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Analgesic Potential of *Opuntia dillenii* and its Compounds Opuntiol and Opuntioside Against Pain Models in Mice

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Abstract: *Opuntia dillenii* (Nagphana) traditionally used against inflammation and also possess analgesic effect. Thus in the present study analgesic properties of *O. dillenii* cladode methanol extract, its fractions obtained *via* vacuum liquid chromatography along with isolated α-pyrones, opuntiol and its glucoside, opuntioside were analyzed. The acetic acid-induced writhes were reduced by *O. dillenii* test agents with opuntioside being most effective (IC_{50} 26 ± 0.9 mg/kg) and equipotent to diclofenac and β-sitosterol. Consistently, it also elicited most potent effect (IC_{50}: 28 ± 1.1 and 24 ± 1.2 mg/kg) during early and late phases of formalin-induced paw licking, producing effect similar to diclofenac and indomethacin. It was also most effective in hot plate test. Naloxone (opioid antagonist) reversed the analgesic effects of extract and fractions but failed to antagonize the opuntiol and opuntioside analgesic effects. In conclusion, edible *O. dillenii* extract, its fractions, opuntiol and opuntioside reduced peripheral and centrally mediated pain *via* opioid dependent and independent systems. Among them opuntioside emerged as most effective analgesic possibly due to the presence of glucose moiety at position 7 of its α-pyrone ring. This is the first report of opuntiol and opuntioside analgesic effect which may serve as lead compounds in designing of new analgesics.

Keywords: *Opuntia dillenii*; opuntiol; opuntioside; analgesic; inflammation; prostaglandins. © 2016 ACG Publications. All rights reserved.

1. Introduction

Pain is the feeling produced due to tissue injury induced by various noxious stimuli either physical (pressure, heat and cold) or chemical (formalin and acetic acid) [1]. Mainly tissue injuries and/or inflammation have been associated with pain induction by the release of several inflammatory mediators such as eicosanoids [2], vasoactive amines [3] and cytokines that not only sensitizes but also amplify nociceptive responses [4]. Currently, available pain relieving therapeutic agents includes steroidal (SAID), non-steroidal anti-inflammatory drugs (NSAIDs) and opioids widely used to treat mild to severe pains. However, their long term use has been associated with several undesirable effects

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such as hypertension [5], gastric lesions, drowsiness and dependency [6]. Over the years, natural products have gained popularity as source of new drugs with novel mechanism(s) of action(s) especially against pain [7] such as acetylsalicylic acid (*Salix species*), morphine (*Papaver somniferum*) and tetrahydrocannabinols (*Cannabis sativa*) [8].

Opuntia dillenii (Nagphana) belongs to family Cactaceae, grows in various regions of Pakistan [9]. The chemical constituents, identified from its cladode includes flavonoids: isorhamnetin, kaempferide, kaempferol, quercetin and 3-*O*-methyl quercetin [10], and isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-galactoside [11]; phenolics [12]; α-pyrones opuntiol and opuntioside [13] and β-sitosterol [14]. It is used in traditional medicine against inflammation [15], diabetes, gastric ulcer [16], asthma, hepatitis, intestinal spasm and ophthalmia [9, 17]. Its cladode demonstrated wide range of pharmacological activities such as hypoglycemic [18], hypotensive [9], hypolipidemic [19], anti-asthmatic [20] and anti-microbial [21]. Anti-inflammatory activity of aqueous fruit extract [17] and flower extract [22] against rat paw edema and more recently against mice ear edema in the methanol extract, fractions, opuntiol and opuntioside *via* inhibition of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) [23] have been reported. The aqueous fruit [17] and alcoholic flower [22] extracts also demonstrated analgesic activities without any information about its active constituents. Therefore, *O. dillenii* cladodes methanol extract, its fractions (T-1 and T-2) and pure compounds (opuntiol and opuntioside) were evaluated for analgesic activity using chemical (acetic acid-induced writhing and formalin-induced paw licking test) and thermal (hot plate test) stimuli.

2. Materials and Methods

2.1. Chemicals

Acetic acid, acetylsalicylic acid and β-sitosterol were purchased from Sigma-Aldrich (Germany), diclofenac sodium and indomethacin purchased from Sigma Co (St. Louis, Mo, USA). Morphine sulphate (trade name: magnus MR, AGP Pvt, Pakistan), naloxone hydrochloride (Sam Chun Dang Pharm Co Ltd, South Korea) and paracetamol (brand name: panadol, GlaxoSmithKline Ltd Pakistan) were obtained from corresponding companies. Analytical grade of all other chemicals and reagents were used during this study.

2.2. Plant Material

Fresh, green cladodes (45 kg) were collected from the University of Karachi campus in the month of October 2001. A voucher specimen (KUH GH No.68218) was deposited in the herbarium of the Department of Botany, University of Karachi, Pakistan.

2.3. Extraction and Isolation

The collected green cladodes (45 kg) were cut into pieces (one inch) and percolated 2x with methanol. The thick residue of methanol extract (200 g) obtained by *in vacuo* (removal of the solvent) and subjected to vacuum liquid chromatography using various combinations of solvents [VLC, silica gel, pet ether (PE), ethyl acetate (EA) and methanol (MeOH)]. Thirty one fractions were furnished including fractions 1-5 (trace constituents), fraction 6-12 (T-1) predominantly contained opuntiol (**1**) soluble in DMSO (10%) whereas fractions 14-17 (T-2) were rich in its glucoside partner opuntioside (**2**) soluble in saline (0.9%). In VLC fractions 6-12 (PE:EA 1:9), deposited crystals were filtered and by thin layer chromatography (TLC) and spectral studies identified as opuntiol. The VLC fraction 15 (EA: MeOH 6:4) having a main spot on TLC was further purified by preparative thin layer chromatography (PTLC) (silica gel, $CHCl_3$: MeOH, 7:3) and a pure compound opuntioside was identified upon its spectral studies (Figure 1).

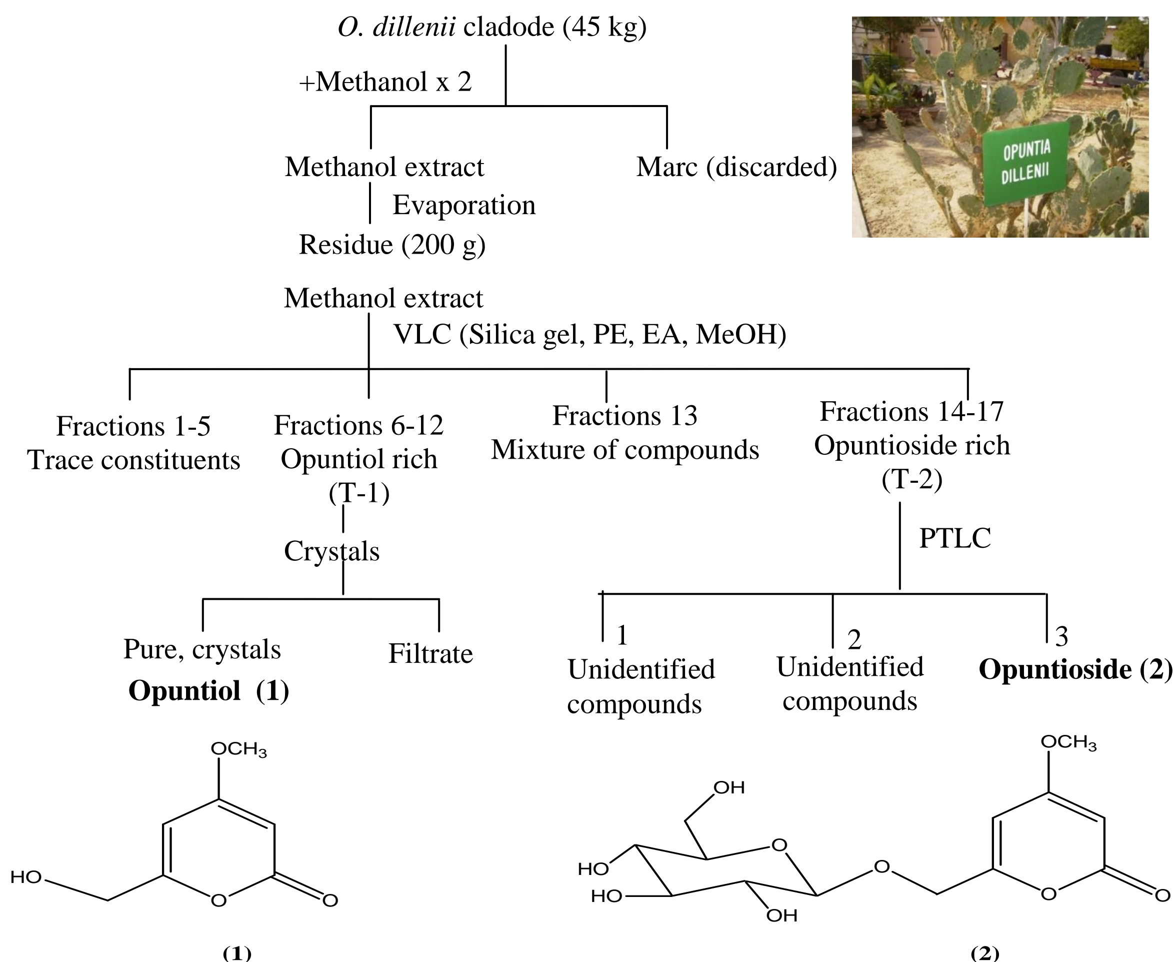

Figure 1. Extraction and fractionation of *O. dillenii* cladode and isolation of pure compounds
Vacuum liquid chromatography (VLC), preparative thin layer chromatography (PTLC), petroleum ether (PE), ethyl acetate (EA) and methanol (MeOH)
The numbers within parenthesis represents the fractions: PE: EA combination (1-5), PE: EA combinations (6-12) and EA: MeOH (14-17).

*Characterization of opuntiol (**1**)*: Crystalline; MP. 180.6-181.1 ºC; UV (MeOH) λ_{max} (log ε): 280, 205;
IR υ_{max} ($CHCl_3$) = 3369, 1701, 1646, 1566 cm^{-1}; EIMS *m/z* (%): 156(59), 128(18), 125(100), 111(7), 97(4), 59(44); HREIMS m/z: 156.0419 (M^+, calculated for $C_7H_8O_4$, 156.0422), 128.0499 (M^+-CO, $C_6H_8O_3$, 128.0473), 125.0247 (M^+-OCH_3, $C_6H_5O_3$, 125.0238), 97.0331 (M^+-OCH_3-CO, $C_5H_5O_2$, 97.0289); ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD): δ (ppm) = 5.32 (1H, d, *J* = 2.1Hz, H-3), 6.02 (1H, td, *J* = 1.0, 2.1 Hz, H-5), 4.19 (2H, s, H-7), 3.71 (3H, s, H-8). ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD): δ (ppm) = 166.8 (C, C-2), 88.5 (CH, C-3), 173.5 (C, C-4), 99.8 (CH, C-5), 166.1 (C, C-6), 60.9 (CH_2, C-7), 56.7 (OCH_3, C-8).
*Characterization of opuntioside (**2**)*: Gummy. UV (MeOH) λ_{max} (log ε): 280, 207; IR υ_{max} ($CHCl_3$) = 3352, 1725, 1707, 1644, 1568, 1253, 1076, 1029 cm^{-1}; EIMS m/z (%): 273(1), 256, 239(1), 205(1), 193, 188(1), 185(6), 184(73), 183(1), 179(2), 163(2), 156(13), 155(15), 140(11), 139(97), 134(5), 125(100), 115(2), 113(10), 112(14), 111(82), 98(15), 97(21), 95(2), 81(18);
HREIMS *m/z*: 318.0896 (M^+, calculated for $C_{13}H_{18}O_9$, 318.0950), 185.0433 (M^+-$C_5H_{10}O_4$+H, $C_8H_9O_5$, 185.0449), 140.0483 (M^+-$C_6H_{11}O_6$+H, $C_7H_8O_3$, 140.0473), 125.0253($C_6H_5O_3$, 125.0238), 111.0479 (M^+-$C_7H_7O_4$-3OH-H, $C_6H_7O_2$, 111.0446); FAB +ve (*m/z*): 319; FAB –ve (*m/z*): 317; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD): δ (ppm) = 5.35 (1H, d, *J* = 2.1 Hz, H-3), 6.17 (1H, td, *J* = 1.1, 2.1 Hz, H-5),

4.46 (1H, brd, *J* =14.7, H-7a), 4.32 (1H, brd, *J* = 14.7, H-7b), 3.71 (3H, s, H-8), 4.25 (1H, d, *J* = 7.6, H-1′), 3.18 (1H, dd, *J* = 7.6, 9.2, H-2′), 3.29 (1H, m, H-3′), 3.29 (1H, m, H-4′), 3.18 (1H, m, H-5′), 3.74 (1H, dd, *J* = 2.8, 12.8, H-6′a), 3.58 (1H, m, H-6′b); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD): δ (ppm) = 165.1 (C, C-2), 88.2 (CH, C-3), 171.5 (C, C-4), 100.6 (CH,C-5), 160.5 (C, C-6), 65.9 (H_2C, C-7), 55.8 (OCH_3, C-8), 102.2 (CH,C-1′), 73.1 (CH,C-2′), 76.0 (CH,C-3′), 69.6 (CH, C-4′), 75.9 (CH,C-5′), 61.2 (CH,C-6).

2.4. Animals

The animal facility of International Center for Chemical and Biological Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan were used throughout the studies. The ethical guidelines for the handling of laboratory animals were followed as recommended by Association for Assessment and Accreditation of laboratory Animal Care International (AAALAC) and *via* clearance of animal use committee of institute (Protocol # 2014-0003). The NMRI mice of both sexes (23-29 g) were kept at a regulated temperature (22 ± 2 ºC) and humidity (50 ± 10%) with 12 h light and dark cycle and fed with standard diet and water *ad libitum*.

2.5. Acute toxicity

Mice (n=10 /group) received either *O. dillenii* cladodes methanol extract (1 and 5 g/kg) or vehicle control (saline, 0.9%) orally consecutively for a period of 7 days as described earlier [24]. During this period the mice were closely observed for agitation, restlessness, sedation, sensitivity to touch and sound, diarrhea, motor activity, gasping and mortality, if any were noted.

2.6. Acetic acid-induced writhes

Mice (n=3/dose) received either DMSO (10%), saline (0.9%) or Na_2CO_3 (1%) as vehicle control (10 mL/kg) or methanol extract (100−350 mg/kg), T-1 (50−250 mg/kg), T-2 (50−150 mg/kg), opuntiol (10−100 mg/kg) and opuntioside (1−30 mg/kg) or reference drugs acetylsalicylic acid (50−150 mg/kg), diclofenac sodium (10−20 mg/kg), indomethacin (5−15 mg/kg), morphine (1−10 mg/kg) and β-sitosterol (10−30 mg/kg) were administered orally. After 30 min acetic acid (0.8%, 10 mL/kg) was injected *i.p* and immediately mice were transferred into transparent observation box (23x12x13 cm). The number of abdominal writhes was counted which is described as a) contraction of abdominal muscles, b) stretching of hind limbs and c) turning of trunk [25-26]. To ascertain the opioid system involvement in its anti-nociceptive effect, 10 min prior to test agents oral administration, naloxone (1 mg/kg) was injected intraperitoneally (*i.p*) in mice followed by acetic acid (0.8%, *i.p*) injection. The inhibition of writhes (%) was calculated as follows:

$$\text{Inhibition of writhes (\%)} = \frac{\text{Number of writhes in control} - \text{Number of writhes in test}}{\text{Number of writhes in control}} \times 100$$

2.7. Formalin-induced paw licking response

Mice (n=3/dose) was pretreated with either DMSO (10%), Na_2CO_3 (1%) or saline (0.9%) as vehicle control or with methanol extract (200−400 mg/kg), T-1 (200−300 mg/kg), T-2 (150−250 mg/kg), opuntiol (25−75 mg/kg) and opuntioside (10−30 mg/kg), diclofenac sodium (15 mg/kg), morphine (10 mg/kg) and β-sitosterol (30 mg/kg) orally. After 30 min formalin (1%, 20 µL) was injected subcutaneously into right hind paw of mice and immediately placed the mice in a observation box (23x12x13 cm) and licking/biting of the treated paw were noted as pain response for 30 min using timer [27-28]. To determine the opioid receptors involvement mice received naloxone (2 mg/kg, *i.p*) 10 min prior to test agents oral administration followed by formalin injection. The licking time of paw was noted and inhibition of licking time (%) was calculated as follows:

$$\text{Inhibition of paw licking time (\%)} = \frac{\text{Licking time in control} - \text{licking time in test}}{\text{Licking time in control}} \times 100$$

2.8. Hot plate-induced jumping response

Mice (n=3/dose) were orally pretreated with either DMSO (10%) or saline (0.9%) as vehicle control or with methanol extract (200−300 mg/kg), T-1 (50−150 mg/kg), T-2 (100−200 mg/kg), opuntiol (20−75 mg/kg) and opuntioside (10−30 mg/kg) or morphine (1−10 mg/kg), paracetamol (500 mg/kg) and β-sitosterol (30 mg/kg). After 30 min of aforementioned test agents administration, each mice was placed on hot plate (Ugo Basile, DS 37, 25x25 cm, Italy) and temperature (50 ± 0.05°C) was maintained. The latency time (jumping or licking or flicking of hind paw) was recorded at various intervals of 0, 30, 60, 90 and 120 min with a cut off time (30 sec) to avoid paw damage [28-29]. Naloxone (2 mg/kg) was injected *i.p* 10 min prior to test agents administration and latency time of response was noted. The increase in latency time with respect to vehicle control was expressed as analgesic effect and calculated as follows:

$$\text{Latency time (\%)} = \frac{\text{Test latency} - \text{base line latency}}{\text{Base line latency}} \times 100$$

2.9. Rotarod test in mice

Mice (n=3/group) capable to grip the revolving bar (16 rpm) for ≥180 sec of a rotarod (Ugo Basile, Model 7650) were selected 24 h prior of experimentation. Thirty minutes after the treatment with saline or DMSO 10% (vehicle control) or methanol extract (400 mg/kg), T-1 (300 mg/kg) and -2 (200 mg/kg), opuntiol (100 mg/kg) and opuntioside (30 mg/kg) the animals were placed on the rod at intervals of 30, 60, 90 and 120 min. The number of falls from rod during the period of 3 min at each interval was noted [30-31].

2.10. Statistical analysis

Data of 3 independent experiments were presented as mean ± standard error of mean (SEM). IC_{50} values were obtained graphically using linear regression analysis (n=3). All results were analyzed using one-way analysis of variance (ANOVA) by post hoc least significance difference (LSD) for the comparison among control and treated groups and Duncan range test to determine inter-group differences. The accepted significance level for the tests was $p < 0.05$ using statistical software package (SPSS v.12.0).

3. Results and Discussion

Acetic acid induced abdominal writhes a popular animal pain model is being used since 1959 Koster et al., [25]. The visceral pain is evoked by the activation of mast cells in peritoneal cavity [3] which releases various endogenous agents such as substance P, bradykinin, serotonin, histamine [32] and cytokines (TNF-α, IL-1) in the peripheral tissue fluids that excite pain through nerve endings [33]. These peripheral nociceptors are sensitive to NSAIDs and opioid analgesics [34]. In the present study, orally administered *O. dillenii* cladodes methanol extract, fractions (T-1 and T-2), opuntiol and opuntioside elicited significant dose dependent reduction in abdominal writhes (Figure 2) as compared to control (50 ± 0.8) with opuntioside (IC_{50} values: 26 ± 0.9 mg/kg) being most effective in antagonizing pain and equipotent to diclofenac sodium and β-sitosterol whereas, opuntiol was similar in response to acetylsalicylic acid (Table 1). Various nociceptive mechanisms involving sympathetic system *via* release of acetylcholine and biogenic amine (serotonin), arachidonic acid metabolites (prostaglandins and leukotrienes) or interaction with opioid (μ, δ and κ) receptors [35] contributes to abdominal writhing. Therefore, to differentiate between peripheral or central mediated analgesic effects by *O. dillenii* additional paradigms such as formalin and hot plate test were also carried out.

The formalin induced biphasic paw licking behavior discriminates the pain into neurogenic (early phase: 1-5 min) and peripheral pain (late phase: 15-30 min) *via* the release of substance P, bradykinin, histamine, serotonin and prostaglandins [36] that stimulates pain by acting on primary afferent sensory Aδ and C nerve fibers [37]. The centrally acting analgesics (morphine) inhibited both phases while NSAIDs acts only on later-phase [38]. The control animal elicited 56 ± 1.9 sec and 106 ± 2.5 sec paw licking response during early and late phases, respectively. However, *O. dillenii* derived

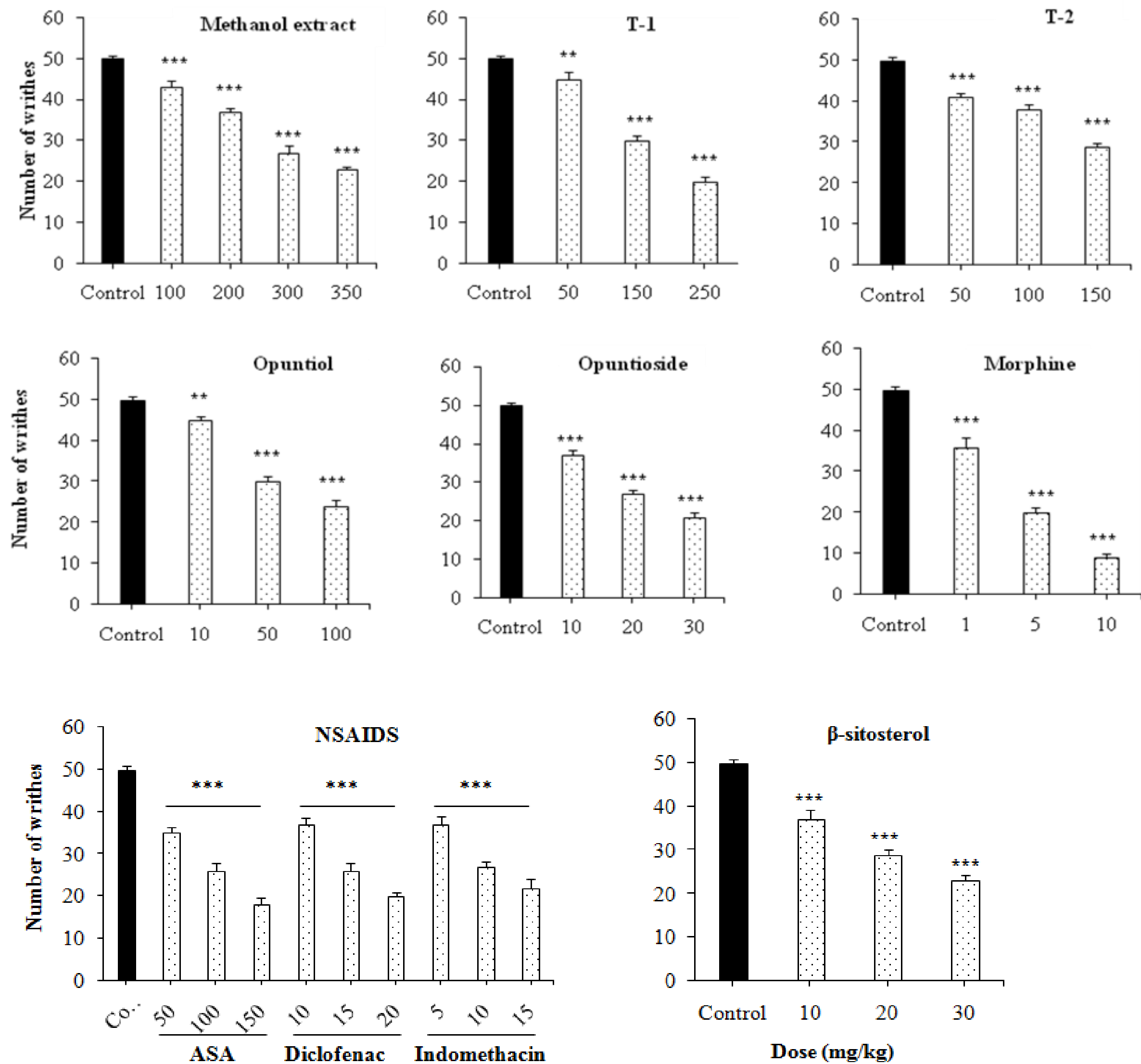

Figure 2. Effect of *O. dillenii* methanol extract, fractions, opuntiol, opuntioside and reference drugs on acetic acid-induced writhes in mice.

Values represented mean ± SEM of number of writhes in control (saline, DMSO 10% and Na_2CO_3; n = 20) and treated group (n = 9) of three independent experiments. T-1 (fractions 6-12); T-2 (fractions 14-17); NSAIDs: Non-steroidal anti-inflammatory drugs.

Asterisks on each bar indicate significant differences in number of writhes (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.005$) while bar without asterisks are non-significant with respect to control.

test agents reduced both phases but more pronounced effect was detectable in the second phase of paw licking (Table 2) with opuntioside being most potent and equipotent in both phases with IC_{50} value of 28 ± 1.1 mg/kg and 24 ± 1.2 mg/kg. It was ~ 6 folds less effective than morphine but similar to β-sitosterol and diclofenac sodium and indomethacin in both early and later phases of paw licking, respectively (Table 3) suggesting that *O. dillenii* derived test agents possess peripheral effects possibly

via inhibition of inflammatory mediators as well as central analgesic activities more likely due to involvement of opioid receptors.

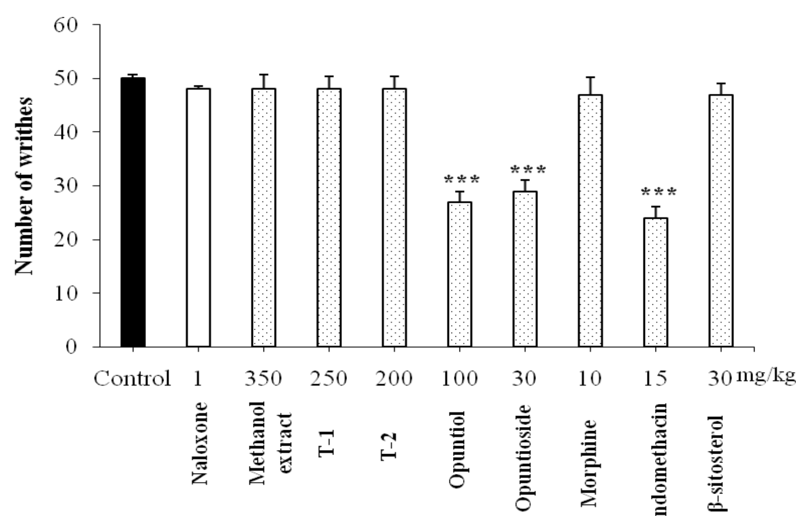

Figure 3. Effect of *O. dillenii* methanol extract, fractions, opuntiol, opuntioside and reference drugs in the presence of naloxone on acetic acid-induced writhes in mice

Naloxone was administered 10 min prior to administration of test agents.
Number of writhes (50 ± 0.8) in control and naloxone treated animals were non-significant.
Each bar represents mean ± SEM of number of writhes in control (n = 20) and treated group (n = 9) of three independent experiments.
Asterisks indicate significant differences in number of writhes (*p < 0.05, **p < 0.01 and ***p < 0.005) with respect to control.

Table 1. IC_{50} values of *O. dillenii* methanol extract, fractions, opuntiol, opuntioside and reference drugs against acetic acid-induced writhes in mice

TREATMENTS	IC_{50} (mg/kg)
***O. dillenii* cladodes**	
Methanol extract	310 ± 10^{f}
T-1	248 ± 6.0^{e}
T-2	150 ± 5.8^{d}
Opuntiol	100 ± 5.8^{c}
Opuntioside	26 ± 0.9^{b}
Reference drugs	
Acetylsalicylic acid	108 ± 4.4^{c}
Diclofenac	16.6 ± 0.9^{ab}
Indomethacin	11.0 ± 1.0^{a}
Morphine	5.0 ± 0.6^{a}
β-sitosterol	27 ± 1.5^{b}

Each value represents mean ± SEM of 50% inhibition of number of writhes (IC_{50}) against acetic acid (0.8%) derived from Figure 1. Dissimilar alphabets (a-f) are significantly different while similar alphabets are non-significant with respect to each other.

Hot plate is another popularly used centrally mediated test based on thermal stimulus with the involvement of spinal reflex response [39]. In this animal model, centrally acting analgesic agents inhibit pain transmission at dorsal horn and prolong the latency time of animals to jumping response [40]. The thermally induced jumping response of control mice exhibited about 12-16 sec from time interval of 0-120 mins. Whereas, *O. dillenii* derived test agents suppressed the centrally mediated pain

Table 2. Effect of *O. dillenii* methanol extract, fractions, opuntiol and opuntioside in the presence and absence of naloxone against formalin-induced paw licking in mice

Treatments	Dose (mg/kg)	Inhibition of licking time (%)	
		Early phase	Late phase
Methanol extract	200	19 ± 1.5[a]	45 ± 2.1[a]
	300	39 ± 1.4[b]	59 ± 3.6[b]
	400	51 ± 0.5[c]	67 ± 3.6[c]
Methanol extract + Nal	400	6 ± 0.1[ns]	3 ± 2.1[ns]
T-1	200	32 ± 1.6[a]	42 ± 1.0[a]
	250	44 ± 2.1[b]	57 ± 5.2[b]
	300	53 ± 2.1[c]	71 ± 4.2[c]
T-1+ Nal	300	7 ±0.9[ns]	4 ± 2.2[ns]
T-2	150	29 ± 1.4[a]	31 ± 5.1[a]
	200	41 ± 1.0[b]	49 ± 2.0[b]
	250	50 ± 1.8[c]	67 ± 2.8[c]
T-2 + Nal	250	5 ± 0.4[ns]	3 ± 1.0[ns]
Opuntiol	25	29 ± 0.5[a]	35 ± 0.3[a]
	50	41 ± 0.4[b]	47 ± 1.8[b]
	75	53 ± 0.4[c]	60 ± 3.0[c]
Opuntiol + Nal	75	40 ± 1.9	42 ± 2.9
Opuntioside	10	25 ± 0.5[a]	28 ± 0.3[a]
	20	39 ± 0.9[b]	43 ± 1.8[b]
	30	51 ± 1.1[c]	57 ± 3.0[c]
Opuntioside + Nal	30	40 ± 1.0	43 ± 3.7
Reference drugs			
Diclofenac	15	9 ± 0.9[ns]	50 ± 4.7
Morphine	10	76 ± 0.9	81 ± 1.2
Morphine + Nal	10	2 ± 4.8[ns]	6 ± 2.5[ns]
β-sitosterol	30	51 ± 0.6	60 ± 4.9
β-sitosterol + Nal	30	4 ± 2.7[ns]	2 ± 1.3[ns]
Control		**56 ± 1.9**	**106 ± 2.5 sec**

Percent reduction during early and late phases of paw licking time (sec) after formalin (1%) injection are presented as mean ± SEM of 3 independent experiments for control (saline and DMSO; n = 20) and treated groups (n = 9) with respect to control. Naloxone (Nal) 2 mg/kg was administered 10 min prior to the administration of test agents.
Non-significant (ns) values are mentioned whereas all the other values were significantly different at ***($p < 0.005$) with respect to control.
Within column identical superscript alphabets (a-c) are non-significant while significant differences have been shown by dissimilar alphabets.

with maximum analgesic response (~70%) at 60 min followed by gradual decline in latency time. Consistently, the effect of opuntioside was most pronounced and comparable to β-sitosterol (Table 5) with 4 folds better anti-nociceptive effect than opuntiol. It is more likely due to the presence of glucose moiety in the α- pyrone ring that helps in faster absorption through gastrointestinal tract [41-42] and hence more effective at low doses as reported for aglycone quercetin which absorbs slowly as compared to its glucoside partner [43]. Similar phenomenon has also been noticed in myricitrin a flavonol glycoside of myricetin that was better analgesic than its corresponding aglycone [44] in chronic animal pain model. Moreover, it has also been noticed that the number and the position of -OH group in aglycone flavonoid plays a crucial role in the gastrointestinal absorption [45] particularly the presence of single -OH in the structure retards the absorption. Thus in our findings, aglycone opuntiol bearing single -OH group in its pyrone ring is more likely to be absorbed slowly hence elicited weaker analgesic response as compared to its glucoside partner opuntioside.

Table 3. Comparison of IC_{50} values of *O. dillenii* methanol extract, fractions, opuntiol, opuntioside and reference drugs against formalin-induced paw licking in mice

Treatments	IC_{50} (mg/kg)	
	Early Phase	**Late Phase**
***O. dillenii* cladodes**		
Methanol extract	393 ± 6.7^a	233 ± 12^a
T-1	268 ± 9.2^b	145 ± 7.6^b
T-2	245 ± 8.7^c	190 ± 10^c
Opuntiol	71 ± 2.2^d	53.3 ± 2.9^d
Opuntioside	28 ± 1.1^e	24 ± 1.2^e
Reference drugs		
Diclofenac sodium	Could not be determined	15 ± 1.2^e
Indomethacin	Could not be determined	20 ± 2.4^e
Morphine	4 ± 0.2^f	5 ± 0.2^f
β-sitosterol	26 ± 1.2^e	23 ± 2.1^e

Mean ± SEM of 50% inhibition concentration (IC_{50}) in the paw licking time against formalin-induced paw licking in mice were derived from Table 2.

In each column dissimilar alphabets (a-f) are significantly different while similar alphabets are non-significant with respect to each other.

Therefore, in all three procedures (acetic acid-induced writhes, formalin-induced paw licking and hot plate-induced jumping response in mice) same doses of methanol extract (100, 200, 250, 300, 350 and 400 mg/kg), fraction T-1 (50, 100, 150, 200, 250 and 300 mg/kg) and fraction T-2 (50, 100, 150, 200 and 250 mg/kg) were used. However, only significant values showing pain relieving effect are presented. This difference in doses may relate to diverse nature of nociceptive stimuli i.e. acetic acid (0.8%), formalin (1%) and increased temperature (50 ºC) responsible to produce activation of variety of nociceptive responses.

Naloxone, a non-selective opioid antagonist for μ, δ and κ receptors [6] blocked the centrally mediated antinociceptive effect of morphine in above mentioned animal pain models and is in agreement as described earlier [28,46]. Likewise, it also completely blocked the *O. dillenii* derived methanol extract, fractions and β-sitosterol-induced analgesic effects against abdominal writhes (Figure 3), paw licking (Table 2) and jumping responses (Table 4) in mice. Thereby, implying that pain relieving effect is neurogenic probably *via* involvement of opioid receptors at spinal/supraspinal level. However, participation of specific receptor(s) in this analgesic action needs to be determined. On the other hand, both opuntiol and opuntioside significantly reduced these behavioral responses and relieved pain by ~50% (Figure 3**,** Table 2 and 4) suggesting it is also partly opioidergic independent response. The non-opioidergic responses possibly governed by other centrally mechanisms such as analgesic effect through COX-2 pathway [47] or GABAergic system [48].

Previously, *O. dillenii* lyophilized aqueous fruit extract described as analgesic against acetic acid and hot plate test in mice *via* inhibitory action of GABA [17]. The centrally mediated analgesic activity of *O. dillenii* flowers against electric stimulus has been associated with high concentrations of flavonoid glycosides (isorhamnetin-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside and kaempferol 3-*O*-α-arabinoside) [22]. The present findings for the first time reports that cladodes of *O. dillenii* methanol extract and its opuntiol and opuntioside (α-pyrones) rich fractions also participates in the analgesic action. The extract and fractions also contains flavonoids, phenolics and β-sitosterol (phytosterol) and their role in analgesic action cannot be ignored. As described earlier β-sitosterol major constituent in *Buddleja globosa* [49] and *Nyctanthes arbortristis* [50] exhibited both peripheral and centrally mediated pain relieving properties [46]. The pyrone ring present in opuntiol and opuntioside, also common in pyrone-rich extracts of *Torresea cearensis* [51] and *Justicia pectoralis Jacq* which are popular in Brazil against pain as well as in umbelliferone (coumarin derivative) possesses peripheral and central analgesic activities [52]. Therefore, in the light of previously reported literature

Table 4. Effect of *O. dillenii* methanol extract, fractions, opuntiol and opuntioside and reference drugs in the presence and absence of naloxone on hot plate-induced jumping response in mice

Treatments	Dose (mg/kg)	Time Interval (Minutes)			
		30	60	90	120
		Latency Time (SEC)			
Methanol extract	200	17.2 ± 1.1^{a}	20.8 ± 1.2^{a}	19.7 ± 0.9^{a}	16.4 ± 1.1^{a}
(MeOH)	250	18.1 ± 1.1^{a}	23.2 ±1.2^{b}	23.0 ± 0.8^{b}	19.5 ± 1.0^{b,2}
	300	21.0 ± 1.4^{b}	25 ± 1.3^{b}	24.3 ± 1.3^{c}	21.0 ± 1.1^{c}
MeOH+ Nal	300	14.0 ± 1.0^{ns}	15.3 ± 1.0^{ns}	16.3 ± 1.1^{ns}	15.9 ± 1.1^{ns}
T-1	50	16.6 ± 0.7^{a}	19.5±0.7^{a}	19.6±0.7^{a}	16.7±0.7^{a}
	100	19.0 ± 1.2^{b}	22.7±1.6^{b}	21.1±0.5^{b}	17.7±1.1^{*b}
	150	20.6 ± 1.4^{c}	24.5±1.0^{c}	24.5±1.3^{c}	20.9±1.4^{c}
T-1+ Nal	150	13.7 ± 1.6^{ns}	15.3±0.8^{ns}	16.6±0.6^{ns}	15.6±0.4^{ns}
T-2	100	16.7 ± 0.7^{**a}	21.2 ± 1.4^{a}	20.3 ± 0.7^{a}	17.7 ± 1.0^{a}
	150	19.3 ± 1.4^{a}	24.1 ± 0.7^{b}	22.2 ± 1.3^{b}	18.2 ± 1.2^{*b}
	200	21.7 ± 1.1^{b}	25.8 ± 1.4^{c}	24.4 ± 1.0^{c}	20.6 ± 1.0^{c}
T-2 + Nal	200	13.8 ± 0.7^{ns}	15.6 ± 0.9^{ns}	16.8 ± 0.8^{ns}	15.7 ± 0.8^{ns}
Opuntiol	25	14.6 ± 0.8^{a}	16.5 ± 1.0^{a}	17.5 ±0.7^{a}	15.9 ± 1.1$^{ns\ a}$
	50	17.2 ± 1.1^{b}	20.8 ± 1.2^{b}	19.7 ± 0.9^{b}	16.4 ± 1.1$^{ns\ b}$
	75	18.1 ± 1.1^{b}	23.0 ± 1.2^{c}	22.9 ± 0.8^{c}	19.5 ± 1.0$^{ns\ c}$
Opuntiol + Nal	75	19.0 ± 1.1	22.4 ± 1.0	21.2 ± 1.1	18.7 ± 0.7
Opuntioside	10	15.9 ± 1.0^{*a}	19.4 ± 0.9^{a}	19.5 ± 1.3^{a}	16.7 ± 0.9^{nsa}
	20	19.2 ± 0.6^{b}	23.3 ± 0.8^{b}	20.5 ± .6^{a,b}	16.6 ± 0.9^{nsa}
	30	21.8 ± 1.0^{c}	25.5 ± 1.0^{c}	23.5 ±1.1^{c}	17.3 ± 1.6^{b}
Opuntioside + Nal	30	15.0 ± 0.8	23.0 ± 0.9	22.7 ± 0.9	19.3 ± 0.4
Paracetamol	500	22.3 ± 0.9	23.1 ± 1.1	21.0 ± 0.7	18.0 ± 0.9
Morphine	10	23.4 ± 1.2	26.4 ± 2.1	29.0 ± 0.3	28.3 ± 0.4
Nal + Morphine	10	14.2 ± 0.7^{ns}	15.4 ± 0.7^{ns}	16.7 ±0.8^{ns}	15.8 ± 0.9^{ns}
β-sitosterol	30	16.0 ± 0.5	22.8 ± 0.8	27.5 ± 0.6	27.0 ± 0.6
Nal + β-sito	30	14.2 ± 0.8^{ns}	15.8 ± 0.9^{ns}	16.7 ± 0.8^{ns}	15.8 ± 1.2^{ns}
Control		13 ± 0.4	15.1 ± 0.5	16.3 ± 0.4	15.6 ± 0.5

Latency time of control (saline and DMSO 10%; n = 22) and treated mice (n = 9) presented as mean ± SEM of 3 independent experiments.
Naloxone (Nal) 2 mg/kg was administered 10 min prior to administration of test agents.
Non-significant (ns) values and significant values with asterisks at *($p < 0.05$, **$p < 0.01$) are mentioned whereas all other values are significantly different at ***($p < 0.005$) with respect to control.
Within column identical superscript alphabets (a-c) are non-significant while significant differences have been shown by dissimilar alphabets.

and present findings it emerged that *O. dillenii* extract and fractions containing flavonoids, phenolics, pyrones particularly opuntiol and opuntioside along with sterols act synergistically towards its analgesic effects.

The neurological effects such as motor co-ordination, unconsciousness and muscle strength of test agents were also screened by placing the mice on rotated rods at constant rotational speed [53]. After the treatment with *O. dillenii* derived test agents all mice stayed on rotarod without falling during 3 min duration of experiment and were similar to that of control implying that *O. dillenii* analgesic effect is independent of sedative or muscle relaxant effects likewise NSAIDs (ibuprofen, naproxen) which relief pain without being causing neurological effects [54].

Table 5. Comparison of IC_{50} values of *O. dillenii* methanol extract, fractions, opuntiol, opuntioside and reference drugs derived from hot plate-induced jumping response in mice

Treatments	Time (Minutes)			
	30	**60**	**90**	**120**
***O. dillenii* cladodes**				
Methanol extract	289 ± 8.6[a]	243 ± 8.8[a]	297 ± 7.6[a]	408 ± 4.4[a]
T-1	133 ± 8.8[b]	100 ± 5.8[b]	155 ± 8.6[b]	213 ± 8.8[b]
T-2	183 ± 6.7[c]	133 ± 8.8[c]	207 ± 6.6[c]	293 ± 11.6[c]
Opuntiol	71 ± 4.7[d]	58 ± 2.7[d]	86 ± 6.8[d]	105 ± 2.9[d]
Opuntioside	24 ± 3.2[e]	19 ± 1.2[e,f]	33 ± 1.4[e]	35 ± 2.6[e]
Reference drugs				
Paracetamol	417 ± 8.8[f]	470 ± 5.8[g]	Could not be determined	Could not be determined
Morphine	7 ± 0.3[e]	5 ± 0.5[f]	5 ± 0.6[f]	6 ± 0.1[f]
β-sitosterol	Could not be determined	27 ± 1.7[e]	8 ± 0.3[e,f]	7 ± 1.4[f]

Each value represents IC_{50} (mg/kg) values mean ± SEM of 50% inhibition of pain derived from Table 4. Within column identical superscript alphabets (a-f) are non-significant while significant differences have been shown by dissimilar alphabets.

According to Saleem et al., [9] no sign of *O. dillenii* toxicity was observed, consistently by methanol extract at 5 g/kg administered daily in mice was non-toxic as no detectable behavioral changes or mortality occurred during 7 days of observations.

Thus present findings demonstrated that edible *O. dillenii* cladodes exhibited analgesic activity at both peripheral and central levels. The *O. dillenii* methanol extract and fractions possess centrally mediated pain relieving effect *via* opioid system while opuntiol and opuntioside act *via* opioid-independent pathways. Among *O. dillenii* test agents, opuntioside being most potent and equipotent to diclofenac sodium and indomethacin at peripheral level and is comparable to β-sitoterol at central level. The better analgesic effect of opuntioside is probably due to the presence of sugar moiety at position 7 of α-pyrone ring which assist in faster absorption, whereas presence of one -OH group in α-pyrone ring of opuntiol (aglycone) makes it a weaker analgesic. Therefore, *O. dillenii* qualifies to be included in the natural product drug discovery program for the development of novel analgesics.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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