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Antinociceptive and Antioxidant Activity of an Aqueous Root Bark Extract of *Daniellia oliveri* (Rolfe) Hutch. & Dalziel (Fam: Leguminosae [Fabaceae]) in ICR Mice

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ABSTRACT

Daniellia oliveri stem bark is used traditionally by the people of Northern Ghana to manage pain. This study therefore sought to validate the antinociceptive property of an aqueous stem bark extract of *Daniellia oliveri* (DOE) using murine hot plate and paw pressure pain models as well as its antioxidant property. Groups of ICR mice were pre-treated with DOE (250, 500, 1000 or 2000 mg kg^{-1}, p.o), morphine (3 mg kg^{-1}, i.p), diclofenac (3 mg kg^{-1}, i.p) or normal saline (2 ml/kg) respectively for 0.5 - 1 h, prior to pain induction. Pain latency period were measured at 0.5 h intervals for 1.5 h. To establish the possible mode of analgesic activity, nociceptive activity of DOE was antagonized by naloxone (2 mg kg^{-1}), glibenclamide (8mg kg^{-1}), and theophylline (5mg kg^{1}). The extract was screened for antioxidant property by its effect on DPPH radical scavenging activity. DOE in both pain models produced significant ($P \leq 0.001$) dose and time - dependent antinociceptive effect comparable to morphine, and diclofenac. The antinociceptive effect of DOE was significantly ($P \leq 0.001$) attenuated by naloxone, glibenclamide, and theophylline. DOE caused a concentration dependent percentage increase in DPPH radical scavenging activity. The aqueous stem bark extract of *Daniellia oliveri* therefore has antinociceptive and antioxidant effect with antinoception possibly mediated through activation of ATP-sensitive potassium channels, as well as opioidergic and adenosinergic receptor pathways.

INTRODUCTION

Pain is a major symptom of many clinical conditions and also one of the numerous reasons why patients consult physicians (Debono *et al.,* 2013). According to International Association for the Study of Pain (IASP) and European Federation of the IASP Chapter (EFIC) one out of every five people globally suffer from moderate to severe chronic pain, and one out of every three people is unable or less able to maintain an independent lifestyle due to pain and about two-thirds of individuals with chronic pain are less able or unable to exercise, enjoy normal sleep, perform household chores, attend social activities, drive a car, walk or have sexual relations (WHO, 2004). Pain relief has long been a dominant concern of humans worldwide although pain serves some important purposes. Nonetheless, Melzack *et al.,* (1983) had argued that pain serves three purposes: firstly, short-lasting pain causes us to withdraw from the source, often reflexively, thus preventing further damage; secondly long-lasting pain promotes behaviors such as sleep, inactivity, grooming, feeding, and drinking that promote recuperation and finally the expression of pain may serve as a social signal e.g. screeching after a painful stimulus may have significant adaptive value by signaling the potential of harm or eliciting certain care-giving behaviors from others such as grooming, defending, and feeding that could mean the survival of the victim. Aside these arguments are the reality of managing pain. It has been recommended that non-pharmacological interventions (e.g., relaxation) be used first to alleviate pain before drug use (Bar *et al.,* 2013) because the known conventional analgesics produce adverse drug reactions (ADRs).

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The most common and effective analgesics are the opioids and Non-steroidal anti-inflammatory drugs (NSAIDs). Opioids such as morphine, codeine and pethidine are most often used to manage acute pain (Walder and Tramer, 2004) or moderate to severe acute pain (Kohn *et al.*, 2007). NSAIDs have traditionally been used more for mild pain and anti-inflammation than for chronic pain. However, newer NSAIDs have significant analgesic effects overlapping with that of opioids (Kohn *et al.*, 2007). But like all other drugs and in their case in particular, the adverse drug reactions produced by the opioids and NSAIDs exceed their therapeutic benefits e.g. these drugs have been reported of a wide range of side effects including respiratory depression, bradycardia, hypotension, constipation, decreased mental capacity, addiction, tolerance, withdrawal, nausea, vomiting, pruritus, urinary retention, intestinal hypomobility, gastrointestinal disturbances, and hepatotoxicity (Walder and Tramer, 2004; Kohn *et al.*, 2007). There is therefore an unmet need for medicinal plants and phytopharmaceuticals with scientifically proven analgesic efficacy. Phytopharmaceuticals with demonstrable efficacies could become a suitable therapeutic alternative to conventional medications for specific indications such as pain. One such medicinal plant with rich potential for exploration of its beneficial pharmacological activities is *Daniellia oliveri*. The plant has been used in various crude forms to manage pain by most tribes in northern Ghana, yet not much scientific investigation has been made regarding its traditional analgesic use. The study therefore sought to validate the antinociceptive property of an aqueous stem bark extract of *Daniellia oliveri* using murine hot plate and paw pressure pain models as well as its antioxidant property.

MATERIALS AND METHODS

Plant collection, identification and authentication

Daniellia oliveri was collected from Dalong in the Northern Region of Ghana. The plant was identified and authenticated by a pharmacognosist at the herbarium unit of the School of Biological Sciences, University of Cape Coast, Ghana, where a voucher specimen (SBS/UCC/D573) was deposited.

Preparation of *Daniellia oliveri* aqueous extract (DOE)

The stem bark of *Daniellia oliveri* was washed thoroughly and air-dried. The dried stem bark was pulverized into fine powder using a hammer mill (Schutte Buffalo, NewYork, USA). A 400 g quantity of the powdered stem bark was mixed with 2L of distilled water and warmed at 40˚C for 20 minutes. It was then filtered first using a white nylon cloth and subsequently using Whatman's No 1 filter paper, to obtain a dark-brown filtrate, which was concentrated over a hot water bath at 60˚C and dried in a hot-air oven at 60˚C. A dark-brown powdery solids, named DOE, (percentage yield: 25.5%) was stored in a desiccator.

Drugs and chemicals

Drugs and chemicals used in the study included: Morphine hydrochloride (Phyto-Riker, Accra, Ghana), naloxone (Troge Medical Gmbh, Hamburg, Germany) and glibenclamide (Sanofi-Aventis, Guildford, UK), theophylline (BDH, Poole, England) and normal saline (Intravenous Infusions Ltd., Koforidua, Ghana).

Phytochemical screening of DOE

DOE was screened to identify phytoconstituents present in the extract using standard phytochemical methods described by Trease and Evans (1989), Sofowora (1993), and Harborne (1998).

Experimental animals

Healthy five-week old ICR mice (25 - 35 g) of either sex were purchased from Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Ghana and maintained at the Animal Experimentation Department of the School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana. The animals were housed in groups of 7.0 in stainless steel cages (34 × 47 × 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema, Ghana), and were given water *ad libitum***.** Animals were maintained under ambient laboratory conditions (i.e. Temperature 24-28 °C, Relative Humidity 60 - 70% and 12h light-dark cycle). All procedures and techniques used in the study were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication No. 85-23, revised 1985).

Hot plate pain model

A hot plate (Stuart Scientific Magnetic Stirrer, SN: 24642, U.K.) maintained at a temperature of 55˚C ± 1˚C was used. The pain latency period i.e. time between pain stimuli application and pain perception, was measured for each animal in each group by placing the animal on the hot plate and noting the time the animal licks its paw or jumps off the plate, before and 60, 90 and 120 minutes post treatment. Mice in groups 1, 2, 3 and 4 were pre-treated orally with DOE (250, 500, 1000, and 2000 g kg^{-1} respectively), groups 5 and 6 were pre-treated with diclofenac (3 mg kg^{-1}; i.p) and morphine (3 mg kg^{-1}; i.p) respectively; and group 7 was treated with the vehicle (normal saline). A cut off time of 20 seconds in the case of unresponsiveness, was selected to prevent thermal injury to animals. The percent maximum possible effect (%MPE) was calculated primarily as the analgesic activity using the equation below:

$$\% \text{ MPE} = \frac{\text{Post-drug latency} - \text{Pre-drug latency} \times 100}{\text{Cutoff latency} - \text{Pre-drug latency}}$$

Paw pressure pain model

In the paw pressure pain model, an analgesiometer (Ugo Basile Biological Research Apparatus, SN: 15776 v 220, Milan-Italy) as described by Randall and Selitto (1957) with modification by Woode *et al.*, (2013) was used. Before the test, animals in each group received 10 minutes of handling. To get used to manipulation, each animal was placed in a soft cotton cloth and carefully immobilized with the same hand used to hold the tested

paw. Baseline measurements were taken for all animals before treatments. A total of 28 ICR mice were randomly put into seven groups of four animals each. Mice in groups 1, 2, 3 and 4 were treated orally with DOE (250, 500, 1000, and 2000 mg kg^{-1} respectively). Mice in groups 5 and 6 were treated with diclofenac (3 mg kg^{-1}) and morphine (3 mg kg^{-1}) respectively. Mice in group 7 were treated with vehicle (normal saline). For each animal, the analgesiometer was used to apply linearly increasing mechanical force (pressure) by the tip of a blunt perspex cone to the dorsal region of the right hind paw until the animal exhibited nociceptive withdrawal. The nociceptive withdrawal threshold was measured by recording the distance covered on the analgesiometer scale at which each mice exhibited nociceptive withdrawal. The nociceptive withdrawal threshold was measured at 60 and 120 minutes post-drug administration. The maximum force applied was limited to 250 g (2500N) to prevent tissue damage. The results were recorded as latency (reaction time) and a maximum cut off time of 20 seconds was set to avoid prolonged nociception and tissue damage. The percent maximum possible effect (% MPE) was calculated primarily as the analgesic activity by using the previously described equation.

Establishment of mode of DOE analgesic activity

An antagonist-agonist receptor interactions involving naloxone, theophylline, and glibenclamide as antagonists and DOE or morphine as agonist drug in the hot plate pain model was employed. Nine groups of mice with four mice in each group were used. To investigate the involvement of opioid receptor activation, one group of mice were pre-treated with naloxone (2 mg kg^{-1}; i.p), followed by treatment with DOE (2000 mg kg^{-1} p.o), 30 minutes after administration of antagonist drugs. The pain latency period (seconds) was measured at 60, 90 and 120 minutes post-treatment. For the involvement of adenosinergic pathways, another group of mice were pre-treated with theophylline (5 mg kg^{-1}; p.o). To investigate the involvement of ATP-sensitive K^+-channels, mice were pre-treated with glibenclamide (8 mg kg^{-1}; p.o) followed 60 minutes later by DOE and latency determined at 60, 90 and 120 minutes post DOE treatment. These procedures were repeated for morphine (3 mg kg^{-1}; i.p) and the latency periods measured at 30, 60 and 90 minutes post-treatment.

Antioxidant property of DOE

Scavenging activity against 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) radical was evaluated as described by Stankovic *et al.*, 2010. A stock solution of DOE prepared in methanol to a concentration of 100 µg ml^{-1} was diluted. Dilutions were made to obtain concentrations of 80 µg ml^{-1}, 40 µg ml^{-1}, and 20 µg ml^{-1}. Each milliliter of the diluted solutions was mixed with 1ml of DPPH methanolic solution (40 µg ml^{-1}). After 30 minutes in darkness at room temperature (23ºC), the absorbance of the test and blank samples were recorded at 517 nm using a microplate reader (Rayto RT-2100C, China). The control sample contained all the reagents except the extract. All experiments were performed thrice and the results were averaged. Percent inhibition was calculated using the following expression:

$$\% \text{ inhibition} = \frac{(A_{blank} - A_{sample}) \times 100}{A_{blank}}$$

Where; A_{blank} and A_{sample} stand for absorption of the blank sample and absorption of tested extract solution respectively. The IC_{50} values which denote the concentration of test drugs required to scavenge 50 % of DPPH free radicals were estimated. Ascorbic acid was used as the standard.

Statistical analysis

Values were presented as Means ± SEM. Significant differences in measured parameters between treatments were established either by; Two-way ANOVA followed by Bonferroni's *post hoc* test, or One-way ANOVA followed by Dunnett's Multiple Comparison *post hoc* test available in GraphPad Prism for Windows Version 5.00 (GraphPad software, San Diego, California, USA). A $P \leq 0.05$ was considered statistically significant for all analyses. The graphs were plotted using Sigma Plot Version 11 for windows (Systat Software Inc. Germany).

RESULTS

Phytochemical screening

Phytochemical screening of DOE showed the presence of some phytocompounds (Table 1).

Analgesic activity

DOE produced both dose and time dependent increase in the pain threshold in the hot plate pain model, with analgesic activity comparable to Diclofenac [DIC], and morphine [MOR] (Figures 1 and 2). At equipotent doses morphine comparatively increased the pain threshold than diclofenac. Although morphine and diclofenac produced a pain threshold greater than DOE at 250 mg kg^{-1}, their effects were lower than that produced by DOE at 500, 1000, and 2000 mg kg^{-1} (Figure 3). Similar observations were made in the paw pressure pain model except that DOE at 2000 mg kg^{-1} produced dose but not time dependent increase in the pain threshold (Figure 4).

Mode of DOE analgesic activity

Naloxone, Theophylline, and Glibenclamide significantly ($P \leq 0.001$) attenuated the antinociception produced by DOE and morphine (Figures 5, 6 and 7). The attenuation of antinociception by naloxone was replicated at all the post-treatment times (except at time zero) for DOE, but was highly significant ($P \leq 0.001$) for morphine (Figure 6). Whiles theophylline attenuated antinociception produced by DOE and morphine at 0, 60, 90 minutes post-treatment time, it did not block morphine at 120 minutes post-treatment (Figure 6). Glibenclamide significantly ($P \leq 0.001$) attenuated antinociception produced by DOE at all post-treatment times, it did not block morphine except at 120 minutes post-treatment (Figure 7).

Table. 1: Phytochemical composition of DOE.

Phytochemical	Results
Terpenoid	+
Saponin	+
Steroids	+
Flavinoid	+
Tannins	+
Phlobatannins	+
Emodels	+
Flavanols	+

+ : indicates the presence of the phytocompound

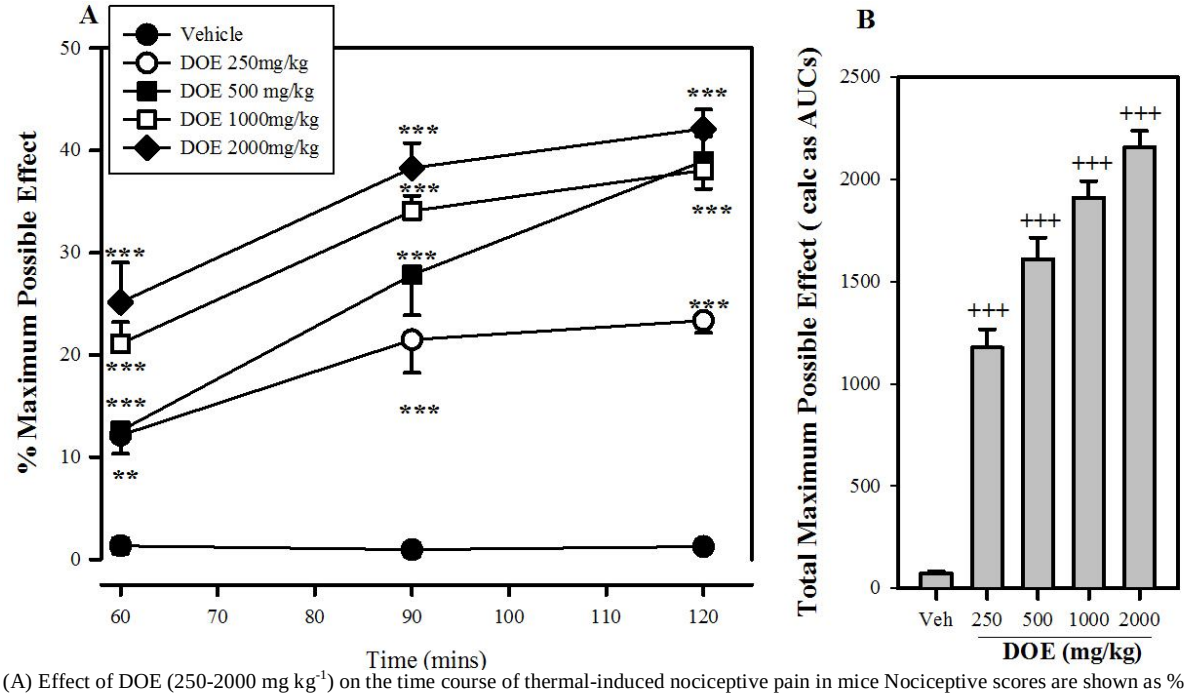

Fig. 1: (A) Effect of DOE (250-2000 mg kg^{-1}) on the time course of thermal-induced nociceptive pain in mice Nociceptive scores are shown as % maximum possible effect (% MPE) at 60, 90 and 120 min. (B) The total maximum possible effect calculated from the AUCs from the time-course curve. Each point represents mean ± S.E.M (n = 4). ***P < 0.001; ** P < 0.01 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's *post hoc* test). ^{+++}P<0.001 compared to vehicle-treated group (One-way ANOVA followed by Dunnett's Multiple Comparison *post hoc* test).

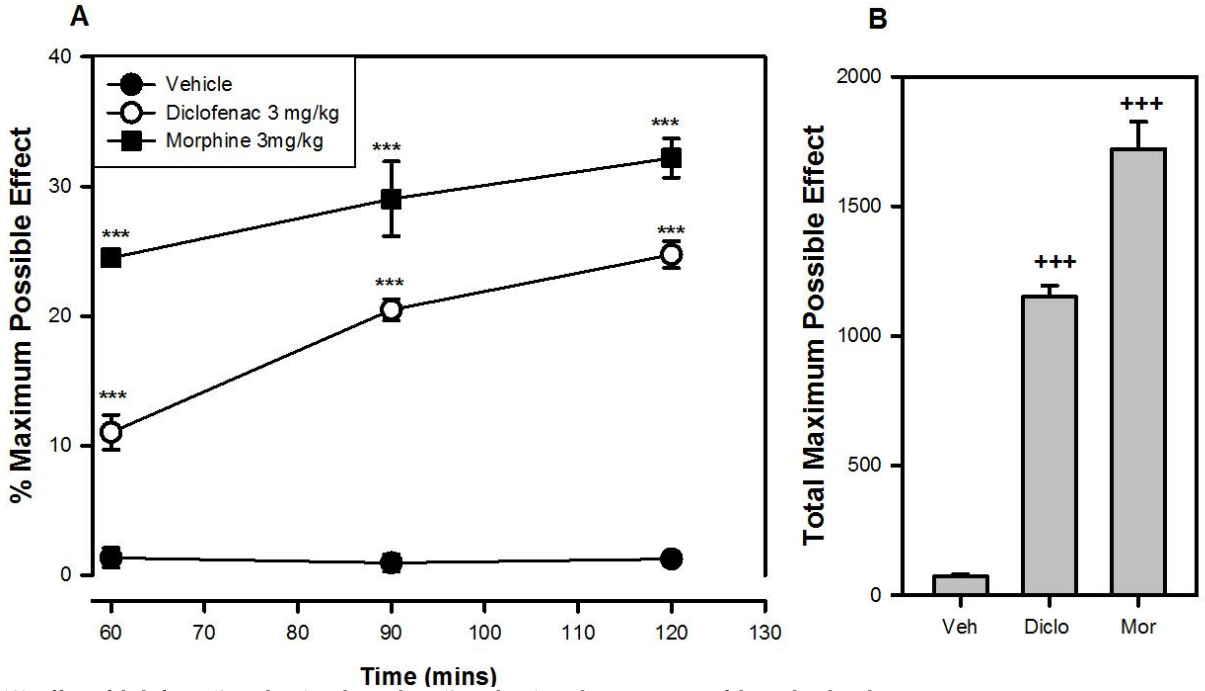

Fig. 2: (A) Effect of diclofenac (3 mg kg-1) and morphine (3 mg kg-1) on the time course of thermal-induced nociceptive pain in mice. Nociceptive scores are shown as %maximum possible effect (% MPE) at 60, 90 and 120 min. (B) The total maximum possible effect calculated from the AUCs from the time-course curve. Each point represents mean ± SEM (n = 4). ***P < 0.001; ** P < 0.01 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's post hoc test). +++P<0.001 compared to vehicle-treated group (One-way ANOVA followed by Dunnett's Multiple Comparison post hoc test)

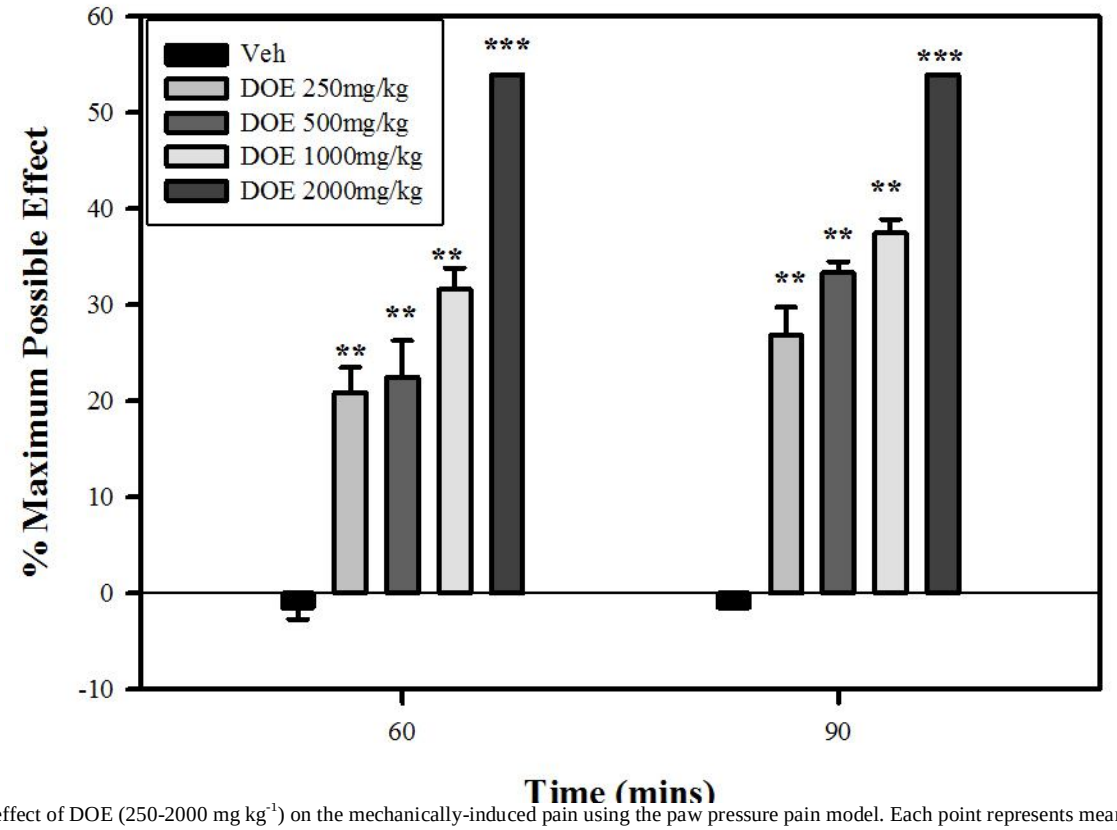

Fig 3: The effect of DOE (250-2000 mg kg^{-1}) on the mechanically-induced pain using the paw pressure pain model. Each point represents mean ± SEM (n = 4). ***$P < 0.001$; ** $P < 0.01$ compared to vehicle-treated group (One-way ANOVA followed by Dunnett's Multiple Comparison *post hoc* test).

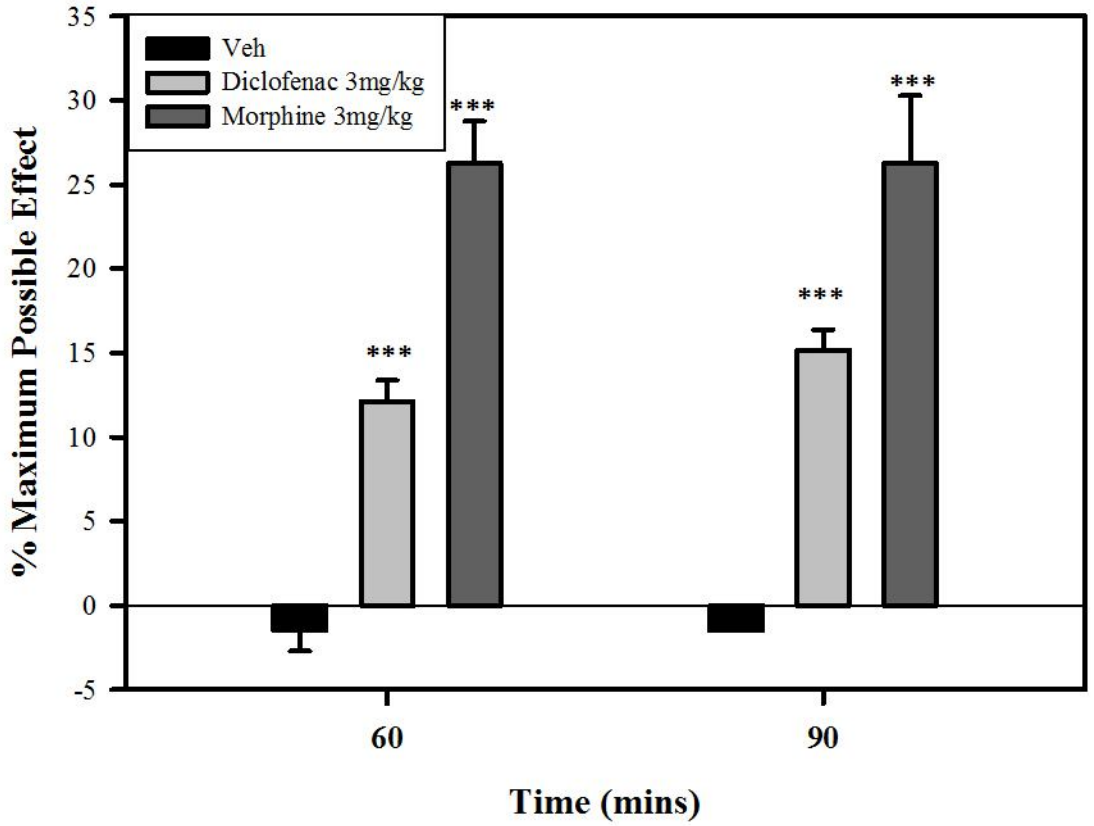

Fig. 4: Effect of diclofenac (3 mg kg^{-1}) and morphine (3 mg kg^{-1}) on the mechanically-induced pain using the paw pressure pain model. Each point represents mean ± SEM (n = 4). ***$P < 0.001$; ** $P < 0.01$ compared to vehicle-treated group (One-way ANOVA followed by Dunnett's Multiple Comparison *post hoc* test).

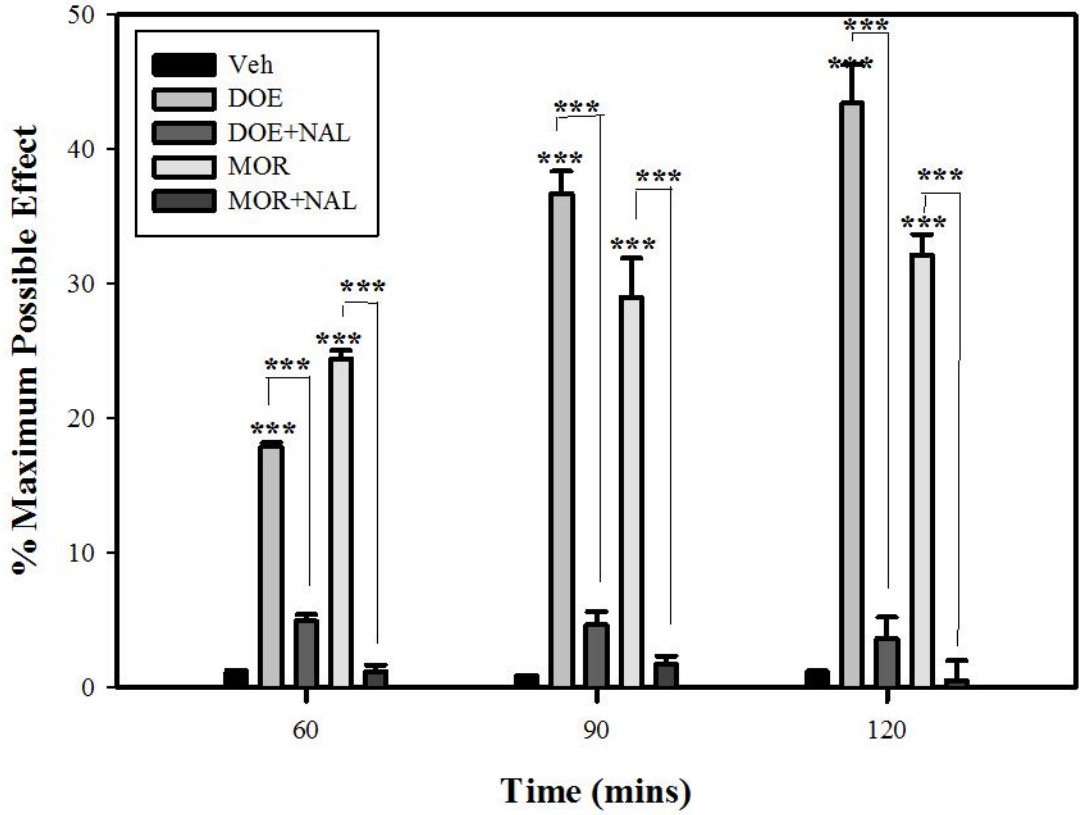

Fig. 5: Effect of naloxone, NAL (2 mg kg^{-1}) on the antinociceptive effects of morphine, MOR (3 mg kg^{-1}) and DOE (2000 mg kg^{-1}) at different post-treatment periods. Each point represents mean ± SEM (n = 4). ****P* < 0.001 comparison of treatment groups to vehicle-control group or between treatment groups (Two-way ANOVA followed by Bonferroni's *post hoc* test). NAL = Naloxone, MOR = Morphine.

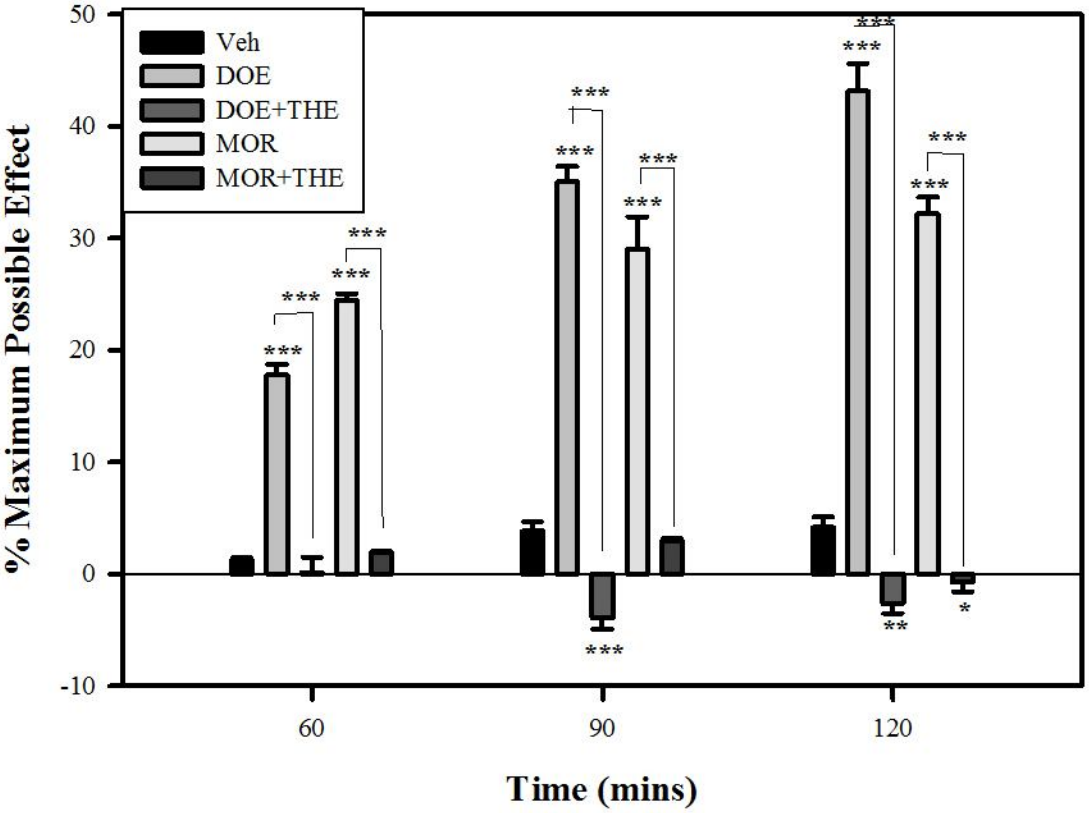

Fig. 6: Effect of theophylline, THE (5 mg kg^{-1}) on the antinociceptive effects of morphine, MOR (3 mg kg^{-1}) and DOE (2000 mg kg^{-1}) at different post-treatment periods. Each point represents mean ± SEM (n = 4). ****P* < 0.001 comparison of treatment groups to vehicle-control group or between treatment groups (Two-way ANOVA followed by Bonferroni's *post hoc* test). THE = Theophylline, MOR = Morphine.

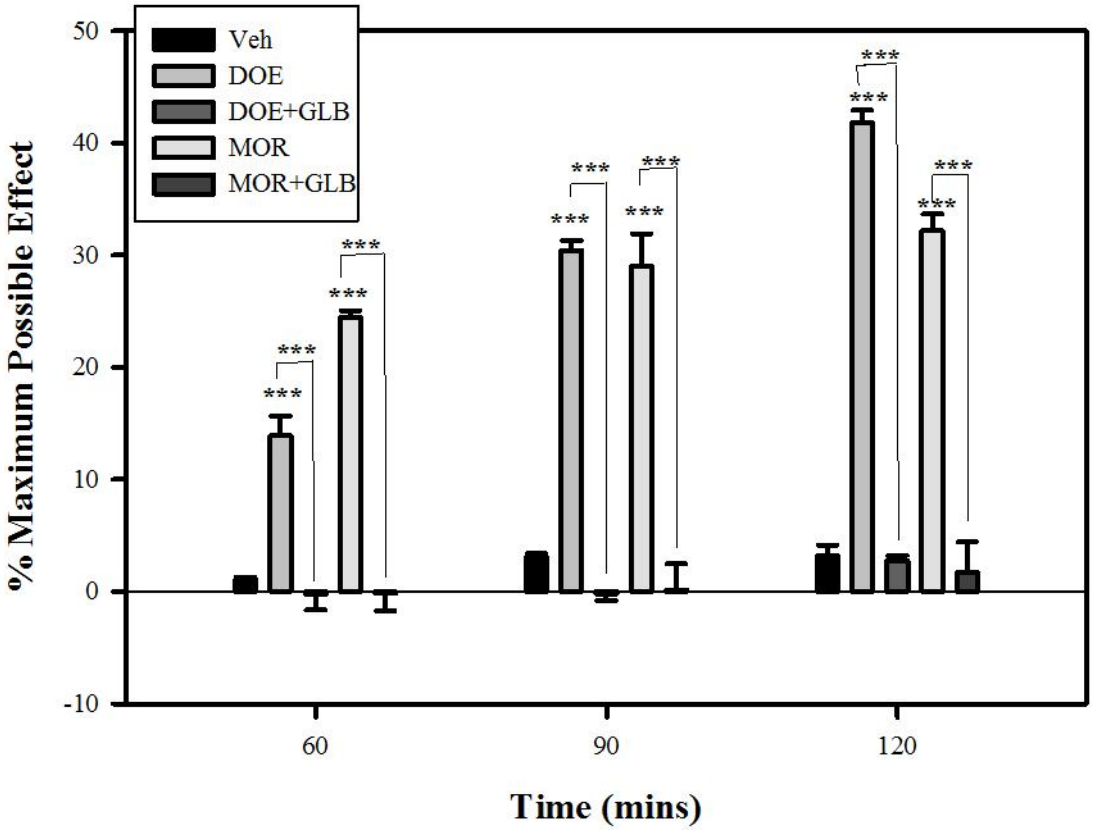

Fig. 7: Effect of glibenclamide, GLB (8 mg kg^{-1}) on the antinociceptive effects of morphine, MOR (3 mg kg^{-1}) and DOE (2000 mg kg^{-1}) at different post-treatment periods. Each point represents mean ± SEM (n = 4). ****P* < 0.001 comparison of treatment groups to vehicle-control group or between treatment groups (Two-way ANOVA followed by Bonferroni's *post hoc* test). GLB = Glibenclamide.

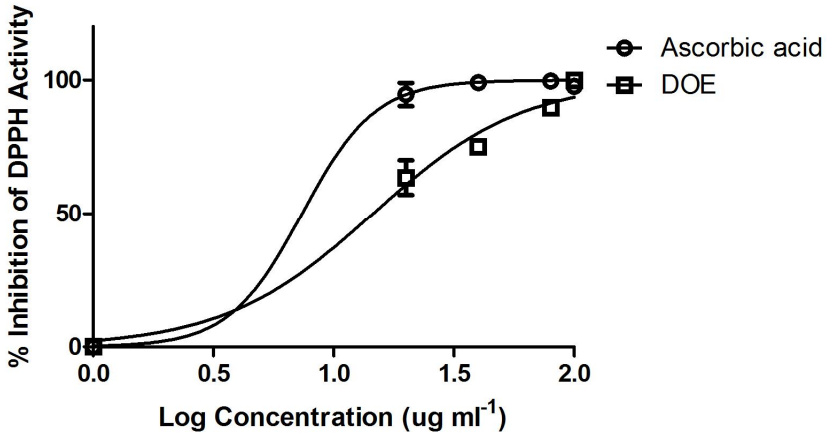

Fig. 8: Antioxidant activities of DOE and ascorbic acid (Standard) expressed as % inhibition of DPPH free radical oxidant activity. Value plotted are mean ± SEM (n = 3). IC50 for Ascorbic acid and DOE are 7.389 and 14.57 µg ml^{-1} respectively.

Antioxidant activity of DOE

The DPPH radical antioxidant test was carried out on both DOE and Ascorbic acid (Standard) indicated that both drugs caused a concentration dependent percentage increase in DPPH radical scavenging activity. However, ascorbic acid had a more significant antioxidant activity as indicated by a comparatively lower IC_{50} value of 7.389 µg ml^{-1}as against that of DOE which was 14.57 µg ml^{-1} (Figure 8).

DISCUSSION

In the present study the hot plate and paw pressure pain models were used to investigate the antinociceptive effect of DOE as a means of pharmacologically characterizing its activity in order to explain its local analgesic use. Our results demonstrate for the first time the dose and time dependent antinociceptive effect of DOE in models similar to morphine and diclofenac. The ability of DOE to attenuate nociception due to heat and pressure suggest it has centrally acting analgesic effect; an observation which agrees with findings by ching *et al.,* (2009) who had intimated that centrally acting analgesic drugs increase pain threshold of animals to heat and pressure. The similarity of the antinociceptive effect of DOE to that of morphine, an opioid receptor agonist which is a known centrally and periperally acting analgesic and diclofenac, a non-steroidal ant-inflammatory drug indicates that DOE could have both central and peripheral analgesic effects. This was supported by the possible mode of anagesic activity of DOE as the ensuing paragraphs explain.

In order to identify some of the possible biochemical pathways involved in the antinociceptive effects of DOE as a means of elucidating the possible mode (s) of action, an antagonism study carried out by using the hot plate test, showed attenuation of antinociceptive effects of DOE and morphine in the presence of three antagonist drugs (Naloxone, glibenclamide, and theophylline). Each of the three antagonist drugs did not produce antinociception when administered alone. However, prior systemic administration of naloxone (a non- selective opioid receptor antagonist) significantly attenuated antinociceptive effects of DOE and morphine in the hot plate pain model. Opioid receptors are G-protein-coupled receptors (GPCRs) and the main receptors involved in the modulation of pain in mammals (Reisine and Bell, 1993; Uhl *et al.,* 1994). The principal opioid receptor subtypes mu (µ), delta (δ), kappa (κ) and nociceptin / orphanin FQ peptide are all expressed in the spinal cord and in the brain as the main modulators of nociceptive transmissions (Pattinson *et al.,* 2008; Erfanparast *et al.,* 2010). In addition, the µ and κ opioid receptors are also expressed in the enteric nervous system. These four opioid receptors mediate many physiological effects of endogenous opioid systems including pain and analgesia, behavior, thermoregulation and immunological responses (Bodnar, 2007). The µ is the preferred receptor for potent analgesics with high potential for abuse, such as normally observed with morphine (Reisine and Bell, 1993). The present observation with DOE strongly indicate for the first time that the antinociceptive effect of DOE is mediated partly through opioidergic receptor activation and that DOE interrupts one or more of the biochemical events involved in opioid receptor activation to bring about antinociception, since naloxone reversed the antinociception produced by DOE.

Our results also demonstrate for the first time that pre-treatment of ICR mice with glibenclamide (A K_{ATP}-sensitive channel blocker) inhibited the antinociceptive effects of DOE and morphine alike in the hot plate test. Evidently, the antinociceptive effects of DOE involve the activation of K^+_{ATP}-sensitive channel opening and activation. On the basis of structure and specific agonists and antagonists, neural K+-channels are typically classified into four classes comprising voltage-gated (Kv), calcium- activated (K_{Ca}), inward rectifier (Kir) and two-pore (K_{2P}) K^+-channels (Hajhashemi and Amin, 2011). It has been established that central K^+-channels especially ATP-sensitive K+-channels (KATP) of the Kir class are involved in the perception of pain (Ocana *et al.,* 2004). Central administration of K^+ channel openers, such as diazoxide, minoxidil, lemakalim and cromakalim, produced antinociception and potentiated analgesic effects produced by opioid and α2-adrenoceptor agonists (Ghelardini *et al.,* 1990; Welch *et al.,* 1993). Furthermore, central administration of ATP dependent K^+-channel blockers, have been shown to attenuate antinociception of many drugs including antidepressants, amitriptyline and clomipramine in a hot plate test (Galeotti *et al.,* 2001). It seems that activation of G-protein coupled receptors (α2-adrenoceptors, opioids, $GABA_B$, muscarinic M_2, adenosine A_1, serotonin 5-HT_{1A} and cannabinoid) by agonists and some nonsteroidal anti-inflammatory drugs (NSAIDs) involve K^+-channels opening and activation (Ocana *et al.,* 2004). The attenuation of antinociception produced by DOE strongly suggest that DOE can block the activation of actual nociceptors and the release of inflammatory mediators involved in the processing and perception of pain as already established (Ocana *et al.,* 2004; Pattinson *et al.,* 2008; Erfanparast *et al.,* 2010). The mechanism of action of DOE falls in line with the fact that opioid receptors and K^+_{ATP}-channels converge in regulating release of neurotransmitters, and initiation of neuronal excitability with both signaling pathways being effective in attenuating perception of pain in animals and in patients (Rodrigues and Duarte, 2000).

In a similar manner, adenosine, a non-selective adenosine receptor antagonist attenuated the antinociceptive effect of DOE and morphine. Specifically, A_1-adenosinergic receptor activation is proposed to be linked to a multi-receptor complex comprising A_1, µ-opioid and α_2-adrenosinergic receptors which have been demonstrated on the basis of cross antagonism, cross tolerance, and cross withdrawal among these receptor systems (Aley and Levine, 1997; Sawnok and Liu, 2003). Suh *et al.,* (1997) had reported that activation of one of these receptor systems may affect the others.

The antinociceptive effect of DOE could possibly have been due to the collective physiological effects of all the secondary plant metabolites i.e. phytochemicals present in the extract which were terpenoids, saponins, steroids, flavonoids, tannins,

phlobatannins, emodels and flavanols working synergistically or additively to produce antinociception. Detection of these phytochemicals in DOE agrees with earlier findings by Kabore *et al.,* (2012). Triterpenoids and steroidal glycosides, collectively referred to as saponins are bioactive compounds present naturally in many plants. Saponins are a major family of secondary plant metabolites containing a sugar moiety glycosidically linked to a hydrophobic aglycone (sapogenin). Many reports had demonstrated the antioxidant and anti -inflammatory pharmacological activities of saponins in both *in vitro* and *in vivo* experiments (Mylonas and Kouretas, 1999; Sur *et al.,* 2001; Muraleeedharannair *et al.,* 2012).

Zhang *et al.,* (2001) had reported the chemoprotective effects of saponins, it was therefore not surprising that DOE which had saponins as a component produced antinociceptive effect since pain has inflammatory component. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized on the basis of their chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. Studies suggest that flavonoids, such as quercetin, may confer pain and inflammation reducing activity by inhibiting cyclooxygenase, lipoxygenase, and phospholipase C (Percival, 1999). Inhibition of oxidases, such as *lipoxygenase* (LO), *cyclooxygenase* (COX), *myeloperoxidase* (MPO), *NADPH oxidase* and *xanthine oxidase* (XO), have also been considered as important mechanisms for the ability of flavonoids to suppress generation of reactive oxygen species (ROS) *in vivo*, as well as organic hydroperoxides (De Groot and Rauen, 1998). Moreover, they have also been shown to inhibit enzymes indirectly involved in oxidative processes, such as phospholipase A_2 (FLA_2) (Lindahl and Tagesson, 1997), while they stimulate others enzymes with recognized antioxidant activity, such as catalase and superoxide dismutase (SOD) (Sudheesh, 1999). For instance, they have been reported to have antinociceptive (Maleki-Dizaji *et al.,* 2008; Campêlo *et al.,* 2011), antioxidant (Bioportfolio, 2013; Rossato *et al.,* 2011) and anti-inflammatory effects (Hussain *et al.,* 2011).

Tannins have been shown to have anti-inflammatory and antioxidant properties for therapeutic use (Muraleeedharannair *et al.,* 2012). Ratnasooriya et al., (2005) had suggested a link between antioxidant activity and antinociception. In this study our results have shown the presence of saponins, flavonoids, emodels, flavanols, phlobatannins which have invariably been shown to have antioxidant activity (Ferreira *et al.,* 2004; Barbosa *et al.,* 2004; Masoko *et al.,* 2010). It is possible these antioxidant agents conferred antinociceptive effects on DOE.

CONCLUSION

We conclude that *Daniellia oliveri* has dose dependent antinociceptive effect in murine pain models and that the antinociceptive effect is mediated through opioidergic, adenosinergic and ATP-sensitive K^+-channel receptor pathways. Also, the antinociceptive effect of *Daniellia oliveri* could be secondary to its phytocompound with demonstrated antioxidant activity. Our results therefore pharmacologically substantiate the local use of the plant as a pain reliever.

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