Analgesic, anti-inflammatory and diuretic activities of *Cicer arietinum* L.

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Abstract: Analgesic, anti-inflammatory and diuretic activities of the methanol extract of two varieties of *Cicer arietinum viz* black or *Desi* and white or *Kabuli* were tested in the doses of 200 and 400 mg/kg. For analgesic effect of the extracts, acetic acid induced writhing, tail immersion and hot plate tests were employed in mice. The anti-inflammatory activity was carried out by carrageenan induced inflammation in rats, whereas the diuretic action was determined using metabolic cages for rats. Animals were divided into six groups (n=7): (1) Control (2) Standard (3) MECAB 200 (4) MECAB 400 (5) MECAW 200 (6) MECAW 400. All extracts and standard drugs were administered orally. Acute oral toxicity of the extracts was also checked in mice up to 2000mg/kg dose, which showed a favorable safety. Significant analgesic and anti-inflammatory effects were observed. The results of diuretic activity were significant at 12^{th} and 24^{th} hrs. Therefore, it is concluded that the methanol extracts of the seeds of *Cicer arietinum* have analgesic, anti-inflammatory and diuretic potential.

Keywords: *Cicer arietinum*, analgesic, anti-inflammatory, diuretic, methanol extract.

INTRODUCTION

Cicer arietinum L. belongs to family Fabaceae and is commonly known as Chickpea in English and *Channa* in Urdu. Based on color and size two cultivated varieties of chickpea are recognized as Microsperma and Macrosperma chickpea which are locally known as *Desi* and *Kabuli Channa* respectively (Al-Snafi, 2016). Thick coated and colored chickpea seeds are called *Desi* type found in several shades and combinations of green, yellow, brown and black. These are small in size and angular with a rough surface. The *Desi* types are account for 80-85% of chickpea cultivation area. Chickpea seeds which are white or beige-colored with thin seed coat are called *Kabuli* type. These are characterized by ram's head shape, and smooth surface. The *Kabuli* type has lower level of fiber and higher level of sucrose. As compared to *Desi* type, *Kabuli* variety has large sized seeds and get higher market value (Jukanti *et al.*, 2012).

Cicer arietinum is grown in temperate, sub-tropical and tropical areas of the world. The specie is originated in the northern Persia and southern Caucasus, southeastern Syria and Turkey (Al-Snafi, 2016). Chickpea is a legume of economical importance and is considered as the 5^{th} valuable legume in the world (Aurelia *et al.*, 2009) and third in global production (Subba *et al.*, 2013). White chickpea is mainly cultivated in the Mediterranean, America, and central Asia whereas black chickpea is cultivated mostly in East Africa and India (Sharma *et al.*, 2013).

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Chickpea is consumed commonly as food in various forms and preparation based on regional and ethnic factors. In subcontinent Indo-Pak, chickpea is made as *Daal* and *Besan* which are used to make different snacks. In India it is very often used as a crash diet and one of the most widely made recipes in Indian kitchen due to its good taste and nutritive values (Singh *et al.*, 2009). The green seeds are eaten as a vegetable (Zia-ul-Haq *et al.*, 2009). Parched seeds or *Batana* and parched seed flour or *Sattoo* are famous snack items. In Asian and African countries, it is used in salads, soups and also as boiled, roasted, salted, and fermented forms which provide many nutritional benefits (Jukanti *et al.*, 2012). The seeds are consumed in raw green and tender form, known as, Malana and also in mature dry seeds in Egypt. Its flour is used in food mixes, biscuits and bread (Alajaji and El-Adawy, 2006). Leaves of young chickpea are also eaten and cooked in the form of green vegetable in some areas of the world. Therefore, it is a valuable dietary source of nutrients, particularly in malnourished population (Kan *et al.*, 2010).

The plant has been reported to have anti microbial, anti oxidant (Al-Snafi, 2016) and anti tumour activity (Jukanti *et al.*, 2012). The roots are useful in diarrhea and fever (Al-Snafi, 2016). Leaves are sour, improve taste and appetite (Mohanty *et al.*, 2012). The leaves are used in constipation, dyspepsia, and snakebite(Afsheen and Jahan, 2013), bronchitis, diabetes, sprains, dislocated limbs, diarrhea while leaf juice is used as stomachic and laxative (Yadav *et al.*, 2009). The ariel parts are reported as an antifungal agent (Al-Snafi, 2016). The seeds of *Cicer arietinum* are reported as diuretic, antifungal,

refrigerant, tonic, anthelmintic (Arora *et al.*, 2013), stimulant, aphrodisiac (Yadav *et al.*, 2009), appetizer (Zia-ul-Haq *et al.*, 2007) and purgative (Tiwari *et al.*, 2013). Seeds are also useful in skin diseases, bronchitis (Yadav *et al.*, 2009) , blood disorders, biliousness (Tiwari *et al.*, 2013), diabetes, hyperlipidemia, ear inflammation, disease of spleen and liver, headache, sore throat, cough, pulmonary, uterine, anal diseases (Bagri *et al.*, 2011), cholera, catarrh, constipation, dyspepsia, diarrhea, flatulence, sunstroke, snakebite and warts (Al-Snafi, 2016). Therefore, the present study was conducted to find the analgesic, anti-inflammatory and diuretic activity of *Cicer arietinum* to authenticate its traditional use.

MATERIALS AND METHODS

Plant material

The seeds of *Cicer arietinum* L. *Desi* and *Kabuli* variety (1kg each) were procured in January, 2016 from a departmental store in Karachi. The seeds were identified by a taxonomist with Voucher specimen No.CAB- 06-15/16 and CAW- 07-15/16.

Preparation of extract

The seeds of *Desi* and *Kabuli* variety were successively extracted by method of cold maceration to prepare methanol extracts. One kg seeds of each variety were grinded into fine powder and were soaked separately in methanol for one week. Then filtered with the help of filter paper and concentrated at 40^{o}C by rotary evaporator.

MECAB = Methanol extract of *Cicer arietinum* black variety.

MECAW = Methanol extract of *Cicer arietinum* white variety.

MATERIALS AND METHODS

Pharmacological studies

Acute toxicity assay

The acute toxicity was assayed in mice. Seven albino mice were selected randomly and were kept fasted for overnight only providing water. The methanol extracts were given orally in a single dose of 2000mg/kg b.w. and mice were kept under observation for the first 4 hours and then up to 24 hours periodically for any toxic sign, symptoms and mortality.

Analgesic activity

Acetic acid-induced abdominal constriction

The analgesic activity of MECAB and MECAW was carried out by acetic acid induced abdominal constriction method (Koster, 1959). The mice were grouped into six of seven animals per group. Normal saline in 10 ml/kg was given to group 1 whereas MECAB and MECAW (200 and 400 mg/kg orally) were given to groups 2, 3, 4 and 5 respectively; group 6 was given acetyl salicylic acid (100 mg/kg). After 30 min the writhes were produced by injecting 0.9% acetic acid and were counted for 20mins.

Tail immersion method

Before experiment the animals were screened by immersing the tip of tail in water at 55±0.5°C which was the initial reaction time(Gupta *et al.*, 2005). After the administration of MECAB and MECAW (200 and 400 mg/kg) the reaction time was taken at interval of 30, 60, 90, 120 and 180 min. Vehicle (normal saline) was given to control group and standard group received acetyl salicylic acid (100mg/kg).

Hot-plate method

The method was used as described earlier (Dar *et al.*, 2005). Prescreened mice were grouped into six (n=7). Group 1 was given normal saline (10ml/kg). MECAB and MECAW were given (200 and 400mg/kg) to groups 2, 3, 4 and 5 respectively; whereas group 6 was given acetyl salicylic acid 100mg/kg orally as standard. Mice were evaluated individually on a hot plate of metal which was maintained at 55±0.05°C. The effect was measured in the form of jumping, licking or withdrawal of the paws. The readings were noted at 0, 30, 60, 90, 120 and 180 min.

Anti-inflammatory activity

Carrageenan induced inflammation

The inflammation was produced by 0.1ml injection of 1 % carrageenan injection in the sub- plantar region of rat right hind paw(Lee *et al.*, 2009). Extracts MECAB and MECAW (200 and 400mg/kg) were given by oral route 1 h before carrageenan injection. Observation of each rat was noted at 0-5h, with plethysmometer. Diclofenac (50 mg/kg) was given as standard.

Acute diuretic activity

Acute diuretic activity was evaluated by the method reported in literature(Kau *et al.*, 1984). The rats were grouped into six (n=7) and remain fasted but with free access to water before experiment. Four groups of rats were administered orally MECAB and MECAW at 200 and 400mg/kg. One group was given orally 5ml/kg of furosemide as 20mg/kg. Control group was given saline (5ml/kg bw). Then animals were kept in metabolic cages immediately. Graduated cylinders were used to collect the urine and volume was recorded at 30, 60, 90 and 120 mins then at 12th and 24thhr after administration (Ribeiro *et al.*, 2015).

STATISTICAL ANALYSIS

Data was analyzed by one way ANOVA test along with Tukey HSD, SPSS version 20 used in the analysis. Values $p<0.05$ (significant) and $p<0.01$ (more significant) and $p<0.001$ (highly significant) were considered.

RESULTS

The acute toxicity assay showed that the *Cicer arietinum* extracts are non-toxic because no any toxic sign, reactions and mortality were found until the end in a dose of 2000

Table 1: Analgesic activity of the methanol extracts of *Cicer arietinum, Desi* and *Kabuli* variety by writhing test

Treatment	Dose (mg/kg)	Number of writhings	% Inhibition
Control (vehicle)	-	28.39 ± 3.67	-
MECAB	200	13.0 ± 0.57 ***	54.20
MECAB	400	10.66 ± 0.00 ***	62.45
MECAW	200	13.01 ± 0.57 ***	54.17
MECAW	400	10.33 ± 0.33 ***	63.61
Acetyl salicylic acid	100	9.01 ± 1.40 ***	68.26

Table 2: Analgesic activity of the methanol extracts of *Cicer arietinum, Desi* and *Kabuli* variety by tail immersion method

Treatment	Dose (mg/kg)	Latency period in sec						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control (vehicle)	-	0.78±0.02	0.79±0.02	0.79±0.00	0.79±0.01	0.80±0.00	0.78±0.00	0.76± 0.00
MECAB	200	0.82±0.78	1.29±0.11	2.09±0.28	2.36±0.32	2.36±0.38**	2.23±0.39**	1.67±0.15*
MECAB	400	1.05±0.06	1.5±0.10	2.3±0.22	2.81±0.96	2.97±0.51***	2.80±0.47**	1.93± 0.20**
MECAW	200	0.93±0.07	1.09±0.06	1.38±0.13	1.77±0.18	1.86±0.13	1.64±0.20	1.33± 0.22
MECAW	400	0.84±0.04	1.04±0.00	1.84±0.07	2.16± 0.34	2.04±0.10	2.16±0.34	1.75± 0.07**
Acetyl salicylic acid	100	0.83±0.03	1.37±0.11	3.27±0.31**	5.01±0.06***	4.93±0.25***	3.22±0.05***	2.01±0.06**

Table 3: Analgesic activity of the methanol extracts of *Cicer arietinum, Desi* and *Kabuli* variety by hot plate method

Treatment	Dose (mg/kg)	Latency period in sec						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control (vehicle)	-	7.33±0.33	7.66±0.33	7.33±0.33	6.66±0.66	7.32±0.33	6.66±0.33	6.66±0.33
MECAB	200	7.30±0.72	8.09±0.58	8.83±0.34	9.59±0.28	9.47±0.19	9.05±0.26*	8.29±0.36
MECAB	400	8.72±0.24	9.28±0.30	11.24±1.34	10.51±1.28	9.77±0.84	9.05±0.04*	9.1±0.11**
MECAW	200	7.57±0.31	8.07±0.34	8.49±0.31	8.8±0.45	8.8±0.34	8.69±0.37	8.23±0.46
MECAW	400	7.93±0.44	8.76±0.60	10.29±1.31	12.52±1.65**	12.97±0.89***	11.25±0.81***	9.41±0.40***
Acetyl salicylic acid	100	7.66±0.57	8.66±0.89	12.00±0.99*	13.33±0.89**	14.33±0.99***	11.00±0.82***	9.33±0.57***

N=7; Each value is presented as the Mean ± SEM

mg/kg. Therefore, both the extracts were safe and doses of 200 ($1/10^{th}$) and 400 ($1/5^{th}$) mg/kg b.w. were used for pharmacological studies(Shah and Alagawadi, 2011).Both methanol extracts of *Desi* and *Kabuli* variety of *Cicer arietinum* in writhing test exhibited highly significant ($p<0.001$) analgesic effect in both doses. The highest percentage inhibition (63.61%) was shown by MECAW-400 where as lowest by MECAW-200. Standard showed (68.26%) inhibition (table 1).In tail immersion method the most significant analgesic activity was observed in MECAB in the dose of 400 mg which was started at 120 min and remains significant till 180 min (table 2). The extract also showed significant effect for the same period at 200 mg/kg. *Kabuli* variety (MECAW) at 400 mg exhibited significant effect only at 180 min, where as no effect was observed at 200 mg/kg.

The methanol extracts of both verities showed significant analgesic effect in 400mg/kg when evaluated by hot plate method (table 3). The most significant effect was showed by MECAW-400 which started at 90 min and remains significant till 180 min whereas same extract did not show analgesic effect at 200mg.

Both extracts started significant anti-inflammatory effect at 60 min which remain highly significant ($p<0.001$) till 300 min in both doses (table 4).

The acute diuretic effect of both extracts of *Cicer arietinum* at 200 and 400 mg did not show any significant diuretic effect till 120 min but found highly significant ($p<0.001$) at 12^{th} and 24^{th}hrs which were comparable to standard furosemide 20 mg/kg (table 5).

DISCUSSION

Non steroidal anti-inflammatory drugs are used to relieve pain, fever and inflammation but these agents have some side effects including bronchospasm, gastric ulcer, cardiac abnormalities and renal damage. Therefore, researchers

Table 4: Anti-inflammatory activity of the methanol extracts of *Cicer arietinum, Desi* and *Kabuli* variety by rat paw edema

Treatment	Dose (mg/kg)	Paw edema volume in ml					
		0 min	60 min	120 min	180 min	240 min	300 min
Control (vehicle)	-	1.5±0.06	2.91±0.05	3.80±0.06	4.56±0.05	5.34±0.12	5.79±0.09
MECAB	200	1.85±0.05	2.14±0.16 **	2.51±0.20 ***	3.03±0.23 ***	3.52±0.12 ***	3.59± 0.11***
MECAB	400	2.09±0.14	2.39±0.15*	2.70± 0.05 ***	3.15±0.03 ***	3.60±0.05 ***	3.74± 0.02 ***
MECAW	200	1.89±0.06	2.20±0.09**	2.52± 0.06 ***	2.81±0.09 ***	3.12±0.10 ***	3.19± 0.10 ***
MECAW	400	1.92±0.26	2.17±0.19**	2.36± 0.23 ***	2.88±0.35 ***	3.15±0.39 ***	3.26± 0.38 ***
Diclofenac	50	1.6±0.02	1.8±0.02***	1.83±0.05 ***	1.75±0.04 ***	1.7±0.03 ***	1.7±0.02 ***

Table 5: Diuretic activity of the methanol extracts of *Cicer arietinum, Desi* and *Kabuli* variety in rats

Treatment	Dose (mg/kg)	Volume of urine in ml					
		30 min	60 min	90 min	120 min	12 hrs	24 hrs
Control (vehicle)	-	0.0± 0.0	0.04±0.004	0.2±0.005	0.4±0.01	0.6±0.006	1.1±0.03
MECAB	200	0.0	0.01±0.002	0.02±0.001	0.05±0.01	3.2±0.49 ***	5.46±0.14 ***
MECAB	400	0.0	0.01±0.001	0.03±0.001	0.06±0.01	2.73±0.21 ***	4.73±0.12 ***
MECAW	200	0.0	0.02±0.002	0.02±0.001	0.08±0.05	3.0±0.11 ***	5.53±0.35 ***
MECAW	400	0.0	0.01±0.001	0.02±0.002	0.33±0.31	2.83±0.20 ***	5.56±0.23 ***
Furosemide	20	0.57±0.01	0.87±0.01	0.9±0.008	1.0±0.03	2.1±0.05 **	4.1±0.06 ***

N=7; Each value is presented as the Mean ± SEM

are now focusing on plants and their products/compounds such as polyphenols, alkaloids, flavonoids, steroids, and terpenes as they have different pharmacological activities including analgesic, antipyretic and anti-inflammatory effects (Shah and Alagawadi, 2011).

In the present study, significant analgesic activity was observed by MECAB and MECAW. Acetic acid induced writhing test is commonly used for peripherally acting agents. In this test pain occurs by the liberation of endogenous substances like arachidonic acid and prostaglandin (Lu *et al.*, 2007). Intraperitoneal injection of acetic acid causes intense pain and acute inflammation in that area. The endogenous substances stimulate nerve endings and causes the indirect stimulation of peritoneal nociceptors (Yu *et al.*, 2012).Writhing is manifested by the abdominal muscles constriction, extension of the forelimbs and elongation of the body. MECAB and MECAW significantly decrease the abdominal constriction. So, it may be suggested that MECAB and MECAW contain some pharmacologically active compounds which block the endogenous substances and arachidonic acid metabolites which cause excitation of the pain nerve endings (Khan *et al.*, 2011).

The mice were protected by the extracts against thermally induced noxious stimuli as MECAB showed significant analgesic activity in tail immersion test which is used to evaluate central analgesic activity while MECAW was only significant in the last hour at 400mg. The activity shown by MECAB was less than standard Acetyl salicylic acid; however it was more than control group and therefore justifies its effect. Hot-plate test was also used to distinguish between peripheral and central effects. As the method is used for the centrally acting agents; therefore, the effect of any extract or compound in this test reveals centrally acting anti-nociceptive activity (Hassan *et al.*, 2015). The results revealed that MECAB and MECAW increased the latency period in the test suggested that it has also central analgesic activity.

Anti-inflammatory activity of MECAB and MECAW was investigated on rat paw edema method. Carrageenan is frequently used to evaluate anti-inflammatory agents and to confirm the anti-edematous action of natural products (Yu *et al.*, 2012). The edema formation is comprised of in two phases; In the first or early phase, within 60 mins after carrageenan injection, bradykinin, histamine and serotonin are released whereas in late or second phase (2-5h) with increased edema formation that remains up to the fifth hour there is a release of prostaglandins. Prostaglandin is released and cause increase in edema and remains for 5th hour. In the second phase there is an elevated production of prostaglandins, and also the induction of inducible cyclooxygenase (COX-2) in the hind paw (Hassan *et al.*, 2015). After one hour of orally given extracts, the rats were injected with carrageenan. The effects were significant (p<0.05, 0.01) by all the tested groups at 1st h. MECAB and MECAW both in 200 and 400 mg/kgdoses, showed inhibitory effects during 1-5 h. MECAB and MECAW showed significant decrease in paw edema volume. This effect of MECAB and MECAW may be due to the inhibition of histamine and/or prostaglandin. Histamine is an important inflammatory mediator and dilates vessels and also increases vascular

permeability. Therefore, the extracts may showed anti-inflammatory activity by the inhibition of synthesis or release or action of histamine, prostaglandin and serotonine which cause inflammation (Shukla *et al.*, 2010; (Shah and Alagawadi, 2011).

Some herbs stimulate the thirst center in the hypothalamus and therefore enhance fluid intake causing diuresis. Similarly, some plants and herbs induce diuresis due to their high salt content(Ribeiro *et al.*, 2015). MECAB and MECAW in both doses caused an increase in the urine output significantly from the 12th hour till the end of study at the 24th hours. The cumulative urinary volume after 24h of the treatment with MECAB and MECAW at 200 and 400 mg/kg were: 5.56±0.14, 4.73± 0.12, 5.53±0.35, and 5.56±0.23 ml respectively which are significantly higher ($p<0.001$) as then the control (1.1± 0.03 ml).

The MECAB and MECAW in 400 mg/kg were more effective than 200 mg/kg, with a 2.4 diuretic index. It was almost similar to the diuretic index (2.5) of Furosemide. Furosemide increases urine volume and excretion of Na+ and K+. Furosemide inhibited electrolyte reabsorption in the thick, ascending limp of the loop of Henle (Adam *et al.*, 2013). At the end of study the effect was found almost similar to Furosemide and therefore MECAB and MECAW may have similar action on the loop of Henle (Ribeiro *et al.*, 2015).

In diuresis urine volume is increased i.e. water excretion and also there is loss of solutes. MECAB and MECAW caused significantly increase in urine output starting from 12th h and remain for 24h. Although the onset of action was slow but there was a significant difference between the group treated with MECAB and MECAW in 200 and 400mg and the control group showing that MECAB and MECAW possesses a favorable diuretic activity.

The effect of the extracts may be due to the decrease in reabsorption of water and electrolytes. Reabsorption of sodium and water is regulated by both hormonal and non hormonal action, which involve renal-angiotensin-aldosterone system and prostaglandins (Chi *et al.*, 2008). The data presented in this study indicates that the compound(s) in the extracts causing diuretic effects due to increase the rate of urine output. These natural compounds may act synergistically or individually causing an initial vasodilatation. Therefore the extracts showed cumulative effect of several substances or due to secondary active metabolite(s)(Adam *et al.*, 2013).

CONCLUSION

The methanol extracts of *Desi* and *Kabuli* variety of *Cicer arietinum* were analyzed pharmacologically. The extracts exhibited analgesic effect in both chemical and thermal pain models and also significant anti-inflammatory and diuretic activities. Further investigations are required to understand the mechanisms of action of the extracts and isolation of chemical compounds responsible for these pharmacological activities.

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