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Analgesic, anti-inflammatory and toxic effects of ethanol extracts of *Cucumis melo* and *Citrullus lanatus* seeds

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Abstract: Plants are vital in drug discovery, since many safe and bioactive molecules have been discovered from plants in past, hence this study was designed to evaluate analgesic, anti-inflammatory and toxic effects of *Cucumis melo* and *Citrullus lanatus*. Seeds of these plants were selected due to their traditional value for medicinal use. Analgesic activity was determined in mice by Eddy's Hot plate and tail flick method, while anti-inflammatory activity was evaluated by hind paw edema method. Both seed extracts produced highly significant analgesic effects comparable to standard drugs at all three doses by both methods. The extract of *C. lanatus* showed significant anti-inflammatory activity at 100 mg while showed highly significant activity at 200 mg between 3 to 24 hours as compared to standard drugs. Both extracts did not reveal any mortality up to 1000mg/kg, while there was also no change in normal the gross behavior pattern of the animals at the dose of 50 and 100mg/kg, however there was increase in passivity, sedation and startle response at 200mg/kg. Analgesic and anti-inflammatory effects of extracts may be due to presence of cucurbitacin A, B or E in both seeds which are thought to inhibit COX 2. Results indicate that seeds of *C. melo* and *C. lanatus* may be effectively used as adjuvant analgesic and anti-inflammatory agents in situation of chronic pain and inflammation.

Keywords: Analgesia, anti-inflammatory, seeds, safety, efficacy.

INTRODUCTION

Pain and inflammation are protective mechanisms activated in response to tissue injury, however, if pain and inflammation persist over a period of time damages the body tissue initiating different types of disorders like diabetes, spondylitis and rheumatoid arthritis. Patient suffering from pain and inflammation tries to overcome these situations through various NSAIDs which are known to cause serious adverse effects like gastric ulcer, chronic kidney diseases and even kidney failure (Burke *et al*., 2016). Despite the availability of NSAIDs to treat inflammatory disorders still there is need for safe, effective and economical analgesic compounds to deal with various situations of pain and inflammation.

The Cucurbitaceae family encompass about more than 900 species of plants collectively known as cucurbits. The seeds of *C. melo* and *C. lanatus* have high contents of cucurbitacin and reported to possesses nutritive and medicinal properties. Seeds of *C. melo* are rich in starch, amino acids, fatty acids, potassium, magnesium, calcium and sodium (Bouazzaoui *et al*., 2016). It also contains cucurbitacin A, B and E, some trypsin-inhibitors like CMeTI-A, CMeTIB along with melonin and cucumisin (Rastogi *et al*., 2001; Waseem *et al.,* 2018). Several important types of terpenoids (Chen *et al.,* 2009; Vidya *et al*., 2014), chromone derivatives beta-sitosterol, beta-amyrin and beta-sitosterol-3-O-betaglucopyranoside (Ibrahim*,* 2010), phenolic glycoside like dibenzoylmultiflor-8-en-3α, benzyl O-β-D-glucopyranoside (Chen *et al.,* 2005; De Marinoa *et al*., 2009) were also isolated.

Seeds of *C. lanatus* possess high percentage of water (85%-92%) along with small amounts of protein, lipid, starch, pro-vitamin A carotene, vitamin C and K, while it also contains riboflavin, iodine, iron and other minerals like calcium, magnesium, potassium, zinc and sodium (Schippers, 2002). Seeds are also rich source of phytochemicals like triterpenoid, phenolic compounds as cucurbitacin E, triterpenes, sterols and alkaloids (Johnson *et al.,* 2012; Lucky *et al*., 2012). It possesses potent analgesic, anti-ulcerogenic, antitussive, antipyretic, antihelmintic and immunomodulatory properties, while also have antibacterial, gastro protective, laxative, anti-giardial and hepatoprotective properties (Chinmay *et al.*, 2015).

The seeds can be used for reducing the risk of coronary heart disease and cancer, while also possess diuretic, antimicrobial and antifungal activities (Ahmed *et al*., 2011). It is also reported to have an anti-oxidant and antiulcer activity (Gill *et al*., 2011a & b; Lucky *et al*., 2012; Rahman *et al.,* 2013). Therapeutic effects were also identified in the management of obesity and diabetes (Sui *et al*., 2012). The seed of *C. lanatus* have also exhibited hepatoprotective and anti-inflammatory effects (Adebayo,

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2014) and anxiolytic activity (Rahman *et al*., 2013). Thus present study was designed to explore anti-inflammatory, analgesic activity and toxic effect of *C. melo a*nd *C. lanatus* extracts.

MATERIALS AND METHODS

The study was conducted after the approval from Board of Advance Studies and Research, University of Karachi and Departmental Research Committee for the use of animals according to guidelines of NIH.

Collection of plant material

The seeds of. *C. melo* (musk melon) and *C. lanatus* (water melon) were purchased from the local market of Karachi. Plants were grown from these seeds which were then verified along with seeds by a botanist at the Herbarium, Centre for Plant Conservation, University of Karachi. The voucher number 94494 was issued for *C. melo* and voucher number 9462 for *C. lanatus*.

Preparation of extracts

Peeled seeds of *C. melo a*nd *C. lanatus* were weighed, washed, and crushed with laboratory grinder followed by 3 consecutive process of maceration in ethanol for 21 days. Seeds in amount 4 Kg were soaked in portions of 6 litres ethanol to obtain enough extracts to fulfil need of the study. Seeds were kept in tightly closed Jars during the period of maceration. After soaking, material was filtered through the muslin cloth followed by filtration with what man filter paper #1and shaken intermittently during filtration. Solvent was removed from the macerated seed materials by rotatory evaporator followed by freeze drying. These concentrated extracts were kept in tightly closed container and stored in refrigerator at 4°C.

Animal selection and grouping

The study was designed on young mice and rats of either sex. Mice and rats were bred in the Animal house, Department of Pharmacology, University of Karachi. Mice weight were ranges between 25-30g, and of rats were 200-250g respectively. Mice and rats were acclimatized one week before the start of studies and their general health conditions were evaluated. Five mice or rat per cage were housed in polycarbonate cages with rodent laboratory chow and filtered tap water ad libitum. During the study, animals were maintained in a controlled room temperature (25 ± 2 °C), under an alternating 12-h of artificial light/dark cycle.

Selection of doses

Each seed extract was given in the dose of 50, 100 and 200 mg/kg. Extract of seeds were freshly suspended in 5% DMSO while control group only received 5% (DMSO) by mouth equivalent to the volume of respective doses according to their body weight. Reference standards group were given aspirin 300mg/kg (Rahman *et al*., 2015) for inflammation and ibuprofen suspension 100 mg/kg (Lalan *et al*., 2015) for nociception.

Toxicity studies

Acute toxicity

Acute testing was carried out according to Lorke's method at the doses of 10mg/kg, 100mg/kg and 1000mg/kg 3 animals per dose. Mice were initially fasted overnight followed by the administration of extracts to next day. The animals were weighed and divided into respective groups having three mice in each group. Mice were continuously checked for 4 h for symptoms of toxicity like motor activity, convulsions, tonic extension, tremors, loss of righting reflex, ataxia, muscle spasm, sedation or hypnosis, lacrimation, diarrhea, salivation, writhing etc. Mice were then kept under observation up to 48 h for any mortality (Lorke, 1983; Riaz *et al*., 2010).

Sub chronic toxicity

Sub-chronic toxic effects of these seed extract were also observed at all three doses i.e. 10mg/kg, 100mg/kg and 1000mg/kg for 30 days, gross behavioral parameters mentioned in acute toxicity studies were noticed at day 8 and day 30. Mortality of mice was also recorded. All gross behavioral changes of treated mice were recorded for each animal.

Anti-inflammatory activity

The Anti-inflammatory method described by Winter, *et al*., 1962 was mainly used with some minor modifications. The Ethanol extracts were administered orally 1 hour before the induction of edema. Edema in the left paw was induced in each rat by sub plantar injection of 0.1 mL carrageenan (1%) prepared in normal saline. The paw volume was measured at intervals of 1, 2, 3, 4, 5 and 24 h by the water displacement method using a digital plethysmometer. Paw edema as a sign of inflammation was calculated as a reduction in paw volume after treatment by measuring change in paw volume from baseline paw volume of each respective animal. The percentage inhibition of paw volume is also calculated by comparing the treated group with the carrageenan control group. Aspirin and ibruprofen were used as a reference drug (Sood *et al.*, 2009, Jamil *et al.,* 2017). Following formula was used for determination of percent inhibition of paw (Suleyman *et al*., 1991).

$$\% \text{ Inhibition} = \left(\frac{\text{Vc - Vt}}{\text{Vc}} \right) \times 100$$

Where, Vc and Vt are mean change in paw volume of control and treated group respectively.

Analgesic activity

Analgesic activities of these extracts were evaluated using hot plate and tail flick method.

Hot plate test

Hot plate test is mainly used to determine the effect of centrally acting analgesic that causes increase in the

latency times of response i.e. licking of paws, jumping or withdrawal of the paws. Only those mice were selected which reacted within 15 s. Extracts and reference drugs were administered 60 min before the performing the test. Mice were placed on a hot plate maintained at 55 ± 2 °C and reaction time in seconds were recorded for the licking or jumping (Turner, 1965). 15s cut off time was used to prevent damage to the paw (Eddy *et al.,* 1950).

Tail flick method

Tail flick method was employed for antinociceptive effect against thermal stimuli according to the method of Di Stasi *et al*., 1988. The latency time in seconds was measured by placing the animals on heated plate at 50°C. Readings were taken at 1h, 2h, 3h and 4h hours after administration of ethanol extracts. Mean increase in latency time after the administration of the extract were used to measure the analgesic effects produced by test and reference standard drugs.

STATISTICAL ANALYSIS

Data was analysed using SPSS software version 20.0 through General linear model with repeated measures and One way ANOVA followed by Post hoc tests like Dunnet and Tukey tests. Results were considered significant when $p<0.05$ and highly significant when $p<0.01$.

RESULTS

Acute toxicity

Table 1 shows result of *in-vivo* oral acute toxicity of the ethanol extracts. Results clearly indicate that these seeds have safer profile. During the entire period of experiment, there were no symptoms of acute toxicity like salivation, lacrimation, laboured breathing, diarrhoea, constipation, polyuria, polyphagia, polydipsia, weight loss, skin eruptions, haemorrhage, sedation, tremors, writhing and respiratory depression.

Table 1: Oral Acute toxicity of ethanol extracts of *C. melo* and *C. lanatus*

Groups / Dose (mg/kg)	10	100	1000
	No. of animals died/total number of mice		
Control	0/3	0/3	0/3
C. melo	0/3	0/3	0/3
C. lanatus	0/3	0/3	0/3

Table 2: Analgesic effects of *C. melo* and *C. lanatus* by Hot plate method

Groups & Doses mg/kg	Reaction time (Sec)				
	0h	1 h	2 h	3 h	4 h
Control	7.10 + 0.27	7.57 + 0.58	7.52 + 0.78	6.67 + 0.31	7.17 + 0.56
C. melo 50	6.50 + 0.17	14.1 + 0.72**	13.71 + 0.93**	11.91 + 0.68**	11.70 + 0.44**
C. melo 100	7.40 + 0.22	13.77 + 0.84**	12.32 + 0.61**	11.84 + 0.43**	11.70 + 0.51**
C. melo 200	7.10 + 0.23	14.5 + 0.69**	13.60 + 0.69**	11.80 + 0.44**	11.40 + 0.50**
C. lanatus 50	7.40 + 0.22	13.77 + 0.84**	12.32 + 0.69*	10.10 + 0.45**	9.19 + 0.52 [b]
C. lanatus 100	7.07 + 0.32	11.38 + 0.49**[a,b]	12.06 + 0.43**	12.21 + 0.78**	9.18 + 0.78[b]
C. lanatus 200	7.61 + 0.34	12.5 + 0.34** [a]	12.38 + 0.45**	11.9 + 0.57**	10.4 + 0.46*
Aspirin 300	7.27 + 0.32	13.73 + 0.35**	12.73 + 0.56**	11.48 + 0.50**	11.61 + 0.58**
Ibuprofen 100	7.02 + 0.45	14.23 + 0.56**	12.35 + 1.00**	11.77 + 0.92**	11.93 + 0.34**

Table 3: Analgesic activity of *C. melo* and *C. lanatus* by Tail flick method

Groups & Doses mg/kg	Reaction time (Sec)				
	0h	1 h	2 h	3 h	4 h
Control	1.81 + 0.10	1.72 + 0.16	1.96 + 0.27	1.45 +0.18	1.68 + 0.25
C. melo 50	1.71 + 0.09	4.48 + 0.25**	4.50 + 0.26**	3.78 + 0.26**[,b]	3.70 + 0.21**
C. melo 100	1.71 + 0.11	5.01 + 0.34**	4.62 + 0.31**	4.67 + 0.25**	3.52 + 0.20**
C. melo 200	1.67 + 0.10	5.10 + 0.33**	4.80 + 0.22**	4.36 + 0.24**	4.37 + 0.45**
C. lanatus 50	1.53 + 0.12	4.02+ 0.13** [a]	4.60 + 0.36**	3.83 + 0.16**	3.65 + 0.25**
C. lanatus 100	1.97 + 0.30	4.19 + 0.06**	4.41 + 0.24**	4.19 + 0.34**	3.46 + 0.26**
C. lanatus 200	1.64 + 0.10	3.8+ 0.23**[a,b]	4.32 + 0.46**	4.08 + 0.31**	3.80 + 0.31**
Aspirin 300	1.60 + 0.14	5.23 + 0.57**	4.16 + 0.43**	3.91 + 0.38**	3.75 + 0.35**
Ibuprofen 100	1.46 + 0.13	4.97 + 0.25**	4.89 + 0.57**	5.35 + 0.59**	3.70 + 0.21**

n=10, Mean ± SEM; *P<0.05 significant; ** P<0.01 highly significant as compare to control; a; significant difference as compare to Aspirin; b: significant difference as compare to ibuprofen.

Table 4: Anti-inflammatory Activity of *C. melo* and *C. lanatus*

Groups & Doses mg/kg	Mean Paw Edema Volume (mL) with % inhibition					
	1 h	2 h	3 h	4 h	5 h	24 h
Control	0.74 ± 0.22	1.85 ± 0.22	2.27 ± 0.15	1.71 ± 0.09	1.52 ± 0.11	0.62 ± 0.16
C. melo 50	0.67 ± 0.12 (17.61%)	1.15 ± 0.12 (42.72%)	1.30 ± 0.14*,a** (45.29%)	1.10 ± 0.16,a** (35.37%)	0.70 ± 0.08** (54.10%)	0.22 ± 0.05* (63.71)
C. melo 100	0.64 ± 0.05 (21.68%)	1.37 ± 0.14 (31.91%)	1.32 ± 0.16*,a** (44.28%)	0.91 ± 0.21*,a (46.64%)	0.72 ± 0.23* (52.79%)	0.22 ± 0.06* (64.52%)
C. melo 200	0.52 ± 0.06 (35.56%)	1.27 ± 0.16 (36.82%)	1.34±0.12**,a** (43.50%)	0.80 ± 0.08**,a (53.12%)	0.54 ± 0.08** (64.79%)	0.22 ± 0.08* (64.19%)
C. lanatus 50	0.74 ± 0.15 (8.83%)	1.69±0.37 a**,b (16.12%)	1.74±0.29 a**,b (26.80%)	1.17 ± 0.26 ,a** (31.29%)	0.83 ± 0.33* (45.31%)	0.36 ± 0.16* (41.61%)
C. lanatus 100	0.56 ± 0.09 (31.01%)	1.61 ± 0.37 a (20.00%)	1.31 ± 0.38*, a (44.68%)	0.88 ± 0.31* (48.35%)	0.42 ± 0.19** (72.59%)	0.10 ± 0.3** (74.52%)
C. lanatus 200	0.69 ± 0.11 (14.74%)	1.25 ± 0.24a (37.38%)	1.05 ± 0.22** (55.56%)	0.65 ± 0.28** (62.00%)	0.41 ± 0.15** (72.98%)	0.14 ± 0.11* (77.74%)
Aspirin 300	0.22 ± 0.02* (72.40%)	0.42±0.15** (79.30%)	0.22 ± 0.05** (90.81%)	0.09 ± 0.03** (94.82%)	0.17 ± 0.14** (88.72%)	0.05± 0.01** (92.58%)
Ibruprofen 100	0.68 ± 0.03 (16.47%)	0.65 ± 0.12* (67.76%)	0.74 ± 0.21** (68.71%)	0.62 ± 0.21** (63.65%)	0.42 ± 0.17** (72.59%)	0.14± 0.03** (92.58%)

n=10, Mean ± SEM; *P<0.05 significant, ** P<0.01 highly significant as compare to control by Tukey and Dunnet test; a; significant difference as compare to Aspirin; ,a** highly significant difference as compare to Aspirin b: significant difference as compare to ibruprofen.

In present study animals did not reveal any mortality up to 1000mg/kg, however Vindhya *et al.,* 2018 showed that methanol and chloroform extracts of *C. melo* were safe up to the doses of 5000mg/kg by oral route. Similarly Damilola and Ajayi, in 2016 also revealed that the methanol extract of *C. lanatus* was safe up to 5000mg/kg by oral route, hence we can conclude that these seeds are safe up to 5000 mg/kg dose.

Sub-chronic toxicity

Gross behavioural parameters were observed following administration of ethanol extract of *C. melo* and *C. lanatus* to mice in the doses of 50, 100 and 200 mg/kg for 30 days. No change was observed in normal behaviour pattern of the animals i.e. there were no tremors, convulsions, vocalization, aggression, fearfulness. There was also no change in body and limb tone, corneal reflex and righting reflex. While passivity, sedation, startle response and balance beam type behaviors were increased in the animals received 200 mg/kg dose. Pain response and touch response were decreased and awareness and staggering gait was also decreased in 100 mg/kg and 200 mg/kg. The effects were compared to 5 mg/kg diazepam.

Hot plate analgesia

Table 2 shows results of analgesic effect of *C. melo* and *C. lanatus* by hot plate method at 50mg/kg, 100mg/kg and 200 mg/kg. There was highly significant increase in reaction time of both extracts. In case of *C. melo* effects persisted up to 4 hours same as that of standard drugs at each dose as compare to control. *C. lanatus* revealed highly significant analgesic effect up to 3 h at all three doses.

Tail flick analgesia

Table 3 shows results of analgesic effect of *C. melo* and *C. lanatus* by tail flick method at 50mg/kg, 100mg/kg and 200 mg/kg. There was highly significant increase in reaction time of both extracts at 1h, 2 h, 3h and 4 h in all doses as compare to control.

Anti-inflammatory activity

Table 4 shows anti-inflammatory effect of ethanol extracts of *C. melo* and *C. lanatus* seeds by rat paw edema method at 50mg/kg, 100mg/kg and 200mg/kg as compare to control. There was a significant decrease in paw edema at 50mg/kg i.e. 45.29%, which persisted up to 24h with 63.71% inhibition in paw edema, while highly significant decrease in paw edema was observed at 200mg/kg dose of *C. melo* producing 64.79% inhibition in paw edema at 5 h. *C. lanatus* at 50mg /kg dose showed significant inhibition in paw edema at 5h and 24 h with 45.31% and 41.61% inhibition in paw edema. While at 100 mg/kg it showed significant decrease in paw edema at 3 h, and 4h, however at 5h and 24h decrease in paw edema was highly significant with % inhibition of 72.59% and 74.52%. Lastly at 200mg/kg highly significant decrease in paw edema was observed at 3h, 4h, and 5h with % inhibition up to 72.98%, however the decrease in paw edema at 24h was significant with 77.74% inhibition in paw edema.

DISCUSSION

The current management to suppress the pain and inflammation in many diseases is the use of steroidal and non-steroidal anti-inflammatory drugs which have severe side effects. Thus in present study, ethanol extract of *C. melo* and *C. lanatus* seeds were tested for analgesic and anti-inflammatory activity, while acute and sub chronic toxicity was also tested. Ethanol extract of *C. melo* and *C. lanatus* seeds did not produce any symptoms of sub chronic toxicity at 50, 100 and 200 mg/kg, while no mortality was observed up to 1000mg/kg, hence these seed extracts could be considered as safe. Literature survey reveals that seeds under investigation are safe for oral administration up to 5000mg/kg (Vindhya *et al*., 2018; Damilola and Ajayi, 2016).

The ethanol extract of *C. melo* exhibited highly significant analgesic activity at all tested doses both by hot plate and tail flick method during the entire period of study from 1 to 4 h, while the ethanol extract of *C. lanatus* seeds also produced the highly significant effect but it continued up to 3 h only as shown in table 2 and 3. This shows that the extract of *C. melo* is more effective analgesic as compare to *C. lanatus*, both seeds have been reported to contain cucurbitacins which have shown to inhibit COX-2 without having any effect on COX-1. *C. melo* mainly contain cucurbitacin B (56.9%), but also contain cucurbitacin E and cucurbitacin D (Yuan *et al*., 2019). However *C. lanatus* only contain cucurbitacin E, thus *C. melo* due to high contents of cucurbitacins (Kaushik *et al*., 2015) may have shown better analgesic effect with long duration of action as compared to *C. lanatus*.

Both hot-plate as well as tail-flick method used in the study to determine analgesic activity includes neuronal stimulation-induced analgesia by thermal stimuli, however spinal reflexes are also involved in the tail-flick method. The hot-plate method is integrated supraspinally (Schmauss and Yaksh, 1984; Suh *et al*., 1992). The antinociceptive effect in the tail flick and hot-plate tests indicates that these extracts has effect on CNS as may inhibit the spinal reflex and supraspinal centers (Dewey *et al*., 1970).

The ethanol extracts of *C. melo* and *C. lanatus* both showed significant anti-inflammatory effect from 3 hours to 24 hours. However the anti-inflammatory effect of *C. lanatus* at 100mg/kg was highly significant at 5 hour which persisted up to 24 hour, while at 200mg/kg *C. lanatus* showed highly significant anti-inflammatory effect at 3 h, 4h and 5h, however at 24 h the effect was significant. The anti-inflammatory effect of *C. lanatus* was found to be better than *C. melo*. This might be due to presence of Cucurbitacin E which have shown to decrease carrageenan-induced rat's paw edema (Abdelwahab 2011).

Administration of carrageenan increases the phospholipase activity of membrane, inducing pain and inflammation through mediators like leukotriene and prostaglandins along leukocytes which lead to increase in paw size due to accumulation of fluid. Therefore decrease in the paw size is indicative of protection against carrageenan induced edema and reflection of the inhibition of some prostaglandin or leukotriene like mediators.

These effects are proposed due to presence of phytochemicals in these plants. Phytochemistry of the *C. melo* and *C. lanatus* reveals the presence of various chemicals like terpenoids, carotenoids, tannins, resins, saponins and phytosterols (Dhiman *et al*., 2012; Schaffer *et al.*, 2016). Terpenoids also called as isoprenoids are one of the class resembles to terpenes, previous studies have reported that anticoagulant anti-inflammatory activities of some of the cucurbitacins are mainly due to the inhibition of the enzyme cyclooxygenase (Miro, 1995, Peters *et al*., 1998 and Yesilada *et al*., 1998). *C. melo* methanol seed extracts had been found to decrease the LTB_4 levels and also inhibits the leukocyte influx generating anti-inflammatory action (Asif *et al* 2014).

CONCLUSION

Results obtained in present investigation are sufficient to conclude that ethanol extracts of *C. melo* and *C. lanatus* seeds possess effective analgesic and anti-inflammatory effects comparable to standard drugs ibruprofen and aspirin. Moreover extracts of these seeds were also found to be safe when tested for 30 days at all three doses. The analgesic and anti-inflammatory effects of these extracts may be due to presence of high Mg contents in these seeds and various phytochemicals like cucurbitacin A, B, E, tannins and flavonoids. The findings of present studies also suggest that these seeds can be effectively used as adjuvant analgesic and anti-inflammatory agents in situation where patients may have chronic pain and inflammation, since are highly safe and effective analgesic and anti-inflammatory agents. Furthermore we may also conclude that the analgesic and anti-inflammatory effects may be mediated by the inhibition of COX-II or central effects of the seeds as discussed above.

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