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Comparative study of analgesic and anti-inflammatory effects of *Commiphora opobalsamum* with diclofenac in rodents

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This study aims to investigate the analgesic and anti-inflammatory effects of *Commiphora opobalsamum* in rodents in comparison with diclofenac, and its ability to enhance the activity of diclofenac in reduced doses. Wister rats or Swiss mice (5 groups/ 6 each) were administered methalonic extract of *C. opobalsamum*, saline and diclofenac 30 min before the test initiation by i.p. route. The analgesic activities were examined utilizing the acetic acid, hot plate and formalin paw lick techniques. The anti-inflammatory efficacy was examined by utilizing the granuloma induced by cotton pelletand paw edema induced by carrageenan *C. opobalsamum* demonstrated a stronger inhibition of writhing compared to diclofenac, and the 500 mg/kg dose completely inhibited the writhing response. In hot plate, *C. opobalsamum* co-administrated with diclofenac exhibited significant prolongation of reaction time compared to diclofenac alone. Furthermore, *C. opobalsamum* (500 mg/kg) significantly shortens the licking time compared to diclofenac at both phases. In addition, the suppression of paw edema induced by carrageenan was significant in comparison to diclofenac at first hour. Interestingly, significant weight reduction of granuloma tissue was perceived at all doses of *C. opobalsamum* in contrast to control group. This study provides a strong evidence of the analgesic and anti-inflammatory activity of extract of *C. opobalsamum*, additionally it has revealed significant anti-inflammatory effect, equivalent to non-steroidal anti-inflammatory drugs (NSAIDs). Moreover, the combination of reduced doses of *C. opobalsamum* and diclofenac with resultant synergistic potentiation of both analgesic and anti-inflammatory effect, necessitates a cautious approach to elucidate its mechanism with the concomitant meticulous study of its safety profile.

Key words: Commiphora Opobalsamum, anti-inflammatory, analgesic and non steroidal anti inflammatory drugs.

INTRODUCTION

Inflammatory diseases represent a major threat to the health of our society (Serhan et al., 2003; Winyard,

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2003). Inflammation is a direct consequence of pathophysiological reaction of mammalian tissues to a wide range of hostile agents comprising of toxic chemical substances, infectious microorganisms, physical trauma or cancerous growth culminating to the localized accumulation of blood cells and plasma fluid (Kidd, 2006; Schlansky and Hwang, 2009; Conaghan, 2012; Harirforoosh et al., 2014).

It is quite well recognized that corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are the mainstream treatments for inflammation and NSAIDs, which are considered globally as the most frequently utilized drugs (Kidd, 2006). Moreover, NSAIDs have been demonstrated to have significant cardiovascular, renal and gastrointestinal toxicity that make this group of drugs contraindicated in some cases as well as discontinued as a result of their adverse effects. (Antman et al., 2007; Scarpignato, 2008; Higuchi et al., 2009; Schlansky and Hwang, 2009; Massó et al., 2010; Nolin and Himmelfarb, 2010; Perazella and Markowitz, 2010; Plantinga et al., 2011; Unzueta and Vargas, 2013; Krijthe et al., 2014).

Although, corticosteroid are designated as *par excellence* for their potent anti-inflammatory action but they have distinct major disadvantages of multiple adverse effects, which limits their utility (Schlansky and Hwang, 2009). Obviously, the exploration of substitute anti-inflammatory drugs is essentially made from natural herbs. Plants still symbolize a huge unexploited resource of structurally novel compounds that might serve as a guide for the development of innovative and valuable drugs, with wide margin of safety (Al-Howiriny et al., 2004; Sultana and Saeed, 2012).

The rationale for studying the pharmacological activities of natural plants as anti-inflammatory is to circumvent the adverse effects of NSAIDs (Santos, 2003; Sultana and Saeed, 2012; Tripathi, 2013). *Commiphora Opobalsamum* (L) Engl., (syn. *Commiphoraglieadensis*) is a member of large and commonly utilized plant family known as "*Burseraceae",* which is a resinous family comprising, among others, the biblical frankincense and myrrh. *C. opobalsamum* has a distinctive and famous odour and it is known in the society as "*Al-besham*", "*Balsam*", "*Balessan*" and "*Balsam of Mecca*" (Miller and Morris, 1988; Williamson et al., 1996; Wood, 1997; Milwright, 2001; Milwright, 2003; Gupta et al., 2006; Iluz et al., 2010; Amiel et al., 2012).

C. opobalsamum is a small tree (5 m in height) that is found in abundance and widespread on mountains around the holy places such as Makkah Al-Mukarama, Al-Madina Al-Munawara (Al-hijaz area, KSA) and Al-Quds (Palestine). In addition, it is native to other areas such as Oman, Yemen and Somaliland. The ancients had used most of its parts for different purposes. They utilize its twigs for tooth brushing, and they make tea from its leaves as well as they eat its fruits. *C. opobalsamum* was used to treat the disease of chest, gastrointestinal tract, kidney, and to offer symptomatic relief in rheumatism (Milwright, 2001; Milwright, 2003; Sultana and Saeed, 2012).

In addition, it is also used in case of common colds, in the treatment of ear aches, and the application of desiccated bark on wounds to act as an antiseptic, whereas ground balsam bark in its tincture form is utilized for the treatment of skin disorders like eczema and inflammation, additionally it is accredited for impressive relief of labor pains (Abdul-Ghani and Amin, 1997; Gupta et al., 2006; Sultana and Saeed, 2012). Furthermore, its local fame is renowned for two distinctive properties to treat injuries and healing of the tissues (Al-Howiriny, 2005; Al-Sohaibani et al., 2005).

Therefore, in this study the methanolic extract of *C. opobalsamum* was tested in experimental animals to evaluate its anti-inflammatory and analgesic activity in comparison with diclofenac, and to assess its ability to enhance the activity of diclofenac in reduced doses.

MATERIALS AND METHODS

Animal

Male Wister albino rats aged 10 to 12 weeks, weighing 170 to 200 g and 10 weeks old male Swiss albino mice (30 to 40 g) provided by the King Fahad Research Center, King Abdulaziz University, Jeddah, Saudi Arabia, were employed. The animals were kept under the optimum laboratory environment (at the temperature 25 ± 5°C, relative humidity of 30 to 70% and 12/12h light and dark automated cycles) for a minimum period of one week prior to the commencement of experiments. The experimental animals were housed in transparent plastic cages (six/cage) with water and food supply ad libitum. The experimental procedures approved by King Fahd Medical Research Center (KFMRC) and conducted according to their guidelines, and permission of the institutional ethical committee were acquired prior to the initiation of the experimental study.

Plant material and its extraction

C. opobalsamum was collected from Al-Selma (a village located between Makkah Al-Mukarama and Taif, Al-Hijaz, KSA) (21.618466, 40.107040) in the summer of 2013, and identified taxonomically by Dr. Jamal, A. Hussein (Department of Natural Products and Alternative Medicine, College of Pharmacy, King Abdulaziz University, Saudi Arabia). Plant extraction was performed according to the method described by Mothana et al. (2009) and Sawant et al. (2014). The collected aerial parts of plant stems were kept in well ventilated, dry place (30 to 40°C) for five days in order to grind with a mechanical grinder to powder (pale orange) and soaked in 99% w/v methanol at room temperature (25±3°C) for 48 h, and submitted to a four-time extraction thereafter. Then, double filtration of the suspension using cotton and filter papers subsequently to remove the fine particles had been done. After that, the filtrate evaporated by rotary evaporator (Buchi, Schweiz) to remove all traces of methanol. Finally, the moist product kept in the freezer (-80°C) for an hour and then dried overnight in the freezer, vacuum dryer (Zirbus, German) set at -85°C. The dark brown solid product stored securely in the laboratory under controlled temperature and reconstituted in 0.9% isotonic saline solution (vehicle) before administration of methalonic extract of *C. opobalsamum* to the animals.

Chemicals

Acetic Acid [CH3CO2H]: Purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA) as ≥ 99% liquid diluted in 0.9% saline solution to 0.6% v/v for the intraperitoneal (i.p.) injection.

Carrageenan: Procured from Sigma-Aldrich Co. (USA) as an off-white powder dissolved in 0.9% saline solution to 1% w/v within an hour before the test for the subcutaneous (s.c.) injection.

Diclofenac sodium (VOLTIC® Ampoules): Purchased from Jamjoom Pharma Co. (Jeddah, KSA) as ampoule 75 mg/3ml solution for the i.p. injection.

Diethyl ether [(CH3CH2)2O]: Acquired from Sigma-Aldrich Co. (USA) as ≥ 99% liquid to be administered to sacrifice the animals through inhalation.

Formalin: Acquired from Sigma-Aldrich Co. (MO, USA) as 10% Neutral Buffered Formalin solution for tissue fixation and diluted in 0.9% saline solution to 1% v/v for s.c. injection.

Methanol [CH3OH]: Purchased from Sigma-Aldrich Co. (MO, USA) as 99% w/v solvent for the plant extraction processes.

Normal saline: Purchased from Pharmaceutical Solutions Industry Ltd. (Jeddah, KSA) as 0.9% w/v sodium chloride solution used as a vehicle to extract.

Pentobarbital sodium [C11H17N2NaO3]: Obtained from Sigma-Aldrich Co. (MO, USA) as white solid dissolved in sterile water to 0.6% w/v within an hour before the test for the i.p. injection.

Experimental methods

Model for evaluation of peripheral analgesic activity - writhing induced by acetic acid in mice

The analgesic response of *C. opobalsamum* extract was evaluated by using writhing induced by acetic acid as described by Ribeiro et al. (2000). This method is designated as a chemical model for visceral pain employed to evaluate the peripheral analgesic effect. Induction of writhing was conducted by administration of 0.6% aqueous solution of acetic acid by i.p. route into thirty male mice with a dose of 10 ml/kg.

Random selection of mice were done and divided equally into five categories, they were fasted overnight prior to test. Half an hour prior to acetic acid injection, the mice pre-treated were as follows: Group 1: 0.9% saline solution 0.1 ml/10g to 10 ml/kg i.p. (Negative control), Group 2: diclofenac 25mg/kg i.p. route (positive control), Group 3: *C. opobalsamum* extract 250mg/kg i.p route, Group 4: CO extract 500mg/kg i.proute and Group 5: *C. opobalsamum* extract 125mg/kg i.p. in combination with diclofenac 12.5mg/kg i.p. route. For the purpose of comparison, diclofenac was used as a positive control, and the dose employed in mice in this study (25 mg/kg) is in accordance with the study of Rahman et al. (2007). After writhing induction, all mice were kept separately in a transparent box to count the number of writhes that occurred within first 30 min post acetic acid injection (writhes comprises of abdominal muscle contraction in concert with hind limb extension).

Data analysis

The analgesic effect and the enhanced activity of *C. opobalsamum* extract versus diclofenac and control were observed as percentage inhibition of pain, and calculated as the diminution in the number of writhes in control compared with the treated groups. The inhibition of writhes calculated by the following equation:

Percentage inhibition of writhes = [(Nc – Nt) / Nc] ×100.
NC and Nt symbolize the mean number of writhes in control and treated groups of animal respectively.

Models for evaluation of central analgesic activity

Hot plate test in mice

Representation of acute thermal pain is frequently done by the tail-flick method and hot-plate test, while persistent pain can be induced by the formalin test. Evaluation done by these methods signifies that the mechanism of analgesic effect has central origin. The central mechanism of analgesic effect is evaluated by the hot plate test, in this method the analgesic effect of *C. Opobalsamum* extract was measured by the response latencies of mice to thermal stimuli (Hunskaar et al., 1986; Santos et al., 1999; Adzu et al., 2003).

Procedure

In this method, hot plate temperature was kept constant at 55±0.5°C and prior to intervention, animals were once accustomed to the hot plate. Those animals showing latency response >30 Sec or <5 S were excluded from the study; hygienic condition of hot plate was taken care off before each test.

Thirty male mice were randomly selected, they were equally divided into five groups, and fasted overnight prior to test. Two groups of mice were administered *C. opobalsamum* extract i.p. in two different doses 250 and 500 mg/kg, while the third group received 125 mg/kg *C. opobalsamum* extract i.p. in combination with 12.5 mg/kg diclofenac i.p, the fourth group received 10 ml/kg isotonic saline alone by i.p. route, and diclofenac in the dose of 25 mg/kg given by i.p route, it was given to fifth group as a reference drug for comparison.

The response is designated as licking or biting of a paw or jumping out of the hotplate on to the table, while the reaction time is calculated as the interval of time between keeping the mice on the hot plate and the response, this is recorded as the latency of response. Each mouse was kept individually on the hotplate, and the latency response was observed prior to the treatment and subsequently at the interval of 30, 60, 90, and 120 min. A latency response of 60 sec was demarcated as complete analgesia and employed as cut off time, to prevent thermal trauma to the mice.

Data analysis

The analgesic effect and the enhanced activity of *C. opobalsamum* extract versus diclofenac and control were evaluated by comparison of latency response between the treated group and the control.

Formalin – induced paw licking method

This method was developed about 30 years back for evaluation of pain and analgesic drugs in laboratory animals (McNamara et al., 2007). This test is performed by injecting a dilute (0.5-5%) solution of formaldehyde into the paw of rats or mice; subsequently pain

related behaviors are evaluated in two distinct temporary phases. Phase I is characterized by paw lifting, licking and flinching, they are scored during the first 10 min, this is followed by a brief reduction in this behaviors and then the Phase II lasts for about 30 to 60 mins. NSAIDs, N-Methyl-D-aspartate (NMDA) antagonists, morphine and gabapentine are capable of inhibiting Phase II but not the Phase I. Furthermore, this test can be used as an indicator of both pain and inflammation depending on the parameter which is measured. Measurement of licking time is required for pain while inflammation can be evaluated by measuring size of edema. The formalin-induced paw licking test described by Hunskaar and Hole, (1987), Correa and Calixto, (1993), Santos et al. (1999), Lucetti et al. (2010) was adopted to appraise the analgesic efficacy of the *C. opobalsamum* extract against persistent pain.

Procedure

Induction of pain in mice was performed by injecting 20 µl of 0.92% formaldehyde prepared in phosphate buffer solution, by using a 30 gauge needle into the sub-planter aspect of the hind paw of the left side of the mouse. Thirty male mice randomly selected, they were equally divided in five groups,and fasted overnight prior to test. Two groups of mice were administered *C. opobalsamum* extract i.p. in two different doses 250 and 500 mg/kg, while the third group was received 125 mg/kg *C. opobalsamum* extract i.p. in combination with 12.5 mg/kg diclofenac i.p, the fourth group received 10 ml/kg isotonic saline alone by i.p.route, and diclofenac 25 mg/kg, intraperitoneally was given to fifth group as a reference drug for comparison. After injecting formalin in intraplanter region of the left hind paw, the mice were instantly kept individually in a slim cylinder made up of glass, 20 cm diameter,and then the duration of time mice spent on licking the injected paw was measured and this was considered as an index of pain. The recording of time was done from 0 to 5 mins (early, neurogenic phase) and subsequently from 15 to 20 mins (late, neurogenic phase)

Data analysis

The analgesic effect and the enhanced activity of *C. opobalsamum* extract versus diclofenac and control were observed as percentage inhibition of pain, determined as the reduction in the licking time duration, licking time between treated and control groups. The inhibition of licking duration was calculated by the following equation:

% inhibition of licking = $[(D_c - D_t)/ D_c] \times 100$.
Where, DC and $D_t$represent the mean duration of licking in control and treated groups, respectively.

Models for evaluation of anti-inflammatory action

Paw edema inductionin miceby Carrageenan

The model of paw edema induced by Carrageenan was used to evaluate the anti-inflammatory efficacy of *C. opobalsamum*, and this test was performed according to the technique of (Winter et al., 1962; Morris, 2003; Lucetti et al., 2010). The edematous effect usually rose to max during the 3rd to 5th h (Su et al., 2012). we performed the evaluation of anti-inflammatory effect after a period of 4 h post induction.

Procedure

For inducing edema, 1% w/v carrageenan was prepared in normal saline 1 h prior to the test; it was subsequently injected into the sub-planter aspect of hind paw of the right side of the mice. Thirty male mice were randomly selected, they were equally divided in five groups, and fasted overnight prior to test. Two groups of mice were administered *C. opobalsamum* extract i.p. in two different doses of 250 and 500 mg/kg, while the third group received 125 mg/kg *C. opobalsamum* extract i.p. in combination with 12.5 mg/kg diclofenac i.p, the fourth group received 10 ml/kg isotonic saline alone by i.p. route, and diclofenac 25 mg/kg by i.p route was given to fifth group as a reference drug for comparison. Hand screw steel micrometer was used to measure the paw edema immediately prior to the edema induction and 1, 2, 3 and 4 h thereafter. The reading expressed in millimeter (mm) and the increment in paw thickness at different intervals determined the inflammatory response observed as the formation of edema in paw. The augmentation of paw thickness was determined by deduction of primary paw thickness from the thickness calculated at each time point and this is regarded as the standard for the evaluation of edema.

Data analysis

The anti-inflammatory efficacy and the enhanced activity of *C. opobalsamum* extract versus diclofenac and control were observed as percentage inhibition of edema, determined as the reduction in paw edema between the treated and control groups. The inhibition of edema performed by the equation:

% Inhibition = $1- (T_t /T_c) \times 100$
Where, Tc and $T_t$signify the average augmentation in paw thickness in the treated and control groups.

Granuloma induced by cotton pellets in rat

Granuloma model induced by cotton pellets in rat is described by Bianchine and Eade (1967) and Sawant et al. (2014), to evaluate the anti-inflammatory efficacy of *C. opobalsamum* extract. Cotton pellets were employed to study the anti-inflammatory effect of *C. opobalsamum* chronic inflammation. After 5 days of therapy, the animals were again weighed and sacrificed with ether(Bianchine and Eade, 1967; Iluz et al., 2010).

Procedure

Surgical implantation of two cotton pellets was done subcutaneously on the ventral aspect of the rats in order to induce granulomatous lesions. This was accomplished by anesthetizing rats by using pentobarbital in the dose of 40 mg/kg by i.p. route, the abdomen was shaved and cleaned, then sterile cotton pellets weighing about 30±1 mg, were implanted by taking all aseptic precautions, beneath the skin and in the pectoral region, and incision was carefully sutured. The sutured wound was inspected and cleaned daily with 70% alcohol. Then, on the subsequent day thirty rats were randomly selected, they were equally divided in five categories and were treated daily for six days in the following manner:

Category I:0.9% isotonic saline solution 10 ml/kg/dayby i.p.route
Category II:diclofenac 10 mg/kg/day i.p.route
Category III: CO extract 250 mg/kg/day by IP route
Category IV:CO extract 500 mg/kg/day i.p.
Category V:CO extract 125 mg/kg/day i.p.in combination with 5mg/kg/day i.p.

Diclofenac 10 mg/kg was used as positive control for the reason of

Table 1. The analgesic effect of methanolic extract of *C. opobalsamum* versus diclofenac on writhing response induced by acetic acid in mice.

Group	Parameter measured	Writhing count
Control (0.9% saline), i.p.	Mean ± SEM	51.33±6.34
	% inhibition♦	-
Diclofenac 25 mg/kg, i.p.	Mean ± SEM	6.67±5.95 **
	% inhibition♦	87.00
CO 250 mg/kg, i.p.	Mean ± SEM	5.17±4.07 **
	% inhibition♦	89.92
gbCO 500 mg/kg, i.p.	Mean ± SEM	0 **
	% inhibition♦	100
CO 125 mg/kg, i.p. + diclofenac 12.5 mg/kg, i.p.	Mean ± SEM	1.33±1.50 **
	% inhibition♦	97.40

Data are expressed as mean±SEM; n = 6 mice; CO:*Commiphora opobalsamum*; ♦compared with the control group; *p<0.05, **p<0.001 compared with the corresponding control group values; by one-way ANOVA and Tukey HSD post hoc test.

comparison (Sawant et al., 2014). On the 7th day of therapy, rats were sacrificed with diethyl ether inhalation, removal of pellets was achieved by careful dissection with encircled granulomatous tissue, pellets were dried overnight at 60°C. Furthermore, the average weights of wet and dry granuloma formed around each pellet were recorded. The exact net weight of wet and dry granuloma tissue was calculated by deducting the baseline weight of individual pellet from dry and wet pellets.

Data analysis

The enhanced activity of *C. opobalsamum* extract and its anti-inflammatory effect versus diclofenac and control were observed as the percent inhibitions of granuloma tissue, calculated as the reduction in granuloma weight between control and treated groups, determined by following equation:

% inhibition of Granuloma = $[(W_C W_t)/ WC] \times 100$.
Whereas, WC and W_t represent the mean weight of granuloma in control and treated groups, respectively.

Statistical analysis

The complete outcome and information acquired in this study was analyzed by using statistical package for the social sciences (SPSS) data version 19.0. Values in the text and tables were symbolized as mean ± SEM. Multiple comparison was accomplished by one-way analysis of variance (ANOVA) and Tukey's post adhoc test. The statistical significant difference between the mean values were considered at a *p value* of less than 0.05 ($p \leq 0.05$) and very significant at a *p value* of less than 0.001 ($p \leq 0.001$).

RESULTS

Writhing in mice induced by acetic acid

Following acetic acid injection, mean number of writhes observed during the first 30 mins is summarized in Table1. The results shows that the *C. opobalsamum* (250 and 500 mg/kg) has significantly reduced the writhes produced by acetic acid in contrast to the control ($p<0.001$) with mean values ± SEM of 5.17±4.07 and 0, respectively. The mean % inhibitions were 89.92 and 100%, respectively. In contrast, pretreatment with *C. opobalsamum* extract with diclofenac 25 mg alone produced 87% writhing inhibition, this reduction was observed to be insignificant ($p>0.05$). In addition to it, there was considerable decline in the number of writhes induced by acetic acid in combination of *C. opobalsamum* with diclofenac group (125, 12.5 mg/kg, respectively) in contrast with control group ($p<0.001$) with mean values ± SEM 1.33±1.50 and mean % inhibition of 97.40%.

Hot plate test in mice

The hot plate thermal responses of mice are shown in Table 2. There was no significant variation in the latency of response of mice to thermal stimuli at baseline ($p>0.05$) between groups of control, diclofenac 25 mg/kg, *C. opobalsamum* 250 mg/kg, *C. opobalsamum* 500 mg/kg and *C. opobalsamum* 125 mg/kg plus diclofenac 12.5 mg/kg. The latency of responses of *C. opobalsamum* treated groups (250 and 500mg/kg) at 30 min post treatment were appreciably enhanced in comparison with control ($p>0.05$) with mean values ± SEM of 21.32±3.54 and 22.46±4.29, respectively, and were significantly prolonged incontrast with diclofenac group ($p<0.5$) which have mean value ± SEM of 11.78±1.88.

Furthermore, the latency of responses of *C. opobalsamum* treated groups (250 and 500 mg/kg) at

Table 2. The analgesic effect of methanolic extract of *C. opobalsamum* versus diclofenac on latency of response to thermal pain induced by hot plate in mice.

Group	Parameter measured	Latency of response (sec)				
		Baseline	30 min	60 min	90 min	120 min
Control (0.9% saline), i.p.	Mean ± SEM	9.79±3.84	9.75±2.52	9.88±3.12	9.13±2.13	9.57±2.54
Diclofenac 25 mg/kg, i.p.	Mean ± SEM	8.58±2.14	11.78±1.88	19.01±3.81*	13.89±4.38	12.84±1.15
CO 250 mg/kg, i.p.	Mean ± SEM	9.89±2.71	21.32±3.54*†	15.86±4.31*	15.58±3.86	14.85±4.61
CO 500 mg/kg, i.p.	Mean ± SEM	8.57±2.72	22.46±4.29*†	19.90±3.71*	15.32±3.74	15.06±3.93
CO 125 mg/kg, i.p. + diclofenac 12.5 mg/kg, i.p.	Mean ± SEM	9.40±2.67	16.10±3.00*	14.46±3.80	13.82±3.65	11.64±1.92

Data are expressed as mean±SEM; n = 6 mice; CO:*Commiphora opobalsamum*; ♦compared with the control group; $^{*}p<0.05$ compared with the corresponding control group values; $^{\dagger}p<0.05$ compared with the corresponding diclofenac group values; by one-way ANOVA and Tukey HSD post hoc test.

Table 3. The effect of methanolic extract of *C. opobalsamum* versus diclofenac on licking duration in response to nociception induced by formalin in mice.

Group	Parameter measured	Duration of the injected paw licking (Sec)	
		0-5 min	15-30 min
Control (0.9% saline), i.p.	Mean ± SEM	97.00±11.48	109.16±9.74
	% inhibition♦	-	-
Diclofenac 25 mg/kg, i.p.	Mean ± SEM	73.33±8.54 **	61.00±10.33 **
	% inhibition♦	24.74	44.03
CO 250 mg/kg, i.p.	Mean ± SEM	41.50±3.78 **††	26.83±9.82 **††
	% inhibition♦	57.73	76.14
CO 500 mg/kg, i.p.	Mean ± SEM	29.83±4.87 **††	7.66±4.55 **††
	% inhibition♦	70.10	93.57
CO 125 mg/kg, i.p. + diclofenac 12.5 mg/kg, i.p.	Mean ± SEM	70.66±12.11 **	70.83±4.83 **
	% inhibition♦	27.83	35.77

Data are expressed as mean±SEM; n = 6 mice; CO: *Commiphora opobalsamum*; •compared with control group; *p<0.05, **p<0.001 compared with the corresponding control group values; †p<0.05, ††p<0.001 compared with the corresponding diclofenac group values; by one-way ANOVA and Tukey HSD post hoc test.

60 min post treatment were significantly longer in comparison with control (p<0.05) with mean values ± SEM of 15.86±4.31 and 19.90±3.71, respectively, and were inconsequential when compared with diclofenac (p>0.05) that exhibited the latency of the response with a mean value ± SEM of 19.01±3.81 which in turn was significantly longer in comparison with control (p<0.05). Moreover, the latency of responses of *C. opobalsamum* treated groups (250 and 500 mg/kg) at 90 and 120 min post treatment were insignificantly longer compared with control (p>0.05)

Finally, the group treated with combination of *C. opobalsamum* and diclofenac (125 and 12.5 mg/kg, respectively) revealed significant prolongation in latency of response to thermal stimuli at 30 post treatment in comparison with control. (p<0.05) While at 60, 90 and 120 min post treatment, the latency of responses in *C. opobalsamum* plus diclofenac group (125 and 12.5 mg/kg, respectively) were insignificant in comparison with control and diclofenac (p<0.05) with a mean value ± SEM of 14.46±3.80, 13.82±3.65 and 11.64±1.92, respectively.

Formalin-induced paw licking in mice

Table 3, demonstrated **a** notable decrease in licking of the injected paw in diclofenac group, *C. opobalsamum* groups (250 and 500 mg/kg) and *C. opobalsamum* plus diclofenac group (125, 12.5 mg/kg, respectively) in comparison with control (p<0.001) at an early phase (0 to

Table 4. The effect of methanolic extract of *C. opobalsamum* versus diclofenac on edema induced in paw by carrageenan in mice.

Group	Parameter measured	Change in paw thickness (mm)			
		1 h	2 h	3 h	4 h
Control (0.9% saline), i.p.	Mean ± SEM	33.00±4.69	29.83±6.11	43.16±5.23	48.50±7.21
	% inhibition♦	-	-	-	-
Diclofenac 25 mg/kg, i.p.	Mean ± SEM	22.33±4.67*	24.16±2.71	16.83±4.66**	23.50±3.92*
	% inhibition♦	32.33	20.00	61.00	51.54
CO 250 mg/kg, i.p.	Mean ± SEM	12.00±2.75**†	13.66±3.90*	16.33±3.74**	15.66±4.03**
	% inhibition♦	63.63	56.66	62.16	67.71
CO 500 mg/kg, i.p.	Mean ± SEM	10.00±2.94**††	10.16±4.41**†	18.50±4.11**	12.66±2.89**
	% inhibition♦	69.69	66.66	57.00	73.90
CO 125 mg/kg, i.p. + diclofenac 12.5 mg/kg, i.p.	Mean ± SEM	9.00±3.62**††	8.83±2.48**†	11.50±3.34**	12.50±3.77**
	% inhibition♦	72.72	70.00	73.25	74.22

Data are expressed as means±SEM; n = 6 mice; CO:*Commiph oraopobalsamum*; ♦compared with the control group; *p<0.05, **p<0.001 compared with the corresponding control group values; †p<0.05, ††p<0.001 compared with the corresponding diclofenac group values; one-way ANOVA and Tukey HSD post hoc test were used.

5 mins post formalin administration**)** with mean values ± SEM of 73.33±8.54, 41.50±3.78, 29.83±4.87 and 70.66±12.11, respectively with mean % inhibitions of 24.74, 57.73, 70.10 and 27.83%, respectively.

Moreover, a significant inhibition in licking of the injected paw of diclofenac group, *C. opobalsamum* groups (250 and 500 mg/kg) and *C. opobalsamum* in combination with diclofenac group (125 and 12.5 mg/kg, respectively) compared with control (p<0.001) at late phase **(**15-30 minspost formalin administration) were observed with mean values ± SEM of 61.00±10.33, 26.83±9.82, 7.66±4.55 and 70.83±4.83, respectively with mean % inhibitions of 44.03, 76.14, 93.57 and 35.77%, respectively.

Notably, *C. opobalsamum* (250 and 500 mg/kg) appreciably reduced the licking of the injected paw both at early and late phases compared with diclofenac (p<0.001). On the contrary, inconsequential difference was observed in licking of combination of *C. opobalsamum* and diclofenac group (125, 12.5 mg/kg, respectively) compared with diclofenac (p>0.05).

Paw edema induced by Carrageenan in mice

Table 4, as revealed that the treatment with *C. opobalsamum* both in the dose of 250 and 500 mg/kg exhibit a substantial reduction of the edema of the paw. *C. opobalsamum* at 250 mg/kg also shows a considerable decrease of the swelling of the paw in contrast with control after 1 h *p value* <0.001, 2 h (p<0.05), 3 h p value <0.001 and 4 h (p<0.001) of carrageenan injection. These suppression of development of paw swelling were significant in comparison to diclofenac (p<0.05) at 1st h post carrageenan injection.

Furthermore, *C. opobalsamum* at a dose of 500 mg/kg demonstrated a substantial reduction of the paw swelling in comparison with control group after a period of 1 h, 2 h, 3 h and 4 h of carrageenan administration (p<0.001). These suppression of development of paw swelling were significant as compared to diclofenac at 1st (p<0.001) and 2nd h (p<0.05) post carrageenan injection.

Moreover, treatment with *C. opobalsamum* plus diclofenac group (125 and 12.5 mg/kg, respectively) considerably restrained the paw swelling compared with control after 1 h, 2 h, 3 h and 4 h of carrageenan injection (p<0.001) with mean values ± SEM of 9.00±3.62, 8.83±2.48, 11.50±3.34 and 12.50±3.77, respectively and mean % inhibition of 72.72, 70.00%, 73.25 and 74.22%, respectively. The reduction in the development of paw swelling were significant as compared to diclofenac at 1st (p<0.001) and 2nd h (p<0.05) after carrageenan adnimistration.

Granuloma in rats induced by cotton pellets

The weights of granuloma tissues formed around each implanted cotton pellet in rats are summarized in Figures 1 and 2, it shows a significant inhibition in formation of granuloma tissues of diclofenac group in *C. opobalsamum* treated groups in the dose of 250 and 500 mg/kg), and *C. opobalsamum* plus diclofenac group in

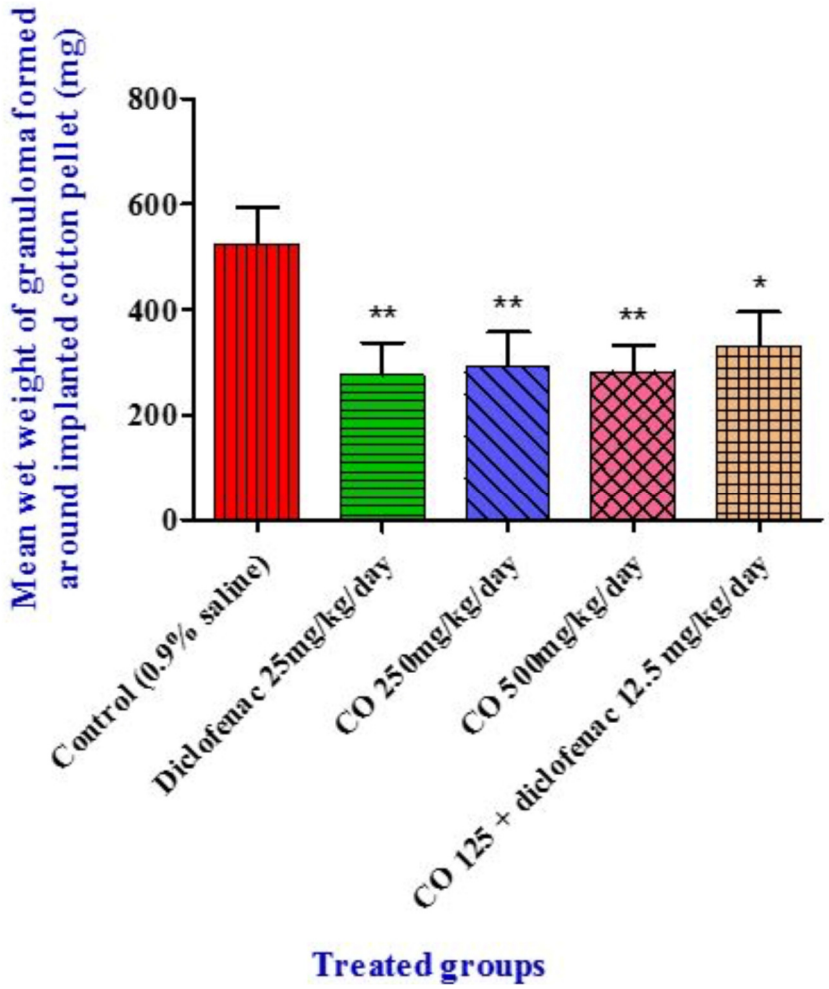

Figure 1. Effect of daily treatment with methanolic extract of *C. opobalsamum* (i.p.) for 6 days versus diclofenac (i.p.) on wet weight of granuloma induced by implanted cotton pellets in rats. $^{*}p<0.05$, $^{**}p<0.001$ compared with the corresponding control group values; by one-way ANOVA and Tukey HSD post hoc test. Data are expressed as mean±SEM; n=6rats; CO:*Commiphora opobalsamum.*

comparison with control (p value <0.001) after 6 days of administration with mean % inhibitions of 88.19, 83.98, 85.99 and 84.19% respectively. In addition, the results show that the inhibitions produced by *C. opobalsamum* (250 and 500 mg/kg) and *C. opobalsamum* plus diclofenac (125 and 12.5 mg/kg, respectively) are insignificant compared with diclofenac (p value >0.05).

In addition, a significant reduction was also revealed in the wet and dry weight of granuloma in *C. opobalsamum* in combination with diclofenac group (125 and 5 mg/kg,correspondingly) in comparison with control ($p<0.05$ and $p<0.001$ respectively) with mean values ± SEM of 330.18±66.03 and 34.55±10.34, respectively and mean % inhibitions of 37.00 and 84.19%, respectively. These reductions were insignificant as compared with diclofenac ($p>0.05$).

DISCUSSION

In view of the well recognized and the intrinsic anecdotal amount of renal, gastrointestinal, cardiovascular and hepatic risk linked with all NSAIDs, their treatment guidelines frequently recommend the use of the minimum effective dose, and if possible avoid their use for a prolong period (Caudill-Slosberg et al., 2004; Zhang et al., 2004; Zhang et al., 2007; Zhang et al., 2008).

Therefore, there is an impending need for the discovery and development of innovative anti-inflammatory and analgesic compound, perhaps with natural origin background characterized by added potency and minimum adverse effects to replace the contemporary drugs or at least minimize the effective dose of NSAIDs and thereby minimize its adverse effect, (Hajhashemi et

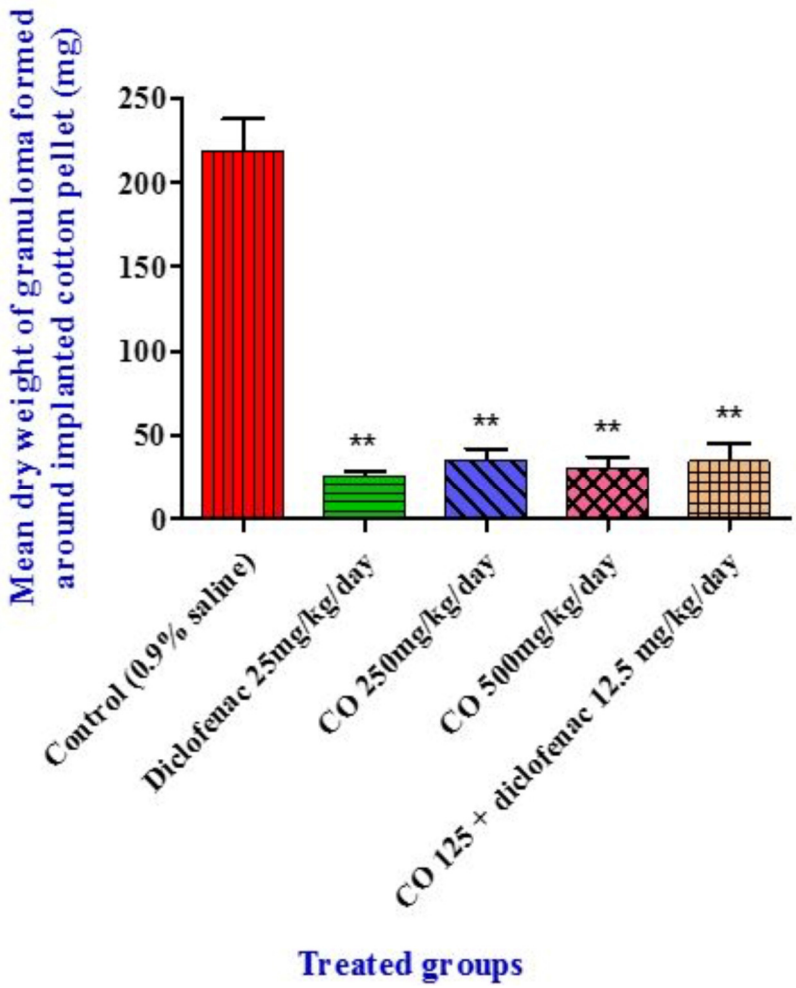

Figure 2. Effect of daily treatment with methanolic extract of *C. opobalsamum* (i.p.) for 6 days versus diclofenac (i.p.) on dry weight of granuloma induced by implanted cotton pellets in rats. $^{*}p<0.05$, $^{**}p<0.001$ compared with the corresponding control group values; by one-way ANOVA and Tukey HSD post hoc test. Data are expressed as mean±SEM; n=6rats; CO:*Commiphora opobalsamum.*

al., 2010) when used in appropriate combination. Several natural products have also been investigated for analgesic and anti-inflammatory potential (Borchers et al., 2000; Kelm et al., 2000; Albert et al., 2002; Santos, 2003; Li et al., 2004; Sultana and Saeed, 2012; Tripathi, 2013). Principally this study was designed to explore the anti-inflammatory and analgesic efficacy of *C. opobalsamum* extract in designated models of animal at different doses in comparison with diclofenac and to evaluate the ability of *C. opobalsamum* extracts to enhance the efficacy of diclofenac in reduced dose as well.

This study, observed a remarkable dose-dependent analgesic effect with *C. opobalsamum* extract. Regarding inhibition of writhing response of *C. opobalsamum* extract, this was observed in totality with the dose of 500 mg/kg, and moreover, this response was better than that observed with diclofenac (25 mg/kg) causing 87% pain inhibition. However, the combination of reduced doses of *C. opobalsamum* and diclofenac (125 and 12.5 mg/kg, respectively) strikingly, not only inhibit the writhing count compared with diclofenac (25 mg/kg) ($p>0.05$) but also exhibit better analgesic effect by a percentage of inhibition of 97.4 and 87% respectively. This result, unequivocally reveals that the combination treatment of Diclofenac in subtherapeutic doses and *C. opobalsamum* extract alone have produced significant analgesic activity.

Furthermore, in this study of hot plate test, the *C. opobalsamum* extract exhibit significant analgesic effect in comparison with control and diclofenac (p value <0.05). Additionally, the combined effect of reduced doses of *C. opobalsamum* and diclofenac (125 and 12.5 mg/kg, respectively) exhibit comparable analgesic effect with diclofenac alone (25 mg/kg) ($p>0.05$). These responses also reflect that *C. opobalsamum* enhances the analgesic

effect of diclofenac.

Moeover, the paw licking model induced by formaldehyde is a reliable and valid test for nociception and inflammatory pain(Shibata et al., 1989). It includes neurogenic response (early phase; 0-5 min) by direct chemical activation of nociceptors leading to the release of substance P and peripheral inflammatory response (late phase response; 15 to 30 min) by the release ofmediators of pain and inflammation like bradykinin, prostaglandins, serotonin (5HT) and histamine (Hunskaar and Hole, 1987; Tjølsen et al., 1992). The results of this study in this model revealed that the *C. opobalsamum* extract (250 and 500 mg/kg) exhibit a significant analgesic effect in comparison with control and diclofenac (p value <0.001) at both early and late phase responses and appear to be dose-dependent. The combination of reduced doses of *C. opobalsamum* and diclofenac, exhibit insignificant pain inhibition compared with diclofenac (25mg/kg) (p>0.05) with comparable percentage of inhibition at both phases. This finding has further revealed and sustained that the *C. opobalsamum* enhances the analgesic effect of diclofenac.

Hence, the results of methanolic extract of CO exhibit significant dose-dependent analgesic effect that might be mediated through central and peripheral pathways of pain perception with the maximum effect shown at a dose of 500 mg/kg. Moreover, the results demonstrated that the *C. opobalsamum* extract can enhance the analgesic effect of diclofenac. Similar results were also observed in a solitary study (Al-Howiriny et al., 2004) emphasizing the *C. opobalsamum* extract have analgesic effects.

On the other hand, the assessment of the anti-inflammatory efficacy of *C. opobalsamum* extract was one of the prime objectives of the present study. Carrageenan induced acute inflammation in rodents is an extensively investigated test and provides extremely reproducible results (Morris, 2003; Lucetti et al., 2010). At the end of 4 h of carrageenan injection with prior treatment of *C. opobalsamum* extract, highest percentage of inhibition of paw edema was perceived. This response was better than that observed with diclofenac (25 mg/kg) causing edema inhibition. Moreover, the combination of reduced doses of *C. opobalsamum* and diclofenac (125 and 12.5 mg/kg, respectively) exhibit inconsequential edema inhibition compared with diclofenac (25 mg/kg) (p>0.05) with almost constant percentage of inhibition over 4 h (72.7%). *C. opobalsamum* extract therefore, seems to be capable of amplifying the anti-inflammatory effect of Diclofenac. The results acquired from this model explicitly signify that the *C. opobalsamum* extract act on both early and late phases of acute inflammation induced by carrageenan perhaps, involving pro-inflammatory mediators and Polymorphonuclear leukocytes (PMNs) migration.

The basic essence of cotton pellet-induced granuloma is the evaluation of both proliferative as well as transudative components of chronic inflammation (Bianchine and Eade, 1967; Sawant et al., 2014). Interestingly**,** the results obtained from this model in this study revealed a significant reduction of wet weight of the cotton pellets in contrast with control (p value<0.001)**.** On the contrary, the effect of the *C. opobalsamum* extract on the cotton pellet's dry weight was significant as in contrast with control (p value <0.001) and the inhibitory effect appears comparable with diclofenac (25 mg/kg/day).

Moreover, the combination of reduced doses of *C. opobalsamum* and diclofenac (125 and 12.5 mg/kg/day, respectively) significantly potentiates and exhibit anti-inflammatory effect on the dry as well as wet weight of the cotton pellets relatively comparable to that of diclofenac (25mg/kg/day). In accordance with the findings of this study, it can be concluded that the extract of *C. opobalsamum* possesses a dose-dependent and noteworthy anti-inflammatory effects both in acute as well as chronic phases of inflammation with the greatest effect shown at a dose of 500 mg/kg.

It is worth mentioning that, except a solitary study of Al-Howiriny et al. (2004) in which Phenylbutazone (an obsolete drug) was used in comparison with *C. opobalsamum*, no such analogous study was found. The clinical significance of the anti-inflammatory and analgesic effect of the extract of *C. opobalsamum* alone, and potentiation of these effects with sub therapeutic doses of diclofenac needs to be considered cautiously. Additional comprehensive experimental studies are required to scrutinize the efficacy of the *C. opobalsamum* extract on PMNs infiltration,TNF-α, MDA and NO activities to explore the mechanisms underlying their anti-inflammatory and analgesic action.

Conclusion

The current study provides a strong indication of the anti-inflammatory and analgesic action of the extract of *C. opobalsamum*, more potent than diclofenac, in both central and peripheral analgesic activity. Moreover, the combination of reduced doses of *C. opobalsamum* and diclofenac with resultant significant potentiation of both anti-inflammatory and analgesic effect perceived in this study necessitates a cautious approach to elucidate its mechanism and meticulous safety profile studies.

Conflict of Interest

The authors have not declared any conflict of interest.

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