INVESTIGATION AND COMPARISON OF ANTI-INFLAMMATORY ACTIVITIES OF DIFFERENT EXTRACTS OF *CYMBOPOGON CITRATUS* USING VARIOUS *IN VIVO* MODELS

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Abstract

    Cymbopogon citratus has been commonly used by practitioners of traditional medicine in inflammatory diseases. The investigation and comparative anti-inflammatory activities were performed with different extracts of *C. citratus* i.e. methanol, chloroform, and n-hexane. The anti-inflammatory effects were determined using carrageenan, serotonin, and histamine. Further *In vivo* models like, xylene-induced ear edema, dextran-induced paw edema, and castor oil-induced diarrhea were also used for the confirmation of anti-inflammatory potential associated mechanism. Phytochemical constituents were determined through GC-MS analysis. Results showed inhibition of carrageenan-induced paw edema with all three extracts. Pre-treatment with plant extracts significantly inhibited paw edema induced by histamine and serotonin which further validated the anti-inflammatory effects. In addition, inflammatory edema induced by dextran and xylene were also found attenuated in experimental groups. We also found significant reduction in castor oil-induced diarrhea in extract treated groups indicating towards the possible inhibition of prostaglandins. Inhibition of these experimental models endorsed the suppression of autacoids as one of the mechanisms for the determination of anti-inflammatory effect. Extracts were also investigated for their potential toxicity, and established a safe dose upto 750 mg/kg body weight. Mono (2-ethylhexyl) phthalate, caryophyllene, and 1,30-triacontanediol were identified in the highest amount through GC-MS analysis. In conclusion, this study showed that *C. citratus* possessed marked anti-inflammatory activity which might be ascribed to the suppression of autacoids.

Key words: Inflammation; Autacoids; Histamine; Lemongrass; Paw distension.

Introduction

    The self-protection replies of body appearing in the form of inflammation helps the body to clear harmful agents and to begin healing process. Different mediators play a significant role in this connection such as; bradykinin, histamine, leukotrienes, prostaglandin and serotonin (Akhtar & Shabbir, 2019; Mobashar *et al.*, 2019). The inflammatory process, if goes uncontrolled, can cause various serious diseases like, rheumatoid arthritis, atherosclerosis, and inflammatory bowel disease etc. (Shabbir *et al.*, 2018).

    The treatment of various chronic and inflammatory diseases is still one of the world's major serious issues. Various serious adverse effects are associated with the currently available anti-inflammatory therapy (Uroos *et al.*, 2017). The herbal medicines are considered to possess minor toxicities and side effects in comparison with allopathic remedies. Most of the ailments of human being and animals have been cured with natural medications obtained from plants, algae, and animal kingdoms. The Ayurvedic medicines remained an important part of remedies worldwide, and it is believed that identification and isolation of new chemical compounds from plants sources provide a foundation for the novel synthesis of medicaments (Dias *et al.*, 2012). People are relying more on natural products globally with the hope of safety and security from herbal products. (Li *et al.*, 2003; Agbaje & Fageyinbo, 2012).

    Cymbopogon citratus (lemongrass) belongs to the family Poaceae. *C. citratus* is widely cultivated in Asia and America, and is native to South India and Sri Lanka (Ojo *et al.*, 2006). This tropical grass grows up-to 6 feet height with short rhizomes. In general, plants are 0.5 to 1 inch wide and about 3 feet long with nice drooping tips, bluish-green color with a pleasant aroma flavor after crushing (Ravinder *et al.*, 2010). The plant has been commonly used in inflammatory disorders by practitioners of traditional medicine (Shah *et al.*, 2011). Previously published review on *C. citratus* mentioned that the plant possessed anti-inflammatory, anti-fungal, anti-oxidant, anti-hepatotoxic, anti-nociceptive, anti-microbial, anti-malarial, anti-diarrheal, and anti-mutagenic properties (Manvitha & Bidya, 2014).

    The focus of current study is on the comparative anti-inflammatory response of various extracts of *C. citratus* using *In vivo* inflammatory models.

Materials and Techniques

Collection of plant and protocol of extraction: The collection of aerial parts of *Cymbopogon citratus* was done from Airport Nursery Sambrial, District Sialkot, Punjab, Pakistan. The herbarium specimen of the plant was deposited in Dr. Sultan Ahmed Herbarium, Department of Botany, Government College University, Lahore, Pakistan and voucher specimen number (GC-Herb-Bot-2407) was obtained.

    C. citratus was dried under shade and ground to powder. Dried powder (1000 g for 7 days) was macerated with 5 L each of methanol, chloroform, and n-hexane, respectively. Bottles containing mixtures were gently shaken after every 12 h during the maceration process. The whole material was initially filtered through muslin cloth

for the removal of course particles and then through whatmann filter paper grade 1. The resulting filtrate was concentrated at 37ºC using rotary evaporator (IKA HB10 Basic, Made in Germany), which produced dark brown extract having semi solid consistency. The percentage yield of each extract was calculated as; 1.52% for n-hexane extract, 3.2% for chloroform extract, and 8.4% for methanol extract. The extracts were stored at 4ºC for further use and doses were freshly prepared when required.

Animals: Healthy either gender Sprague-Dawley rats having weight range 200-250 g were taken from animal house of the University of Lahore, Pakistan. All animals were kept at appropriate temperature (22 ± 2°C) and humidity (55–65%) conditions, for a continuous 12 h rotations of light and dark environment. Rats had free access to water *ad libitum* and were fed with pallet diet on specified times. The approval of current *In vivo* experimental protocol (IAEC-2016-17) was taken from Institutional Animal Ethics Committee, University of Lahore (Shabbir *et al.*, 2016).

Assessment of anti-inflammatory activity of *Cymbopogon citratus*

Rat paw edema induced by Carrageenan: This model comprised five groups of rats and each group had 6 animals. Vehicle (3 ml/kg) and indomethacin (10 mg/kg) were given to control and reference groups, respectively. Experimental groups were orally given 100 mg/kg solution of methanol, chloroform, and extracts (in 10% dimethyl sulfoxide) of *C. citratus*. One hour later, 0.1ml (1% w/v in normal saline) carrageenan suspension was injected subcutaneously into the right hind paw of every rat from all groups for the induction of edema. Paw edema was measured with digital Vernier caliper; firstly, at zero and then after 30 minutes of carrageenan administration. Then the readings were taken hourly for four hours. Rise in paw edema was measured by deducting the initial values from the subsequent figures (Sowemimo *et al.*, 2013).

Paw edema induced with histamine: Rats were grouped into five having six-animal each. Three experimental groups were given (100 mg/kg) methanol, chloroform, and n-hexane extracts orally. Reference group was administered with indomethacin (10 mg/kg) orally. One group was assigned as control group which received only 3 ml vehicle (10% DMSO). After 30 minutes, all animals were injected 0.1ml (1%) histamine at sub-planter region (Amann *et al.*, 1995). Paw volume was determined with digital Vernier caliper at zero minute and subsequently after every hour for 4 hours.

Serotonin-induced paw edema: This model had 30 healthy rats divided in 05 groups having 06 animals each as in carrageenan model. 0.1ml serotonin was administered (1% normal saline) for induction of edema. Similar procedure as described in carrageenan protocol was followed for dose administration and treatment of all rats. Determination of paw edema was conducted hourly starting at 0 time till 3 hours using digital water displacement plethysmometer (Panlab, Harvard Apparatus).

Paw-edema model induced with Dextran: To further investigate the anti-inflammatory activity, rats were divided into groups in similar fashion as in previous models. Induction of edema was also carried out similar to other paw edema models. 0.1ml 1% dextran was injected to induce paw edema in all animals. 30 minutes after dextran injection, inflammation was measured with digital water displacement plethysmometer at 0, 1, 2, 3, and 4 h interval.

Ear-edema model induced with xylene: Division of rats in groups was done in same fashion as previously described. Experimental groups were treated with extracts (n-hexane, chloroform and methanol) in an oral dose of 100 mg/kg. Vehicle (10% DMSO) was orally administered (3 ml/kg) to negative control group and dexamethasone injection (1 mg/kg) was administered (intra-peritoneally) to the reference group. After thirty minutes, a drop of xylene was spread properly on right ear of all animals while, left ear served as control. The rats were killed after 15 minutes, removed both ears, and weighed immediately. Weight differences were measured between both ears of each animal (Núñez Guillén *et al.*, 1997).

Castor oil-induced diarrhea: Twelve hours fasting rats were grouped into five having 06 rats per group. A clean white sheet was spread down the cages for feces collection. Experimental groups were given 100 mg/kg body weight of *C. citratus* extracts (n-hexane, chloroform and methanol). Reference group was treated orally with 10 mg/kg loperamide. Rats of negative control group were give vehicle only. After about 30 min, all groups were fed castor oil (10 ml/kg b.w.) to induce diarrhea (Aniagu *et al.*, 2005; Suleiman *et al.*, 2008). Treated animals were observed for four hours after castor oil administration for diarrheal characteristics.

Acute toxicity study: Overnight fasting 45 rats were equally divided into 09 groups with 05 animals each. First 03 groups were given 250 mg/kg of each extract (n-hexane, chloroform and methanol). Next three received 500 mg/kg dose, and last 03 groups received 750 mg/kg of each extract, respectively. Symptoms of morbidity and mortality were observed after 4 hr, 24 hr, and 07 days of dosing.

Qualitative phytochemical analysis: Qualitative phytochemical analyses were performed using different previously published methods (Balandrin & Klocke, 1988; El-Olemy *et al.*, 1994; Ojo & Anibijuwon, 2010; Hindumathy, 2011; Perveen *et al.*, 2020).

GC-MS analysis: GC-MS analysis was conducted according to the protocol mentioned in our previous publication with slight modification in oven temperature (Uroos *et al.*, 2017). Initially temperature was set for 2 min at 110ºC and then raised to 200ºC at a rate of 10ºC per minute rise in temperature. Finally, temperature was kept at 280ºC.

Results

Treatment of carrageenan-induced paw edema with *C. citratus*: At 1st hr: The significant ($p<0.001$) fall of paw edema was determined in methanol extract (0.9827 ± 0.0063), chloroform extract (1.011 ± 0.0267), n-hexane (0.1758 ± 0.1429) extract, and indomethacin (1.002 ± 0.0105) groups against control (1.688 ± 0.2072) group (Fig. 1A).

At 2nd hr: The data presented substantial ($p<0.001$) declined of edema in rats pretreated with extracts like, ethanol (0.9702 ± 0.0089), chloroform (0.9212 ± 0.0339), and n-hexane (0.9443 ± 0.0332). Indomethacin also displayed (0.9992 ± 0.0112) inhibition on comparison with control (1.959 ± 0.2018) (Fig. 1B).

At 3rd hr: Paw edema was found inhibited ($p<0.001$) with methanol (0.9483 ± 0.0126), chloroform (0.8985 ± 0.0351), and n-hexane (0.9520 ± 0.0293) extracts as compared with control group (1.839 ± 0.1673). Reference drug also displayed ($p<0.001$) reduction (0.9883 ± 0.0071) of paw edema (Fig. 1C).

At 4th hr: We determined suppression ($p<0.001$) of edema in all test groups which were pre-treated with plant extracts i.e. methanol (0.9542 ± 0.0064), chloroform (0.9058 ± 0.0368), and n-hexane (0.3473 ± 0.2035) against control (1.840 ± 0.1697). Similar decrease in edema was also determined with indomethacin (0.9832 ± 0.0131) (Fig. 1D).

Higher inhibition of paw edema with n-hexane extract: n-Hexane extract exhibited higher suppression of paw-edema after 1st and 4th hrs of carrageenan injection comparative to other extracts (Fig. 1A, 1D).

Inhibition of histamine-induced paw edema with *C. citratus*: At 1st hr: Paw edema was found attenuated ($p<0.01$) in chloroform (0.4583 ± 0.0742), n-hexane (0.6083 ± 0.0661), and indomethacin (0.7467 ± 0.0174) administered groups in comparison to control (0.8867 ± 0.0372) group (Fig. 2A).

At 2nd hr: All treatment groups showed attenuation of paw edema i.e. methanolic extract (1.063 ± 0.0342), chloroform (0.4083 ± 0.08518), n-hexane (0.5300 ± 0.0275), and indomethacin (0.6200 ± 0.0403) against control (1.233 ± 0.0447) (Fig. 2B).

At 3rd hr: Statistical analysis showed an alleviation of edema with chloroform (0.3483 ± 0.0685), n-hexane (0.4400 ± 0.0118), methanol (1.027 ± 0.0298), and indomethacin (0.5033 ± 0.0168) when compared with control (1.490 ± 0.0326) (Fig. 2C).

At 4th hr: Groups of rats treated with chloroform (0.2617 ± 0.0578), methanol (0.4500 ± 0.04328), and n-hexane (0.4867 ± 0.0406) extracts, and indomethacin (0.4483 ± 0.0181) showed reduced paw edema against control (1.627 ± 0.0275) group (Fig 2D).

Chloroform extract showed higher inhibition: This comparative analysis showed that chloroform extract caused higher suppression of edema in comparison to other extracts (Fig. 2A-D).

Reduction of serotonin-induced paw edema with *C. citratus*: At 1st hr: Pretreated rats with extracts of n-hexane (0.1467 ± 0.0122), methanol (0.2400 ± 0.0189), and chloroform (0.2783 ± 0.0197) exhibited decline in paw edema (Fig. 3A).

At 2nd hr: The suppressive effect on paw edema was determined with methanol (0.3217 ± 0.0365), chloroform (0.2100 ± 0.0339), and n-hexane (0.2600 ± 0.04973), extracts against control (0.4850 ± 0.0401) (Fig. 3B).

At 3rd hr: Data displayed the drop of paw-edema with methanol (0.2100 ± 0.0324), chloroform (0.0150 ± 0.0034), and n-hexane (0.1533 ± 0.0105) extracts, and indomethacin (0.2433 ± 0.0381) against control (0.6017 ± 0.0667) group (Fig. 3C).

Chloroform extract displayed the highest suppression: Chloroform extract produced higher inhibition of paw edema to other treated groups at 3rd hr (Fig. 3A-C).

Inhibition of dextran-induced paw edema: All extracts significantly attenuated ($p<0.001$) paw edema during 1st to 4th hr of evaluation. Methanol extract showed superior inhibition to all other extracts (Fig. 4A-D).

C. citratus significantly suppressed xylene-induced ear edema

Evaluation after 30 minutes pretreated with extracts: Ear edema was found significantly attenuated with n-hexane (18.33 ± 4.128), chloroform (38.83 ± 5.918), and methanol (45.00 ± 1.065) extracts in comparison to control (67.00 ± 7.179) group. Similar results were found with dexamethasone treatment (3.667 ± 0.8819) (Fig. 5A). The highest attenuation was found with n-hexane extract.

Treatment with *C. citratus* significantly prevented castor oil-induced diarrhea

C. citratus significantly reduced total number of feces: The data showed significant reduction in total number of feces after pre-treatment with methanol (0.8333 ± 0.1667) extract, chloroform (2.500 ± 0.4282) extract, n-hexane (2.667 ± 0.3333; $p<0.05$) extract and loperamide (0.1667 ± 0.1667) in comparison to control (4.000 ± 0.2582) group (Fig. 5B).

C. citratus significantly attenuated total number of wet feces: The data showed reduction in wet diarrheal feces with methanol (0.3333 ± 0.2108), chloroform (1.500 ± 0.2236), and n-hexane (2.167 ± 0.1667) treated groups in comparison to control (3.667 ± 0.2108) group (Fig 5B).

Methanol extract caused superior inhibition of diarrhea: Methanol extract showed significantly higher reduction of number of feces and total number of wet feces in comparison to other treated groups.

Acute toxicity study: Acute toxicity was checked with all extracts i.e. methanol, n-hexane, and chloroform extracts at a dose of 750 mg/kg, and found safe for any morbidity and mortality issues.

Qualitative phytochemical examination: The phytochemical constituents identified by various qualitative analyses are specified in Table 1.

GC-MS investigation: The list of all identified compounds along with their structures, retention times, molecular formulas, molecular weights, and phytochemical class in methanol and chloroform extracts are mentioned in Table 2 and Table 3.
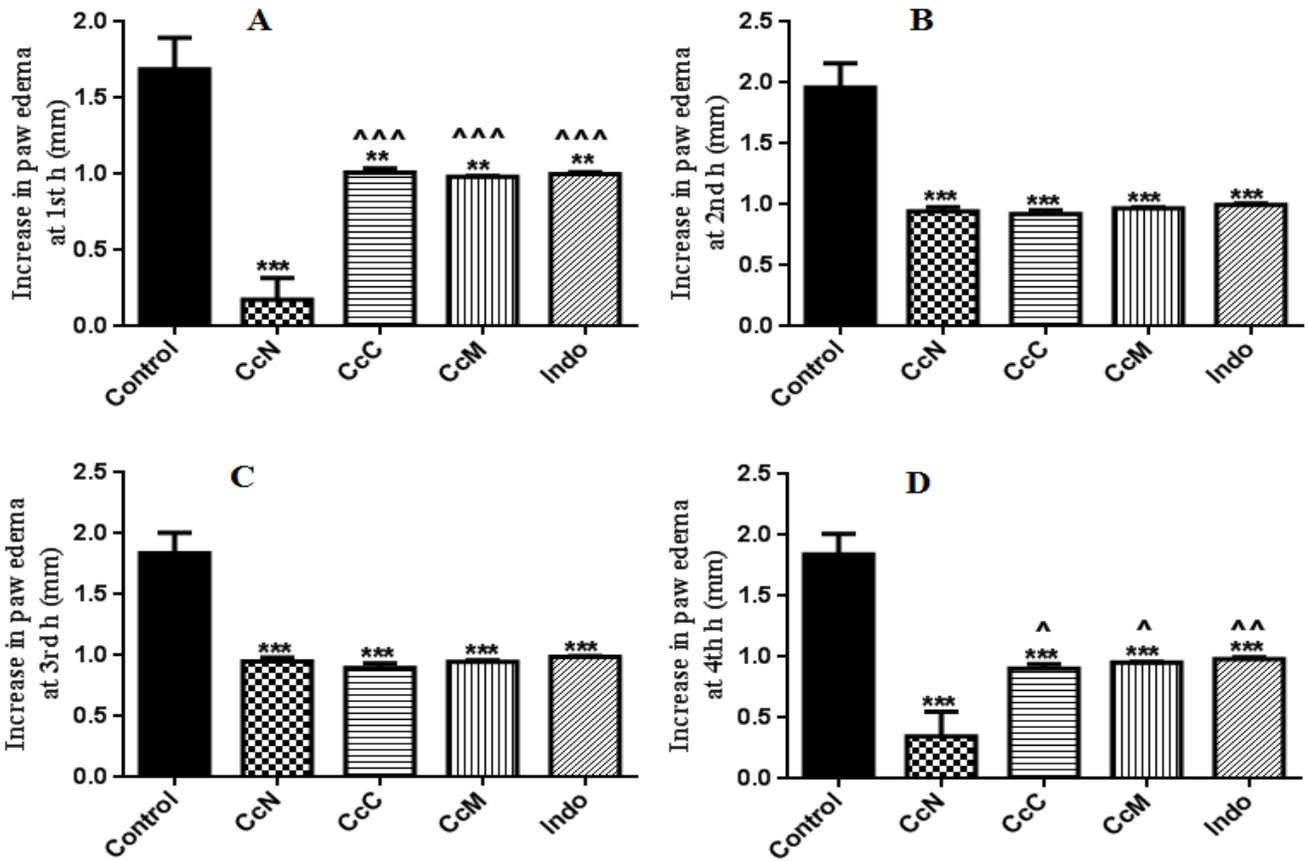
Fig. 1. Treatment with *C. citratus* significantly inhibited paw edema when observed at different hours (A-D) of carrageenan administration. N-hexane extract showed significantly higher inhibition to other treatment groups at 1st h and 4th h.
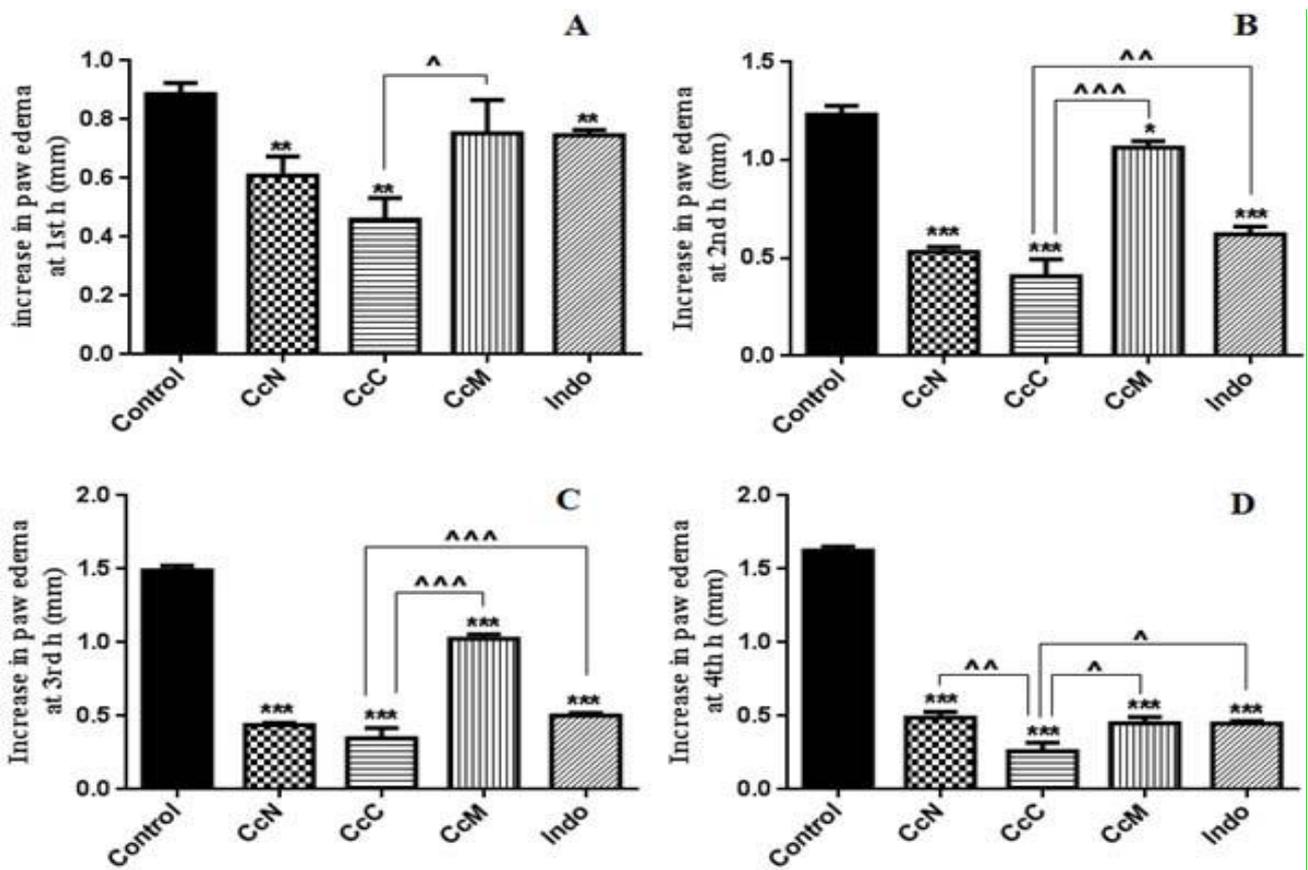

Fig. 2. Treatment with *C. citratus* significantly prevented the histamine induced paw edema when observed at different hours (A-D). The inhibitory effect of chloroform extract was found significantly higher as compared to other treated groups.
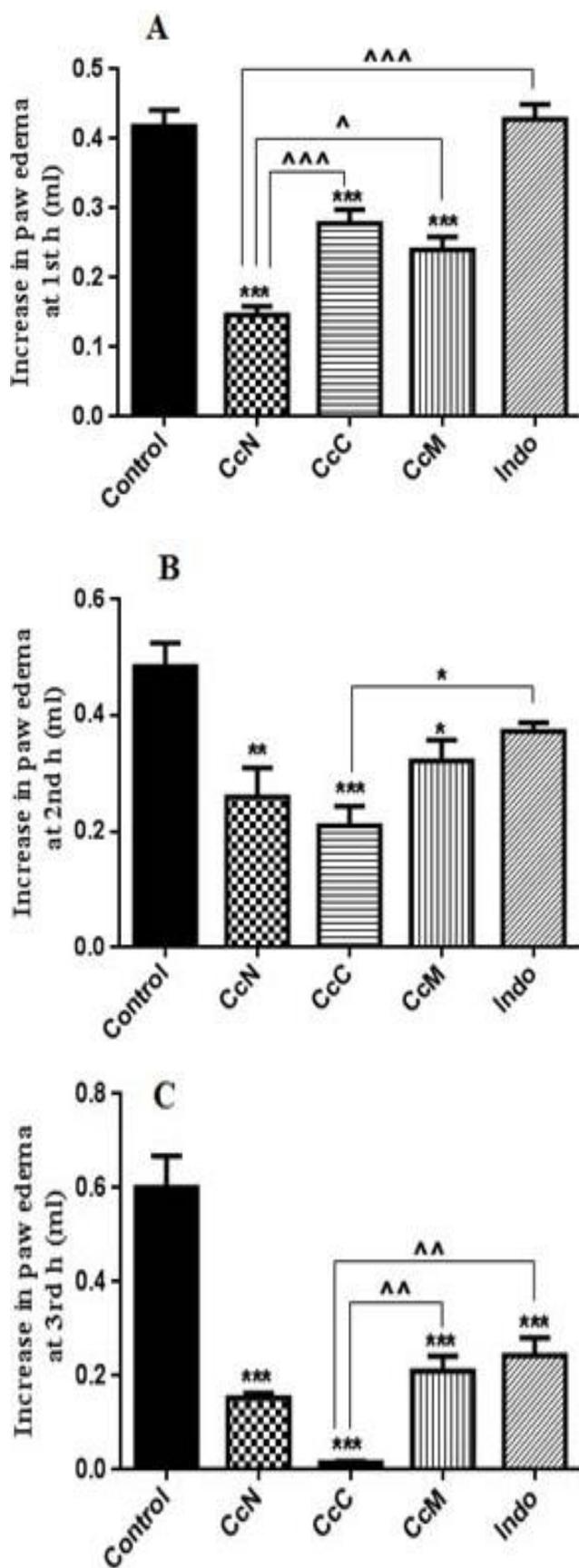
Fig. 3. Treatment with *C. citratus* significantly suppressed paw edema when observed at different hours (A-C) of serotonin administration. Initially, n-hexane extract showed the highest inhibition among all the treated groups. However, inhibition in the chloroform extract treated group was found highest at 2nd and 3rd h.
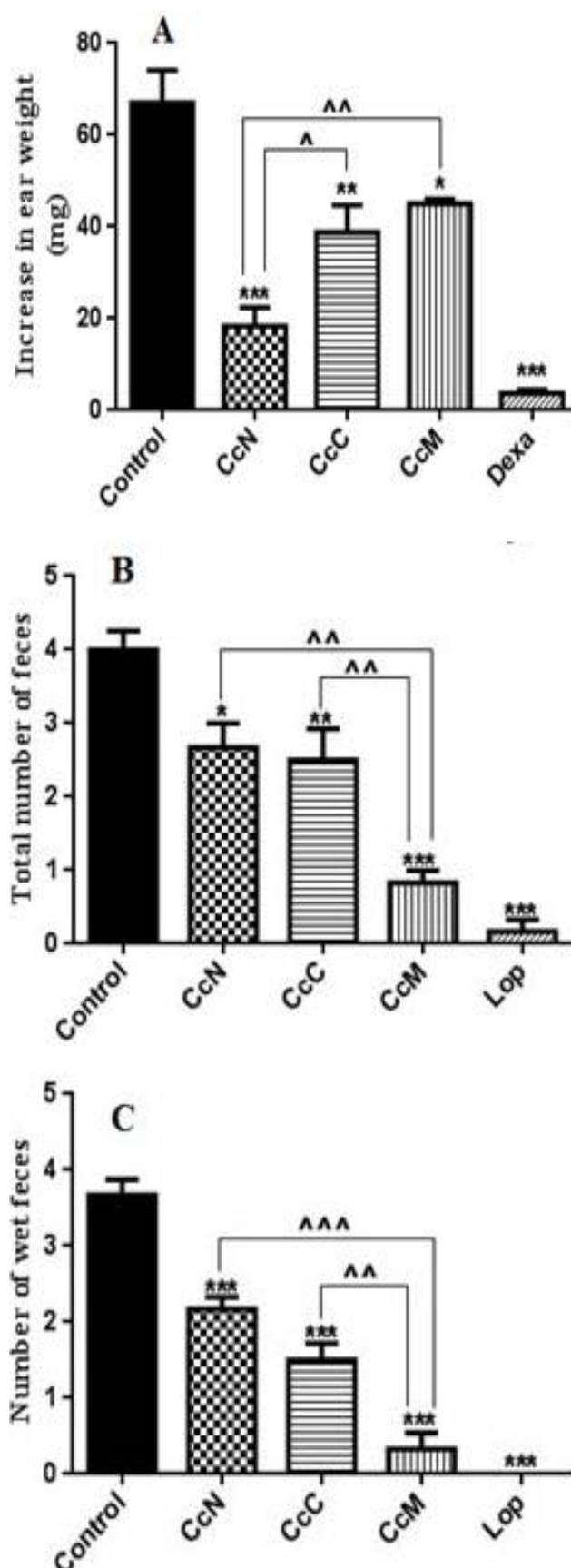
Fig. 5. n-Hexane extract produced greater suppression of ear edema as compared to other extract treated groups (A). *C. citratus* also significantly inhibited total number of feces (B) and number of wet feces (C). Methanolic extract produced significantly higher inhibition as compared to other extract treated groups.
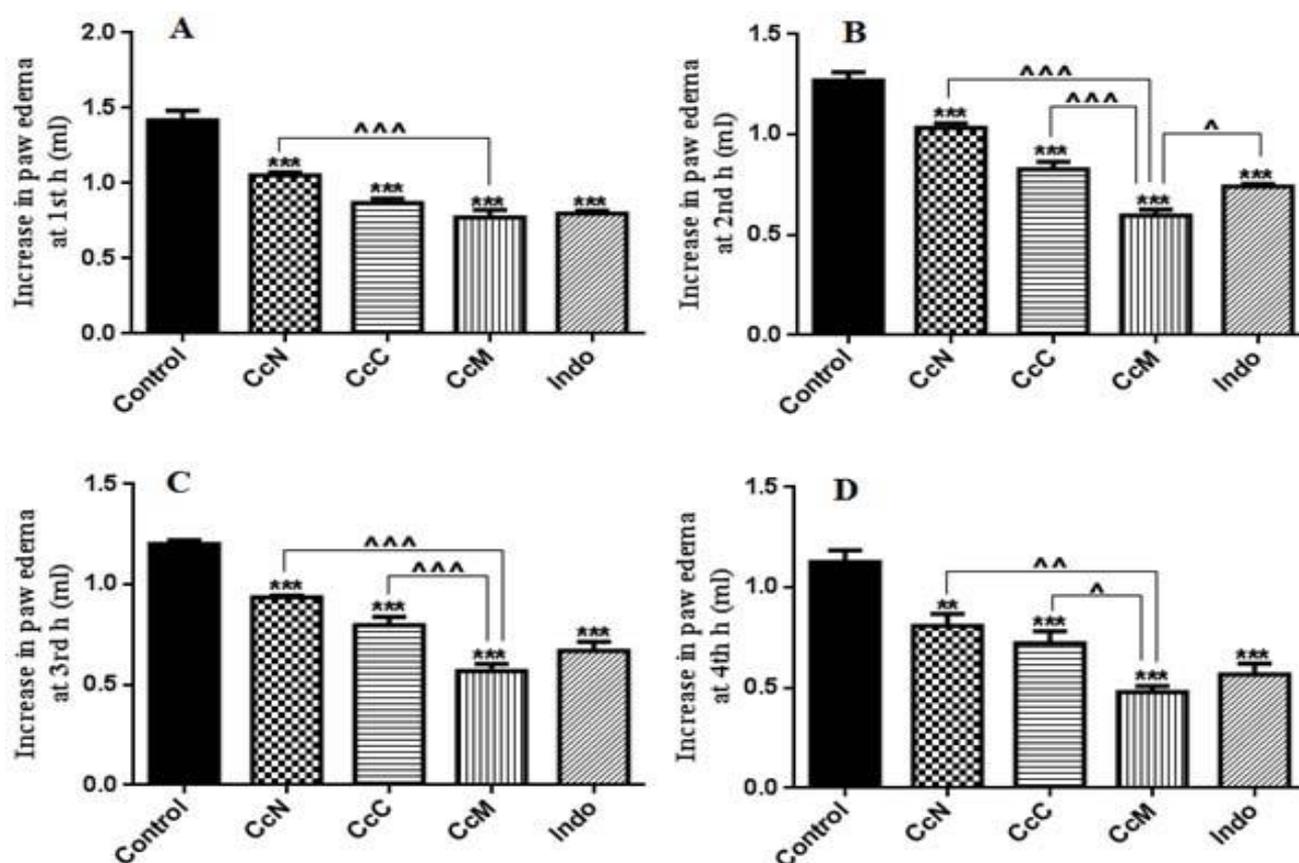
Fig. 4. Treatment with *C. citratus* significantly inhibited the paw edema when observed at different hours of dextran administration (A-D). Inhibition with methanolic extract was found superior to other extract treated groups.

Table 1. Qualitative phytochemical analysis of *C. citratus*.

Phytochemicals	Test name	Crude powder
Alkaloids	Dragendorff's test	+
Anthraquinones	Magnesium acetate test	+
Carbohydrates	Benedict's test	+
Flavonoids	Sodium hydroxide-test	+
Phenol related compounds	Ferricchloride test	+
Saponins	Froth test	+
Steroids	Acetic anhydride test	+
Tannins	Ferric chloride test	+

Discussion

Inflammation is a defensive response against injurious stimuli evoked in the body by different harmful agents such as; infections, antibodies, and physical factors. Various enzymes and mediators play active role in inflammatory process which connected the production of classical signs of inflammation such as pain, swelling, redness and loss of sensation (Sowemimo *et al.*, 2013). Herbal medicines are becoming increasingly popular for the treatment of inflammatory disorders and are being prescribed frequently (Igbe *et al.*, 2010). The current studies were focused on assessment of anti-inflammatory property of various extracts of *C. citratus* using different acute inflammatory models.

Lemongrass is an important plant in folk-medications for the management of various inflammatory disorders (Shah *et al.*, 2011). The anti-inflammatory activities of newly discovered compounds or plant extracts are commonly screened through the measurement of their potential suppressive effects on inflammatory edema induced using different irritant drugs (Agbaje & Fageyinbo, 2012). Previous studies also revealed the anti-inflammatory activities of essential oils of *C. citratus* in different experimental models of inflammation (Boukhatem *et al.*, 2014). The current was study focused on the investigation and comparison of anti-inflammatory effects of different extracts of *C. citratus* using various acute inflammatory animal models.

We used carrageenan to induce inflammatory edema in rat paws for the assessment of anti-inflammatory potential, which was the most commonly used *In vivo* model for the evaluation of newly screened anti-edematous agents (Igbe *et al.*, 2010). Various mediators like prostaglandins, leukotrienes, and bradykinin are released as a result of carrageenan administration. The edema could be controlled effectively by the inhibition of production and release of said-mediators (Asongalem *et al.*, 2004). The progress of edema by carrageenan was biphasic; the first stage occurred within initial 1-2 hours, caused by the release of serotonin and histamine from mast cells. The later stage was associated with the release of prostaglandins and leukotriens. All three fractions of *C. citratus* significantly suppressed the edema in 1st phase, which might primarily be credited to the drop of release and synthesis of serotonin and histamine. Therefore, serotonin- and histamine-induced edema models were developed for further confirmation of suppression of inflammatory response with plant extracts during 1st phase (Gupta *et al.*, 2005; Ravi *et al.*, 2009; Unnisa & Parven, 2011; Sowemimo *et al.*, 2013).

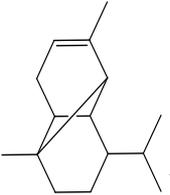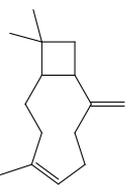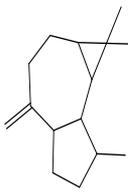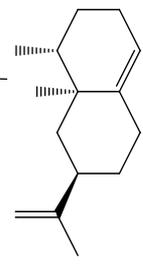**Table 2. Constituents identified using GC-MS analysis in methanol extract.**

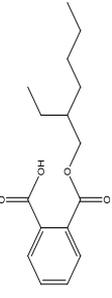| Retention time (minutes)	Total%	Name	Formula	Molecular weight g/mol	Phytochemical class	Structures
11.120	6.001	"Copaene" 1,3-dimethyl-8-(1-methylethyl)-tricyclo[4.4.0.02,7]dec-3-ene)	$C_{15}H_{24}$	204	Sesquiterpenoid	
11.826	18.623	Caryophyllene	$C_{15}H_{24}$	204	Bicyclic Sesquiterpene	
12.131	7.898	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	$C_{15}H_{24}$	204	Triterpene	
13.014	5.134	Naphthalene, ,2,3,4,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,[1R-(1.alpha.,7.beta.,8a.alpha.)]-	$C_{15}H_{24}$	204	Carotenoid	
3.978	6.056	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	$C_{15}H_{26}O$	222	Homoterpene	
18.757	5.952	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Fatty acids	
21.167	5.580	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	Fatty acids	
26.978	6.351	13-Docosenoic acid, methyl ester	$C_{23}H_{44}O_2$	352	Fatty acids	
27.657	20.894	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278	Phthalic acid	
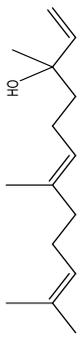
Table 3. Constituents identified using GC-MS analysis in chloroform extract.

Retention time (minutes)	% of total	Name	Formula	MWg/mol	Chemical class	Structures
9.782	1.984	Epoxy-linctialooloxide	$C_{10}H_{18}O_3$	186	Monoterpenes	
11.656	1.968	6-Nonenal, 3,7-dimethyl-	$C_{11}H_{20}O$	168	Medium chain aldehyde	
13.292	5.718	Phenol 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	Phenol	
27.732	40.617	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	Benzoic acid esters	
30.019	4.656	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	Benzoic acid esters	
32.585	10.412	1-Pentatriacontanol	$C_{35}H_{72}O$	508	Cyanolipid	
33.115	4.925	17-Pentatriacontene	$C_{35}H_{70}$	490	Lipid	
34.384	17.409	1,30-Triacontanediol	$C_{30}H_{62}O_2$	454	Fatty alcohol	
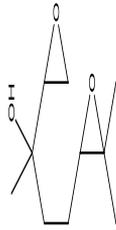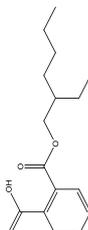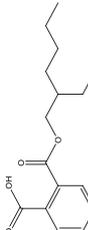
Experimental data revealed that *C. citratus* significantly prevented the edematous condition caused by histamine. When come in contact to noxious substances, mast cells degranulate and preformed histamine is released causing vasodilation and increase in capillary permeability. The main contribution of all these events is the development of Inflammatory signs (Benly, 2015). Serotonin is another important mediator which endorses the vasodilation and increases capillary permeability during the progression of acute inflammatory symptoms. In addition, it causes smooth muscles contraction (gut), coagulation and vasoconstriction (Linardi *et al.*, 2000; Cuman *et al.*, 2001). We found attenuation of serotonin-induced paw edema by *C. citratus* when comparison of data was done with control group.

Furthermore, another important model i.e. dextran-induced edema was also employed. The plant significantly suppressed the inflammatory paw edema which confirmed the suggested mechanisms behind anti-edematous effect by previous experiments i.e. interference in the synthesis and/or release of histamine and serotonin. It has been previously reported that dextran causes the discharge of serotonin and histamine from the mast cells which leads to induction of paw edema (Igbe *et al.*, 2010).

The anti-inflammatory potential of *C. citratus* was also determined using a different inflammatory model than paw edema i.e., ear-edema induced with xylene. This model is moderately concerned with the secretion of substance-P from sensory-neurons causing elevation in the permeability of blood vessels and promotion of plasma diapedesis. Subsequently, development of neurogenic-inflammation leads to edema (Agbaje & Fageyinbo, 2012). Other strong inflammatory mediators, such as histamine, kinins and fibrinolysins are also partially involved in xylene-induced edema (Guo *et al.*, 2011). Neurogenic inflammation is inter-connected with both of these biochemical reactions, as substance-P stimulates the mast cells to release histamine and simultaneously histamine discharges substance-P (Rosa & Fantozzi, 2013). Current study showed significant reduction in ear edema which might be attributed to alleviation in neurogenic inflammation. These results are consistent with the suppression of histamine-induced paw edema by the plant extracts. It is possible that inhibition of neurogenic inflammation might be partially ascribed to the suppression of histamine synthesis/release along with inhibition of substance P.

The mechanism of anti-inflammatory outcome during phase 02 was also investigated by the effect of plant extracts on prostaglandins synthesis, was further confirmed using castor oil-induced diarrheal model. The initiation of evacuation of watery stools in this model is based on the activation of prostaglandin receptors that increase biosynthesis of prostaglandins (Tunaru *et al.*, 2012). Previously, suppression of castor oil-induced diarrhea by plant extracts has been associated with the prostaglandin suppression by different studies (Agbaje & Fageyinbo, 2012). The data showed that *C. citratus* expressively attenuated the diarrhea compared to the control group. From these results it was concluded that *C. citratus* effectively inhibited the prostaglandin response and further strengthened this concept that inhibition of carrageenan-induced paw edema during 2nd phase was associated with prostaglandins which largely involved in the maintenance of edema during 2nd phase.

The current GC-MS analyses of *C. citratus* showed the existence of 15 constituents in methanol extract and 19 compounds in chloroform extract. Mono (2-ethylhexyl) phthalate was found in the highest concentration in both methanol and chloroform fractions. While, caryophyllene and 1,30-triacontanediol were detected in the second highest concentration in methanol and chloroform extracts, respectively. Mono (2-ethylhexyl) phthalate is known to exhibit both pro- and anti-inflammatory properties in different models. Its anti-inflammatory property is attributed to the activation of peroxisome proliferator activated receptor-α (PPARα). It has been documented that activation of PPARα causes the inhibition of NF-κB signaling which resultantly leads to the inhibition of NF-κB-mediated TNF-α production (Rakkestad *et al.*, 2010). Similarly, previous studies have shown that caryophyllene significantly suppressed rheumatoid arthritis in a model of chronic inflammation (Vijayalaxmi *et al.*, 2015). Medicinal plants containing 1,30 triacontanediol were documented to assist the development of anti-inflammatory and anti-oxidant potential (Huang *et al.*, 2011; Abarca-Vargas *et al.*, 2016). Previously, lemongrass was found to be a chief source of essential oils (Velluti *et al.*, 2003) which were considered to possess marked anti-oxidant and anti-inflammatory characteristics (Miguel, 2010). The large concentration of Mono (2-ethylhexyl) phthalate, caryophyllene and 1,30 triacontanediol found in plant extracts might be responsible for the anti-inflammatory properties that are observed in our current studies; however, future studies are required for isolation and confirmation of particular configuration associated with this property.

Conclusion

In conclusion, the *C. citratus* leaves significantly suppressed the edema in carrageenan model. The anti-edematous effect of lemongrass might be accredited to the suppression of autacoids as shown by suppression of edema in serotonin and histamine models. The inhibition of castor oil-induced diarrhea indicated towards the inhibition of prostaglandins which also validated the inhibition of autacoids as a possible anti-inflammatory mechanism. The further support regarding the anti-inflammatory potential of *C. citratus* was grown by the attenuation of ear edema through xylene-induced model. The anti-inflammatory potential might be ascribed to bioactive compounds such as, mono (2-ethylhexyl) phthalate and caryophyllene etc. Further studies are essential for isolation and documentation of active constituents, and evaluation of further anti-inflammatory and immunomodulatory mechanisms.

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