Pharmacological validation of the folkloric uses of *Cyperus rotundus* L. in different ailments: *An in vivo and in vitro* research

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Abstract: *In vivo and in vitro* research study was conducted on *Cyperus rotundus* to evaluate the sound mechanistic background in the treatment of gastrointestinal, bronchial and vascular disorders as well as in pain, emesis, pyrexia and bacterial infections. Results showed that crude extract of *Cyperus rotundus* (Cr.Cr) exhibited the dose-dependent spasmolytic effect in rabbit jejunum by inhibiting the spontaneous and K⁺ (80 mM)-induced contractions. Pretreatment of tissue with Cr. Cr caused the rightward shift of calcium concentration response curves, similar to verapamil. Cr. Cr also caused the relaxation of K⁺ (80 mM)- and carbachol (1 µM)-induced contractions of trachea preparations, similar to that of verapamil. Moreover, Cr. Cr also relaxed the contraction induced by the K⁺ (80 mM) and phenylephrine (1 µM) of aorta preparations. Data show that *C. rotundus* possess the spasmolytic, bronchodilator and vasodilator activities possibly through calcium channels blockade; validating its folkloric use in diarrhea, dyspepsia, bronchitis, asthma and hypertension in addition to antibacterial, antiemetic, antipyretic and analgesic activities.

Keywords: Spasmolytic, bronchodilation, vasodilatation, Ca²⁺ antagonist, antiemetic.

INTRODUCTION

*Cyperus rotundus* Linn. (*Cyperaceae*) is known by vernacular names of purple nut-sedge and nut-grass (English), muthaghas (Bengali), motha (Hindi) and mustaka (Urdu). *Cyperus rotundus* also known as "Worlds worst weed” is widely distributed throughout India and Pakistan (Anonymous, 1990). Rhizomes are dark brown and woody, while stems are trigonous, green and smooth.

The roots and rhizomes of *C. rotundus* are useful in diarrhea, dyspepsia, cholera, inflammation, dysentery, skin rashes and excess bleeding while fresh tubers in the form of paste or plasters are applied on the breast, scorpion sting and spreading ulcers (Kirtikar and Basu, 2002; Sharma and Gupta, 2007). The tubers of *C. rotundus* are traditionally used as; antiemetic, anthelmintic, antipyretic, hypertensive and smooth muscle relaxant (Nadkarni and Nadkarni, 1996; Kirtikar and Basu, 2002; Khare, 2007). The plant has also been reported to possess antimalarial, hepatoprotective, tranquillizing activity and reduce the obesity by suppressing appetite centre (Gupta, 2003).

Photochemical investigations revealed the presence of sesquiterpene 4a-, 5a-, cyperene-1 (tricyclic sesquiterpene), oxidodecesm-11-en-3a-ol, cyperenone, α-cyperone, cyperene-2 (bicyclic sesquiterpene hydrocarbon) (Anonymous 1990, 1992), β-selinene, sugetriol triacetate (sesquesterpenoid), mustakone (sesquesterpenketone), sugenol (sesquesterpenketol) (Rastogi and Mehrotra, 1969); the essential oil including eugenol, copadiene,a-and β-rotunol, epoxyguaieronertund one, calamenone, cyperron, ciperolone, isocyperol, ciperol, d-cadinene, kobusone and isokobusone (Anonymous, 1992); a flavonol glycoside, β-sitosterol rhamnosyl-(1-4) rhamnopyranoside (Gupta,2003); myrtolen, α-cyperone, caryophyllen oxide, β-pinene, flavonoids, alkaloids, ascorbic acid and poly-phenols (Lawal and Oyedeji, 2009).

Despite the *C. rotundus* ancient history of use for GIT, respiratory and vascular system disorders, as well as its use as antipyretic, analgesic and antiemetic remedy, it’s *in vivo* anti-diarrheal study has been performed (Uddin et al., 2006), just validating its pharmacological effectiveness but no further studies have been reported with respect to its underlying pharmacological mechanism of actions. The present study was carried out to rationalize and to explore mechanistic background validating the folkloric uses of *C. rotundus*.

MATERIALS AND METHODS

Collection and extraction of plant

Rhizomes and roots of *C. rotundus* were collected during the month of May-June, 2012 and were identified by Curator of Department of Botany, G.C University, Lahore, with voucherspecimen labeled as GC. HERB.BOT. 206. Triple maceration process was adopted for the extraction of coarsely powdered material (#40) using 70% methanol-aqueous mixture (Hussain et al., 2018).
2014) and resulting in dark brown thick syrup consistency; named Cr. Cr, with an approximate yield of 18.25%.

**Animals and housing conditions**
Animals including, rabbits (1.0-1.5kg, 6-9 months old), Swiss albino mice (38-48g) and chicks (7-10 days old, 70-90g) of either sex were locally purchased; provided with tap water ad libitum and standard diet under controlled environmental condition (25-28°C), while all chicks were housed at room temperature with 12h/12h dark-light cycle. Whereas, *Staphylococcus aureus*, *Bacillus pumilus*, *Escherichia coli* and *Citro bacterfreundii* were collected from National Institute of Biotechnology & Genetic Engineering, Faisalabad. All experiments were performed by following the ruling of Institute of Laboratory Animal Resources, Commission on Life Sciences National Research Council 1996).

**Standard drugs, chemicals, and solvents**
All Standard drugs, chemicals and solvents such as carbachol, potassium chloride, EDTA, verapamil, phenylephrine, chlorpromazine, copper sulphate, dimethyl sulfoxide, glucose, magnesium sulphate, calcium chloride, sodium bicarbonate, dichloromethane, potassium dihydrogen phosphate, polyoxyethylene-sorbitan mono-oleate (tween-80), sodium dihydrogen phosphate, and methanol were of research grade.

**Determination of phytochemical constituents**
The crude extract was initially subjected to screening with different reagents to detect the presence of possible important constituents, according to a standard method (Tona *et al*., 1998; Evans, 2006).

**In vitro experiments**

**Isolated rabbit jejunum**
The crude extract was tested on jejunum preparations for assessment of spasmylocytic activity (Hussain *et al*., 2015a; Hussain *et al*., 2016). A weight of 1g was applied to jejunum segments (2-3 cm) hanged in tissue bath filled with 15 mL of Tyrode’s solution, aerated with carbogen (pH 7.4;37°C) and tissue sensitivity was recorded via displacement transducers attached with PowerLab data acquisition system (Janbaz *et al*., 2012). For spasmylocytic activity, the crude extract was applied to the spontaneous contractions in a cumulative fashion, without prior addition of any agonistic agent (Janbaz *et al*., 2013).

**Assessment and conformation of calcium channel blocking activity**
To assess whether the spasmylocytic response was mediated through calcium channel blockade (CCB), the tissue preparations were depolarized by exposing to K+(80mM) as KCl, resulting in appearance of sustained contraction (Farre *et al*., 1991), where as control calcium concentration-response curves (CRCs) were constructed for the confirmation of calcium channels blockade (Gilani *et al*., 2005; Janbaz *et al*., 2013; Hussain *et al*., 2015b).

**Isolated rabbit trachea**
For the assessment of the bronchodilator effect of crude extract, isolated rabbit trachea preparations (2-3mm width) were hanged in tissue baths filled with 15mL of normal Kreb’s solution, aerated with carbogen (pH 7.4;37°C) (Gilani *et al*., 2005; Janbaz *et al*., 2013). Afterwards, tissue preparations were exposed to K+(80 mM)- and carbachol (1µM)-for stabilization (45 min). The crude extract was applied to stabilized tissues to obtained sustained contractions for possible relaxant effect. For the confirm of the possible mechanism of action, verapamil (standard Ca2+ channel blocker) was tested on K+(80mM)- and carbachol (1µM)-induced spastic contractions (Hussain *et al*., 2015c).

**Isolated rabbit aorta**
The vasodilator/vasoconstrictor effect were studied by application of crude extract to tissue bath containing isolated rabbit thoracic aorta preparations (2-3 mm wide) in a cumulative manner, already exposed to K+(80 mM)- and phenylephrine (1 µM)– for stabilization with a dose interval of 45 min (Janbaz *et al*., 2014).

**Antibacterial potential assessment**
For the assessment of antibacterial potential, disc diffusion method was used by measuring the zone of inhibition around sample disc (crude extract), positive (Gentamicin) and negative (DMSO) control (Taylor *et al*., 1995). The relative percentage of inhibition (RPI) with respect to positive control was derived from following equation (Ajay *et al*., 2002).

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RPI = \left( \frac{A - B}{C - B} \right) \times 100
\]

In this equation, A, B and C represent the total area of inhibition of sample discs, negative control and positive control respectively.

**In vivo experiments**

**Antiemetic potential assessment**
Chick emesis model was slightly modified for the assessment of the antiemeticpotential of crude extract (Akita *et al*., 1998; Ahmed and Patrricia, 2013). Chicks were placed in 4different groups and each group has 6 chicks. Each chick was set aside in a large beaker for the 15 min to acclimatize. Chicks of control group (group 1), experimental group (group 2 and 3) and standard group (group 4) were subjected to administration of normal saline (0.9% NaCl; 10mL/kg), crude extract (10 mL/kg of 100 and 150 mg/kg) and Chlorpromazine (150 mg/kg), respectively. After 10 min, Copper sulphate (50 mg/kg of body weight) was given orally to chicks of all groups in order to stimulate the peripheral nervous system for emesis, then numbers of retches were counted for next 15 min and %age inhibition was calculated by this equation:
Retching inhibition (%) = [(A-B)/A] x 100
A and B represents the frequency of retching in control and experimental groups respectively.

**Antipyretic potential assessment**
Antipyretic activity was performed on albino rabbits by the slight modification of previously reported method (Grover, 1990; Naveed et al., 2012). Albino rabbits were divided into 3 groups of 6 each. Pyrexia was induced by i.p. injecting brewer yeast (0.5 mL/kg) to each group. After 2 h of pyrexia, rabbits of group 1, 2 and 3 were subjected to i.p. administration of 2 mL/kg normal saline (0.9% NaCl), 2 mL of 150 mg/kg of crude extract properly dissolved in saline and 2 mL of 10 mg/kg of Aspirin properly mixed in normal saline respectively. After administration of the doses, rectal temperature was measured for 1 to 4 h. Decrease in rectum temperature (%) was noted by using this equation:

Decrease in rectum temperature (%) = [(B-Ca)/(B-A)] x 100

In this equation, A, B and C represent the normal rectum temperature, rectum temperature after 2 h of injecting brewer yeast and rectum temperature after 1st, 2nd, 3rd, and 4th h of treatment respectively.

**Phytochemical screening**
Phytochemical study of *C. rotundus* showed the presence of saponins, tannins, alkaloids, flavonoids, terpenes and sterols.

**Antibacterial activity of C. rotundus**
The crude extract of *C. rotundus* (150 mg/mL) showed zone of inhibition (mm) of 20.12, 18.25, 17.65, and 18.95, while Gentamicin at the dose range of 20 μg/disc showed the zone of inhibition (mm) of 22.45, 20.84, 19.41 and 22.67 with relative percentages of inhibition (RPI) of 80.35, 76.73, 79.41 and 69.90 against *S. aureus, B. pumilus, E. coli* and *C. freundii* respectively (table 1).
In vitro experiments

Antiemetic activity of *C. rotundus*

In the chicks of control group (treated with 10 mL/kg of normal saline followed by Copper sulphate) numbers of retches were 70±0.17, while in the chicks of group 2 (100 mg/kg of Cr. Cr), group 3 (150 mg/kg of Cr.Cr) and group 4 (150 mg/kg of Chlorpromazine), the number of retches were reduced with %age inhibition of emesis of 51.45, 78.60 and 45.75 respectively (table 2). It is clear from the results that all tested *C. rotundus* have significant antiemetic potential (*p*<0.05) which were comparable to Chlorpromazine in Copper sulphate induce chick emesis model.

**Antipyretic activity of C. rotundus**

The crude extract of *C. rotundus* (150 mg/kg) decreased the rectum temperature of pyrexia induced rabbit from 40.73±0.98 to 38.35±0.76°C (77.27%) after 4 h.
comparable with Aspirin, which decreased from 40.69±0.94 to 37.90±0.76°C (90.95%). However, normal saline solution induced no prominent response on the elevated rectal temperature of the rabbit. There was a significant rise in temperature in all groups after brewer yeast administration but extract and Aspirin treatment resulted in a significant decrease ($p<0.05$) in rectum temperature equal to normal rectum temperature thus expressing that *C. rotundus* has a significant antipyretic effect (table 3).

**Analgesic activity of C. rotundus**
Prior to administration of *C. rotundus*, deflection time was 3.15±1.11 sec but after i.p. administration of *C. rotundus* (200 mg/kg) and Aspirin (75mg/kg) to mice of group 2 and 3, deflection time was increased up to 9.15±0.7 and 9.95±1.11 sec respectively. Tail deflection time of *C. rotundus* was comparable to aspirin ($p<0.05$), thus indicating the significant analgesic effect (table 4).
**Acute toxicity of C. rotundus**

Acute toxicity of crude extract of *C. rotundus* was tested at 1, 3 and 5 g/kg; there was no behavioral changes and mortality up to 5g/kg which was higher than the normal therapeutic dose, thus indicating that *C. rotundus* is safe.

**DISCUSSION**

The crude extract of *C. rotundus* was tested for different phytoconstituents and it was found to contain saponins, tannins, alkaloids, flavonoids, terpenes and sterols.

*C. rotundus* (Linn.) has traditionally been used to treat diarrhea, dyspepsia, bronchitis, and hypertension, as well as having antibacterial, antipyretic, antiinflammatory and analgesic activities but presently, the crude extract of *C. rotundus* is subjected to evaluate and validate the folkloric uses and underlying possible mechanisms of aforementioned activities. Spontaneously contracting jejunal segments were used to study the spasmyloytic (inhibitory) mechanism of *C. rotundus,* without the addition of any spasmogen (Farre *et al*., 1991). Contraction of jejunal smooth muscle is mediated through activation of contractile elements caused by increased level of cytoplasmic free Ca²⁺ (Karaki and Weiss, 1983). The increase in intracellular Ca²⁺ occurs either an influx of Ca²⁺ through voltage-dependent Ca²⁺ channels (VDCs) or release of Ca²⁺ from sarcoplasmic reticulum which results in spontaneous rhythmic movement of smooth muscles by periodic depolarization (influx of Ca²⁺ via VDCs) and repolarization (Brading, 1981). Thus, the spasmyolytic (inhibitory) effect of *C. rotundus* may appear due to calcium channel blockade (CCB) mediated possibly due to the interference of Ca²⁺ influx through VDCs. Calcium antagonistic activity was further confirmed when pretreatment of tissue with *C. rotundus* shifted the calcium concentration-response curves (CRCs) to the right, in a manner similar to verapamil.

Further research study was performed on tracheal preparations for the assessment of bronchodilator effect. Like in gut preparation, *C. rotundus* produced a concentration-dependent inhibition of K⁺(80 mM)- and carbachol (1 µM)-induced contractions, similar to that of verapamil, thus indicating that bronchodilator effect was mediated possibly through Ca²⁺-antagonism (Chad and Eckert, 1984).

Moreover, *C. rotundus* also relaxed phenylephrine (1 µM)- and K⁺(80 mM)-induced contractions; elaborating calcium channel blockade (Gillani *et al*., 1994), as K⁺(80 mM)-induced contractions are mediated through activation of calcium channel as well as release of Ca²⁺ from endoplasmic reticulum (Janbaz *et al*., 2012). Whereas phenylephrine (1µM)-induced contractions is mediated through the activation of α-receptors and subsequent Ca²⁺ influx. As *C. rotundus* relaxed the both phenylephrine (1µM)- and K⁺(80mM)-induced contractions, indicating the calcium channel blockade.

The presence of flavonoids, saponins, and tannins (Kai *et al*., 1998; Zhu *et al*., 2005) support the calcium channel blockade effect of *C. rotundus,* which might be responsible for its traditional use in diarrhea, asthma and hypertension, while additional mechanism (s) cannot be ruled out.

Bacterial infections may result in fever, chill, headache, nausea, vomiting, and organ failures that may lead to physical disabilities, health problems, and mortalities. Almost, all known bacteria have developed resistance to antibiotics, whereas, antibiotics are associated with serious unwanted effects such as antibiotic-associated diarrhea, hypersensitivity, depletion of the gut normal flora, allergic response and immune-suppression (Al-Jabri, 2005). *C. rotundus* exhibited significant response by inhibiting the growth of bacteria on most regulatory levels such as DNA, RNA, peptidoglycan, and protein synthesis that may be due to the presence of secondary metabolites such as tannin, alkaloids, and flavonoids that are responsible for antibacterial activity (Trease and Evans, 1983).

Emesis is caused by the activation of vomiting centre located in the medulla oblongata, either activation of the motor pathway or the following input from four principal areas such as chemoreceptor trigger zone (CRTZ), GIT, cortex (and thalamus), cerebral and vestibular region. The CRTZ is in the proximity to the medulla and it is not surrounded by blood brain barrier (Becker, 2010). So, it can be hypothesized that the antiemetic effect of the *C. rotundus* can be likely mediated through inhibition of chemoreceptor trigger zone.

Pyrexia is caused by the approach of prostaglandins to the thermo-regulator hypothalamic neurons, which stimulates the set point and result in the elevation of body temperature through the retention and/or active generation of heat (Loux *et al*., 1972). *C. rotundus* exhibited a significant antipyretic effect (febrifuge) as it reduces the elevated body temperature of the experimental animals, that may be due to presence of flavonoids and alkaloids which are known inhibitors of prostaglandins through inhibition of cyclo-oxygenase (Hajare *et al*., 2000; Rajnarayana *et al*., 2001; Ray *et al*., 2006).

Pain is generated by the interaction of prostaglandins with nociceptor (Geusens *et al*., 2013; Kanda *et al*., 2013), whereas, prostaglandins are biosynthesized by the action of the cyclooxygenase 1 & 2 on ω-3 and ω-6 polyunsaturated C-20 fatty acids (Lone and Taskén, 2013). *C. rotundus* exhibited a significant analgesic response in mice by tail flick method that may be mediated through the inhibition cyclo-oxygenase resulting...
in unavailability of prostaglandins for action on nociceptors.

An oral dose of *C. rotundus* did not produce the lethality among the treated groups of animals up to 5 g/kg, which is higher than the therapeutic dose. However, chronic and sub-acute tests are to require to justify the safety of *C. rotundus*.

**CONCLUSION**

It can be inferred that relaxant effect showed by the *C. rotundus* on isolated rabbit jejunum, trachea and aorta preparations may be attributed due to blockade of calcium channels, which provide sound mechanistic background to validate the folkloric uses of *C. rotundus* in the management of gastrointestinal, respiratory and vascular disorders, though additional mechanism(s) cannot be ruled out. The observed results also validate its uses in bacterial infection, emesis, pain and pyrexia.

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