultra sonicator for about 3 h. Very few drops of the nano suspension were situated on a segment of micro glass slide, which offered metal lattice enclosed by carbon cover and dried out gradually at room temperature. The discharge was then covered and envisaged with a JEOL-JSM 6480 Scanning Electron Microscope to assess the dimension, outline and proportion of the particle [25].

X-ray diffraction examination

The X-ray diffraction (XRD) was used to determine the disposition and range of the ZnO nanoparticles, the section shot was suspended in sterile water and continued the procedure constant for 3 times by the identical solution pursued by centrifugation. The pellet was sealed and allowable to dry. The fine particles getting from the section was enclosed with XRD lattice, where the spectrum were recognised in 35 kV and a current of 30 mA with K alpha and K beta radiation with XRD (XRD-7000-Shimadzu). The thinned potency was established from 10 to 70 °C at 2 theta angles. The crystal surroundings of intermingled nanoparticles were examined from the wideness of the XRD peak by means of Debye formula [26].

Acetic acid persuaded nociception examination

Experimental animals divided into five clusters consisting of six animals in each, control I was treated with tween 80 (1%), group II to IV animals were treated with ZNO-NP of V. amygdalina (2.5, 5.0, 7.5 mg/kg) and group V animals treated with diclofenac (10 mg/kg) as a standard. After the 1 h treatment
of *V. amygdalina* and diclofenac sodium, the animals were challenged with 1% of acetic acid through intraperitoneal route and then all experimental animals were located in the observation cabin [27]. The numbers of writhing like stretching movement, elongation of body and hind limbs were noted. The number of writhes done by experimental animals within 30 min was recorded.

**Glutamate induced nociception check**

The similar experimentation was continued for glutamate-induced nociception analysis. The animals were pre-treated with ZNO-NP of *V. amygdalina* (2.5, 5.0, 7.5 mg/kg) and 10 mg of diclofenac sodium before 10 min of the experiment. These mice were subsequently induced with glutamate (10 µm) by injecting slowly it to the abdominal plane of left posterior foot of the animal and examined for surveillance for 20 min while control received the saline [28]. The amount of licks achieved by the mice were counted and documented.

**Capsaicin provoked foot licking test**

The animals were submitted to capsaicin induced nociceptive trial to spot the consequence of ZNO-NP from *V. amygdalina* adjacent to neuropathic nociception. Sooner than 30 min, the experimentation performed the mice were pre-treated with dissimilar absorption of *V. amygdalina* and diclofenac sodium. Capsaicin (15 µl) suspended in 95% phosphate-buffered saline and ethanol (5%) was introduced to the appendage of the mice as each base with 1.5 µg capsaicin while control received only saline. The standard control animals received the diclofenac sodium [29]. The animals were kept under examination for 10 min and the moment of licking on inserted paw be verified for the occurrence of nociception.

**Formalin stimulated appendage licking test**

Formalin provoked foot licking tests were achieved to affirmed the antinociceptive effectiveness of ZNO-NP from *V. amygdalina* [30]. The animals were pre-treated with normal (Tween 80) for control; *V. amygdalina* (2.5, 5.0, 7.5 mg/kg) & diclofenac sodium (5 mg/kg) as positive control (group V) ahead of 30 min via subcutaneous shot. Subsequently pre-treatment of 3% of formalin was injected to the right back mitt plantar plane and kept back for scrutiny cavity for half an hour. The quantity of lickings carried out by mice from the first stage to the arising of neuro ache (0–5 min) and subsequent stage (20–30 min) of tenderness was recorded.

**Peritoneal hollow leukocyte permeation test**

Leukocyte permeability into the peritoneal movement subsequent to induction of pre-treatment & anti-inflammation effectiveness of ZNO-NP from *V. amygdalina* was noticed by the technique of Vinegar et al. [31]. The experimental animals were pre-initiated with Tween 80 for control mice, ZNO-NP from *V. amygdalina* (2.5, 5.0, 7.5 mg/kg) and last group mice were induced with diclofenac sodium, considered as positive control (5 mg/kg) previous to 30 min of experimentation. The animals were sacrificed according to ethical guidelines, and then the peritoneal void was cleaned and washed with PBS, which contains 1 mM EDTA to collect cells. The eludication was centrifuged and scrutinised whole leukocyte in addition to discrepancy cell count up. The total leukocytes mono-nuclear and polynuclear cells were counted by using the Neubauer chamber in blood mixed with 3% of acetic acid and 1% of crystal violet in 5:44:1 ration. The total amount of entire leukocytes, mono & polymorphonuclear cells were documented.

**Consequence of ZNO-NP from V. amygdalina on inflammation promoting cytokines**

The animals were subjected with anaesthesia and the backside fur was shaved, 5 ml of disinfected space was subcutaneously infused two times at similar spot at an intermission of 3 days to shape a pocket [32]. The animals with sacks were separated into 6 groups and induced with Tween 80 (1%) (Normal mice), 0.5 ml Carageenan (control), diverse absorption of *V. amygdalina* (2.5, 5.0, 7.5 mg/kg) and dexamethasone (optimistic control). The experimental animals were sacrificed by means of cervical displacement following 60 min, the pouch was pierced and 1.5 ml saline was injected within the hollow and sucked backside to collect the cells. Mass of cells was centrifuged and pellets were submitted to investigate proinflammatory cytokine like IL-6, IL-1β and TNF-α.

**Open field assessment**

The tranquiliser consequence of ZNO-NP from *V. amygdalina* was evaluated by executing open field test. The animals were subjected with dissimilar attention of ZNO-NP from *V. amygdalina* (2.5, 5.0, 7.5 mg/kg) and affirmative control, diclofenac sodium (5 mg/kg). Subsequent to 1 h, the experimental animals were introduced in to the open turf equipment which was a container with all sides of 50 cm each. Then, the package was alienated into 25 equivalent cubes. Later, the mice were allowed to ascertain the open ground device for few minutes, the quantity of squares traversed by the mice by all the foot was documented. The equipment used was kept clean by using of alcohol every time prior to performing the experimentation with new mice.

**Statistics**

All the obtained data were significantly examined by means of graph prism software and interpreted as mean ± standard deviation. Dunnet’s post hoc testing was executed for one way Analysis of Variance to evaluate momentous dissimilarity among the cluster. *p* values were represented as *p* < .05, *p* < .01 correspondingly.

**Results**

**UV-Visible spectroscopy exploration of V. amygdalina**

The reduction of zinc oxide ions in the *V. amygdalina* was confirmed by UV-Vis Spectrophotometer, shown in
The UV-Vis incorporation of ZnO NPs was at the crest maximum at 350 nm that was compared to the range of zinc oxide nanoparticles. Subsequent incubation, the colour was faded because of the excited state of Surface Plasmon in the ZnO NPs. The decline of zinc was confirmed by UV-Vis Spectrophotometer. Combination spectra of ZnO NPs twisted in the response medium which has crest at 320 nm; improvement of peak state that the rudiments are detached. The promptness and width of the surface plasmon absorption depend on the quantity and shape of the nanoparticles and as well on the insulating constant of the metal itself and the adjacent intermediary [33]. *Allium cepa* extract and zinc nitrate changed the colour of the ZnO NPs. The uppermost strength at 350 nm was tentative which designate entire refuse of zinc ions. The range of ZnO NPs by UV-Vis established a critical absorbance at 350 nm, which assign an estimated reliable accumulation of the nanoparticles.

**Electron microscope examination of V. amygdalina**

The external shape and structure of the nanoparticles was illustrated by Scanning Electron Microscopy Figure 1(B). *Vernonia amygdalina* extract mediated ZnO NPs were initiated as nano shaped (Figure 1B). The organisation, segment and form of amalgamated product were examined by the SEM study. The SEM result demonstrated the cylindrical nanoparticle shaped with thickness of about 20–40 nm.

**Fourier transform infrared spectroscopy study of V. amygdalina**

FTIR study established to distinguish the biomolecules, which responsible for diminishing metal ions into ZnO NPs in association with *V. amygdalina* extract and also demonstrated various integration peaks ranged from 3500 cm\(^{-1}\) to 500 cm\(^{-1}\) (Figure 2). The biological constituents originated in the *V. amygdalina* extract were responsible for the arrangement of a mixture of nanoparticles. The FTIR array of *V. amygdalina* demonstrated various integration peaks ranged from 3500 cm\(^{-1}\) to 500 cm\(^{-1}\). The range of incidence from 3321 cm\(^{-1}\) crest represented the O–H stretching vibration as well indicated the presence of amino acids and carbohydrates. The peak of 2467 cm\(^{-1}\) denoted the C–H extension particularly, lipids. The occurrence of range varied from 1634 cm\(^{-1}\) peak represented proteins by Amide I: C=O length. Also, ranges from 1322 cm\(^{-1}\) peak symbolised sulphur compounds and the range from 1037 cm\(^{-1}\) illustrated the C–N stretching: amino acids.

**X-ray diffraction analysis**

The distribution of the dissimilar peaks in the XRD model depicted crystalline zinc oxide with hexa organisation in the network parameters (Figure 3). In the whole band of 2θ...
values, the XRD outline established different absorption peaks ranges from 10° to 70° for the V. amygdalina. The V. amygdalina produced ZnO NPs were indexing in 100, 002, 101, 102, 110, 103, 112, and 201. The zinc was indexed and excited at 101 and 100.

Figure 2. FTIR spectra of ZnO nanoparticles of V. amygdalina. V. amygdalina extract were answerable for the configuration of a variety of nanoparticles. The FTIR range of V. amygdalina illustrated several assimilation peaks ranged from 3500 cm⁻¹ to 500 cm⁻¹. Figure 2 shows the representative FT-IR spectra obtained from V. amygdalina.

Figure 3. X-ray diffraction analysis of ZnO-NPs synthesised from V. amygdalina. The XRD pattern showed dissimilar concentration peaks in the entire band of 2θ values between range from 25° to 75° for the V. amygdalina extract. The V. amygdalina synthesised ZnO NPs were indexed as 100, 002, 101, 102, 110, 103, 112, and 201. The zinc was indexed and excited at 101 and 100.

Figure 4. Antinociceptive upshot of ZNO-NP in the nociception induced by acetic acid in mice. All values are illustrated as mean ± SD of six animals. The statistical significance level was calculated by one-way ANOVA followed by the Dunnet’s post hoc test; note: #p < .05 when compared with control group and *p < .05 when compared with ZnONPs administered groups.

Antinociceptive activity of ZNO-NP from V. amygdalina

Acetic acid stimulated test

The enteric bending assessment was used to examine the efficiency of V. amygdalina to ease out pain. Animals except control were given acetic acid to persuade intestinal twisting and integer of writhes and the intestinal elongation performed by the mice was counted. The number of writhes was considerably (p < .05) reduced (34 and 46) in V. amygdalina managed mice as compared with control. But, the 7.5 mg of V. amygdalina treated animals displayed condensed twist (25) that was close to the effect of diclofenac (18) treated mice (Figure 4).

Glutamate stimulation outcome

Figure 5 demonstrated the anti-nociceptive effects of V. amygdalina in glutamate induced mice. There was lessen amount of licks indicated the anti-nociceptive effects of V. amygdalina. Also, the V. amygdalina (7.5 mg) oral routed animals achieved 58.57 ± 1.11 licks (p < .05) which was equivalent to the regular diclofenac, which showed 50.38 ± 1.12.
licks. But in contrast to control animals, the 2.5 mg and 5.0 mg of *V. amygdalina* managed mice that illustrated reduced ranges of licks subsequent to glutamate insertion.

**Capsaicin encouraged paw licking analysis**

During the post inoculation phase, mitt licking actions took almost 5 min in the aggrivated capsaicin introduced mice. By contrary, the regular drug of diclofenac (48 ± 2.3 lick) and 7.5 mg of *V. amygdalina* introduced mice performed fewer counts (*p < .05*) of licks (43 ± 1.65 licks). The highest quantity of clicks was found in normal mice, while it was appreciably reduced in 2.5 and 5.0 mg of *V. amygdalina* induced animals in Figure 6.

**Paw licking test induced by formalin**

Figure 7 explained the sedative pain medicine in the formalin-induced model to know the consequence of *V. amygdalina* and morphine. Formalin provoked mitt licking trial explicated and confirmed the anti-nociceptive upshot in *V. amygdalina* at two stages. In comparison to normal and all other dosage of *V. amygdalina*, pre-initiated mice licking discharge were extensively (*p < .05*) declined in two stage of study. The *V. amygdalina* treated mice was increased at Stage B (15–30 min) that was similar to Stage A (5 min) hammering. And the Morphine managed animals revealed remarkable drop off in licking, which contrast to control and *V. amygdalina* introduced mice.

**Peritoneal void leukocyte permeable test**

The amount of whole leukocytes, mononuclear and polymorphonuclear invaded cells in the serous membrane hollow space in control animals, *V. amygdalina* (2.5, 5.0, 7.5 mg) and morphine pre-trial animals were completely represented in Figure 8. The *V. amygdalina* and morphine treated animals showed (*p < .05*) declined number of leukocyte invasion when compared to control mice. Also, there was negligible invasion of leukocytes was observed in the morphine pre-treated mice. As well, the minimum permeable of leukocytes were observed in 75 mg of *V. amygdalina* treated mice, which was similar to the morphine treated.

**Outcome of *V. amygdalina* on inflammatory cytokines**

The inflammatory cytokines such as TNF-α (Figure 9(A)), IL-1β (Figure 9(B)), IL-6 (Figure 9(C)) were examined in carageenan alone, *V. amygdalina* and dexamethasone treated mice that achieved in space sacks, In contrast, carageenan alone introduced experimental animals were considerably increased the above mentioned markers plane. Among all the three dosages, the absorption of *V. amygdalina* and dexamethasone were repressed (*p < .05*) the attentiveness of TNF-α (Figure 9(A)).

**Open field examination**

The tranquiliser efficacy of *V. amygdalina* was evaluated by the behaviour changes of experimental animals in open field equipment. When contrast to normal mice, the *V. amygdalina* (2.5 and 5 mg) induced mice does was not displayed any considerable (*p < .05*) modifications while the integer of squares was crossed by the *V. amygdalina* (7.5 mg) induced mice. But, the morphine treated mice exposed diminished behavioural alterations (Figure 10).

**Discussion**

The chemicals, thermal, and electrical induced inflammatory pain models were extensively used in the preclinical researches [36]. These models can be regarded as a way to mimic the inflammatory pain symptoms noted in the clinical in the case of tissue inflammation, where symptoms of alldynia and mechanical allodynia are observed in the patients [37]. In the current study, we examined the dose-dependent
anti nociceptive and anti-irritation effects of *V. amygdalina* in diverse animal models that explained the effect was completely contrast with the regular drugs. The anti-inflammatory exploit of *V. amygdalina* was well established by evaluation of leukocyte invasion in abdominal lining and also by the plane of inflammatory cytokines. The UV-Vis absorption range of ZnO nanoparticles by *Nyctanthes tristis* established an absorbance at 369 nm [38]. Also, the FTIR series of ZnO nanoparticles exhibited the crest at 417.52 cm$^{-1}$, denoted the assimilation of ZnO connection and the extensive absorption of peak at 3438 cm$^{-1}$ confirmed the typical enclosure of hydroxyl group [39]. As well the peak at 667.29 cm$^{-1}$ indicated the expanding observance of ZnO nanoparticle which was correlated with the present outcome [40]. Additionally, in XRD studies had no extra peaks that allegedly described the macromolecule amalgamation of ZnO NPs, derived elevated limpidness. The crystal-like gathering was established at 21 nm [41]. The scanning Electron Microscope depicted the ZnO NPs synthesising by *Trifolium pretense* that had constituent dimension displayed from 100 to 190 nm [42]. This correlated with our current SEM records with particles dimension arrayed from 50 to 100 nm.

Pain is the reflective response to the stimulus such as, infection, injury, etc. The nociceptive assessments were executed to evaluate the curative effect of sample agents to reduce the pain. The numerous assays were performed to such as mechanical, electrical, thermal, and chemical stimulus [43]. The acetic acid-stimulated abdominal constriction is a visceral pain model, usually executed to screen the antinociceptive or anti-inflammatory effects of sample analgesic agent [44]. The formalin injection to the experimental animals stimulates the biphasic pain response [45]. The first phase corresponds to the neurogenic pain caused by the direct effect of formalin and the second phase is the development of an inflammatory response and the release of nociceptive mediators like serotonin, prostaglandins, bradykinin in the peripheral tissues [46]. The tail immersion induced pain model is executed to examine the acute pain and it is a helpful assay to differentiate the central opioid like analgesics from peripheral analgesics [43]. The open field test was performed to confirm the anti-nociceptive action of sample analgesic agents related to non-specific disturbances in the locomotor activity of the experimental animals [47].

The greenery synthesis of zinc oxide nanoparticles using *Moringa oleifera* had rough and delicate crest from Zn and O atom and weak peaks were found form the elements like calcium and potassium [48]. The study of nociceptive was executed to review the beneficiary properties of drug to lessen soreness, which carried out by geothermal, automatic or electrical incentive [49]. Past research described the *V. amygdalina* was one of the potential therapeutic to analyse toxicity test [50]. The Carrageenan provoked rat was a better model to estimate the anti-oedematous effects of herbal yield with biphasic in nature [51]. The primary phase involving in the
discharge of serotonin and the expressing of histamine in the second phase (after 1 h) was mediated by prostaglandins, cyclooxygenase harvest and the stability lying between two phases was offered by kinins [52].

The *V. amygdalina* is rich in flavonoids, steroids, necessary oil and tannins, which preferably used in animal models to predict nociception [53–56]. The certain stimulus of nociceptors can be reviewed by the initiation of inflammation that similar to acetic acid. These can be used to calculate writhes, which was ground to examine stomach renunciation and stretching of hind limb. The acetic acid model was performed to perceive the outcome of painkiller by the levels of prostaglandins in CNS, which usually elevated in the nociceptors [57]. The current results demonstrated that *V. amygdalina* established repressed in the synthesis of prostaglandins E2 and prostaglandins D2 [58]. Since the carrageenan induced inflammation was an important prognostic test for anti-inflammatory mediator sensitive inflammation, these outcomes suggested that the *V. amygdalina* was an efficient in severe inflammatory turmoil. In this study, the nanoparticles

Figure 9. The consequence of ZNO-NP on TNF-α, IL-1β, and IL-6 fabrication in the air pouch test. All values are illustrated as mean ± SD of six animals. The statistical significance level was calculated by one-way ANOVA followed by the Dunnet’s post hoc test; note: #p < .05 when compared with control group and *p < .05 when compared with ZnONPs administered groups. (A) TNF (pg/ml) (B) IL-1β (pg/ml) (C) IL-6 (pg/ml).

Figure 10. The effects of ZNO-NP in the open field test. All values are illustrated as mean ± SD of six animals. The statistical significance level was calculated by one-way ANOVA followed by the Dunnet’s post hoc test; note: #p < .05 when compared with control group and *p < .05 when compared with ZnONPs administered groups.

![Graphs showing TNF, IL-1β, and IL-6 levels](image-url)
from the *V. amygdalina* showed the potent anti-inflammatory activity against the carrageenan induced inflammation in mice [52,59,60].

In the present finding, the reduction in quantity of writhes was achieved by dissimilar absorption of *V. amygdalina*. This revealed the *V. amygdalina* also would inhibit the production of prostaglandins that related to sensitisation of nociceptors. Glutamate transmits the reaction by two sorts of receptors i.e. N-methyl-D-aspartate and non-NMDA receptor which activate the peripheral neurons by liberating proinflammatory cytokines [61]. Correspondingly, our study of *V. amygdalina* pre-treated mice demonstrated glutamate-induced soreness by counting of licking number. The earlier reports about the acetone extract of *V. amygdalina* showed notable decline in the integer of paw licking, which induced by formalin as compared to the control [50]. The two phase discharge of prostaglandins E2 stimulated nociceptive inside formalin nociception rat replica, and initiation of tenderness generated by inflammatory cytokines on centre sensation neurons [62]. As correlated with this current study, the *V. amygdalina* drastically reduced the formalin-induced licking numbers in both neuro and inflammatory phases that explained as anti-nociceptive agent. The Naringenin restricted the carageenan pro-voked leukocyte invasion in intestinal lining hollow space. The increase of leukocyte production may direct the Myeloperoxidase action in animal foot edema by reactive oxygen species produced by carageenan [63].

Spinal supportive nerve cell exude powerful pro inflammatory cytokines such as TNF-α, IL-1β and IL-6 which respond to the pain [64,65]. The present results showed anti tenderness of *V. amygdalina* and considered as space pocket model trial. The plane of inflammation promoting cytokines was down regulated in *V. amygdalina* treated mice that contrast to control mice. The open field examination explored the improved behavioural transformation in *V. amygdalina* induced mice than standard drug, which brought effective anti-nociceptive drug with minimal side effects. Therefore resistance of inflammatory cytokine production by *V. amygdalina* might be the motive for its anti-nociceptive possessions.

**Conclusions**

The present study exhibited proficient antinociceptive and anti-inflammation action of the combination of Zinc oxide nanoparticle with the extract of *V. amygdalina*. Further results demonstrated the beneficial effects Zinc oxide nanoparticles from the *V. amygdalina* through ameliorating the anti-inflammatory and antinociceptive activities. It explained the systematic base on pain improvement and managing inflammatory disarray. In dissimilar nociceptive and swelling mice models, the *V. amygdalina* was found as strong antinociceptive and antisoreness drug by exposing the performance of pro-inflammatory cytokines. Altogether, all the outcomes revealed the effective anti-inflammatory of zinc oxide nanoparticles from *V. amygdalina* in mice models.

**Authors’ contribution**

Xiao Wang – conceived the study design, commented on both the manuscript and figures, and approved the manuscript; Hairui Liu and Peipei Kang – analysed data; Ying Liu, Yifan An and Yanting Hu – composed this manuscript and prepared the figures; Xiuyan Jin, Xin Cao, Yunfei Qi and Thiyagarajan Ramesh – proof reading the manuscript.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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