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# Analgesic and anti-inflammatory potential of four varieties of bell pepper (*Capsicum annum* L.) in rodents

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**Abstract**: The aim of study was to evaluate the analgesic and anti-inflammatory activity of four different colored (green, yellow, orange and red) sweet bell peppers (*Capsicum annuum* L.) available in the local market of Karachi Pakistan. Their 95% ethanol extracts at 200 and 400 mg/kg were prepared and compared with commonly used analgesic (aspirin) and anti-inflammatory agents supporting its traditional use. The analgesic effects of 95% ethanol extracts of *Capsicum annum* L. were investigated by acetic acid induced writhing, tail immersion and hot plate test. The anti-inflammatory activities were observed using carrageenan-induced edema of hind paw in rats. Animals were divided into 10 groups (n=7): (1) Control (2) CAG 200 (3) CAG 400 (4) CAR 200 (5) CAR 400 (6) CAO 200 (7) CAO 400 (8) CAY 200 (9) CAY 400 and (10) Standard. All the extracts were given orally. Acute toxicity was also determined by increasing the dose till 3000 mg/kg, which showed no evidence of mortality. All extracts of *Capsicum* significantly increased the hot plate pain threshold, moreover remarkably reduced the carrageenan-induced rat paw edema. Results obtained were compared with corresponding control group revealed that the fresh fruits extract of all four kinds of bell pepper (200 mg/kg and 400mg/kg) possess anti-inflammatory and pain suppressing activities possibly mediated via PG synthesis inhibition.

**Keywords**: *Capsicum annum*, Capsaicin, di-hydrocapsaicin, Phenolic compounds, anti-nociceptive.

## INTRODUCTION

Epidemiologic evidences show that by increasing consumption of vegetables there are constructive effects on health. Plants contains multiple varieties of phytochemical constituents which have valuable therapeutic properties that can be utilized for the therapy of human ailments (Atolani *et al*., 2020, Akinpelu *et al*., 2008). Studies conducted *in-vivo* and *in vitro* with animals show role of food in maintenance of health and on the reducing risk of diseases (Bastos *et al*., 2009).Native from the America, sweet bell pepper is regarded as *Capsicum annuum* L. species belonging to the Solanaceae family which is widely consumed worldwide with increasing popularity. Another factor of attraction is it availability in an extensive multiplicity of colors (green, yellow, orange, red, and purple), shapes, sizes and its characteristic flavor (Castro *et al*., 2008). Notwithstanding, there are roughly 20 wild species that have been archived (Heiser Jr, 1973). The color of each *Capsicum* assortment within the full-ripe stage is dependent on its capacity for synthesizing carotenoids and for holding chlorophyll pigments (Collera-Zúñiga *et al*., 2005). Significance of *Capsicum* prevails since the 7500 BC (Nadeem *et al*., 2011). Non-sharp sweet chilies are named as 'Capsicums' which are local to Mexico. In American English, it is by and large known as the Chili Pepper or Bell Pepper. Whereas, Bell pepper is regarded as pepper in British English, although in Australian and Indian English, there is no utilized name and the name Capsicum is being usually utilized for peppers solely. *Capsicum* is locally regarded as *Shimla Mirch in Pakistan* (Grubben and Denton, 2004). Pepper is one of many crops that is sensitive to high temperature and grown in the mid-latitudes and said to have sensitivity to high temperatures. (Erickson and Markhart, 2002).

*Capsicum* is a vital agricultural crop due of its economic significance, as well as for its generally high antioxidant content, they are extraordinary source of ascorbic acid, natural colors and other antioxidant compounds (Ghasemnezhad *et al*., 2011). Carotenoids, phenolic compounds and other phytochemicals have been identified from many species of *Capsicum* (Asnin and Park, 2015). Some of the new metabolites have recently been reported from pepper such as tetrahydropentoxyline, acid colnelenic, blumenol C glucoside and gingerglycolipid (Guevara *et al*., 2021). Carotenoid pigments produced during ripening are responsible for producing yellow, orange, and red coloration of bell peppers. More than 30 different plant pigments have been isolated and identified in pepper until date. (Ghasemnezhad *et al*., 2011). Chemical analysis of fruits also indicates the presence cinnamic acid and flavonoids derivatives(Materska and Perucka, 2005). Peppers are a good source of vitamins E and vitamin A and one pod of

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chili can provide 5% of vitamin E whose RDA is around 8-10 mg and contributes to antioxidant activities (Do Rêgo *et al*., 2012, Krinsky, 2001). Since decades Capsicum pain-killing properties have been identified and as a result, they are utilized for compounding of different ointments (Perucka and Materska, 2001). Capsaicin is additionally the dynamic compound that is liable of therapeutic potential of *Capsicum*. It has been utilized as a pain relieving agent for joint inflammation and for analgesia (Sora *et al*., 2015). Chili is supposed to have higher antioxidative capacity than other vegetables like ginger and garlic(Shobana and Naidu, 2000, Mňahončáková *et al*., 2021) and has a beneficial effect on metabolic processes in the human organism and can decrease the risk of cardiovascular diseases (Sanati *et al*., 2018) Besides the use of bell pepper as culinary ingredient, it is also used in traditional system especially for treating symptoms such as stomach ache, diarrhea and dysentery (Tchiegang *et al*., 1999). Postpartum abdominal and back pain is found to be resolved by using of decoction of fruits (Coe, 2008). In Indian central Himalaya, Oil of *Capsicum annum* L. is used in dog bite (Samal and Dhyani, 2006). Fruits of *Capsicum* have evidence of use as anti-hypertensive (Pieroni *et al*., 2004). Smoke bath/steam bath of leaves are found to be effective against headache, epilepsy, blood vomit and breathing difficulties (Valadeau *et al*., 2010). Stem is used for dental and oral health care (Kayode and Omotoyinbo, 2008).

The current experimental study was conducted to determine the analgesic and anti-inflammatory potential of four varieties of bell pepper to authenticate their traditional use.

## MATERIALS AND METHODS

### *Plant materials*

Four varieties of fruit of *Capsicum annum* L. were procured in Karachi by local market. Multiple varieties were distinguished by a taxonomist and stored with voucher numbers *Capsicum annum* variety [Green bell pepper (CAG)CAGG/(G) 04-15, red bell pepper (CAR) CAGR/(R) 05-15, Orange bell pepper (CAO) CAGO/(O) 06-15, Canary belt (CAY) CAGC/(Y) 07-15. All four fruits 1 kg each were soaked in 98% EtOH for 3 days, Filtration of the EtOH extract was done to obtain concentrated crude extract, Percentage yield 5-7% of each extract.

### *Animals*

The guidelines and regulation were observed during the conduction of all experiments in accordance with the Ethical Committee of University of Karachi. Either sex male or female Swiss albino mice of (20-25g) and Wistar albino rats (150-180g) were used. These animals were obtained from animal house maintained at the Department of Pharmacology, University of Karachi. All animals were acclimatized for 7 days in cages. They were provided food and water at 25±1$^{o}$C with a 12-hour light-dark cycle. Institutional guidelines were followed for the use of animals. The animals were assigned to different groups to be treated in experiments.

### *Ethical approval*

Ethical approval number: IBC KU-210 / 2021.

### *Chemicals*

The following substances were used: Carrageenan, Acetic acid (Merck & Sigma Chemical Co.), Aspirin (Reckitt Benkiser Pakistan), Diclofenac Sodium (Glaxo Smith Kline)

### *Preparation of extracts*

All four varieties (1Kg) were individually washed thoroughly and sliced into small pieces followed by soaking in 2L98% ethanol solvent separately for three days. The resultant mixtures were filtered with Whatman's filter paper. The filtrates were dried at 40$^{o}$C under vacuum using rotary evaporator. All ethanol extracts were stored at 8°C and warm at room temperature for performing pharmacological activities on animals. (Walum, 1998)

### *Acute toxicity test*

Acute toxicity of the extracts was performed in mice as described earlier (Walum, 1998). Seven mice were selected randomly and were fasted for overnight. Mice in each group were treated with doses 1000, 2000 and 3000 mg/kg of the ethanol extracts orally. Control group of mice was treated with normal saline (10ml/kg) PO. Toxicity was observed for 6h and the mortality was observed for 24 h.

### *Analgesic activity*

*Tail immersion test*

Mice were divided into ten groups of seven animals in each group. Before experiment, hot water was maintained at 56$^{o}$C in which 3cm of mice tail was immersed (Bannon and Malmberg, 2007). The time measured for tail with-drawl from hot water was measured in seconds was considered as the reaction time and was noted 1h before and after the oral administration of the extracts CAG, CAO, CAR, CAY (200 and 400mg/kg). Acetyl salicylic acid (100mg/kg) was given orally 30min before the test to the positive control group and physiological saline (10 ml/kg) to the control group.

### *Hot plate test*

A hot plate test was performed according to a previously reported method (Li *et al*., 2011). Mice were grouped into 10 groups (n=7 per group), Control group received saline (10ml/kg) and the positive control group received 100 mg/kg, of the acetyl salicylic acid. Other groups were given 200 and 400mg/kg of CAG, CAO, CAR, CAY

extracts. Mice were observed individually on the hot plate which was maintained at the temperature of 55±0.05°C. The time in seconds for which mice remained on hot plate without licking or flicking of hind limb and jumping was considered as latency time. Time was noted at 0 and after 30, 60, 90, 120, 150 and 180 seconds of administration.

***Anti-inflammatory activity***

*Inflammatory paw edema in rats*

Carrageenan-induced rat paw edema method was employed to determine the anti-inflammatory potential of the extracts (Morris, 2003). Wistar albino rats (150-180g) were divided into ten groups with seven rats in each group. Each rat was treated orally with CAG, CAO, CAR and CAY (200mg/kg and 400mg/kg) extract, 1h before carrageen an injection. Carrageenan (0.1ml of a 1% suspension prepared in 0.9% NaCl) was injected into the sub-plantar region of right hind paw of rats. Positive control group of rats was treated orally with 50mg/kg of diclofenac sodium as a standard medicine. Vehicle control group received saline (10ml/kg). The paw volume was measured by Plethysomometer after carrageen injection at 0-300 min.

The difference between the initial and subsequent paw volume reading gave the actual oedema volume.

The percent inhibition of inflammation was calculated using the formula:

$$\% \text{ inhibition} = 100 \quad \left(1 \frac{V_t}{V_c}\right)$$

Where *Vc* represents oedema volume in control and *Vt* the oedema volume in the group treated with the tested flavone or diclofenac.

## STATISTICAL ANALYSIS

Data were reported as mean ± standard error of mean (SEM). Statistical analysis was conducted using Statistical Package for the social sciences (SPSS), version 20 (SPSS Inc Chicago, IL, USA). Data were analyzed by one-way analysis of variance (ANOVA) along with Tukey HSD. Values were considered significantly different when *=p<0.05 (significant), **=p<0.01 (more significant), *** = p<0.001 (highly significant)

## RESULTS

***Acute toxicity results***

The acute toxicity assay determined that *Capsicum annum* extracts are non-toxic because no mortality was observed till the end at a dose up to 3000mg/kg. So, both the extracts were found to be safe and 200 and 400mg/kg body weight has been used for the comparative pharmacological activities.

***Analgesic activity***

*Tail immersions method*

Ethanolic extracts of Capsicum showed positive results in all the varieties of bell pepper extracts at the doses of 200 and 400 mg/kg (table 1).

As compared with the control, the most significant analgesic effect of CAG 200 mg/kg was found to be at 150 min (P<0.01). the results were also significant at 60- and 90-min P<0.01 (tables 1 and 2).

By increasing the dose up to 400 mg/kg the significant value exhibited from 30 min and remain significant at 150 min (P<0.01), with the maximum effect at 120 seconds, which is closely related to the significance of aspirin at 60 seconds (P<0.01).

CAR 200 mg/kg, exhibited significant effect only at 90 min (P<0.01), at the doses of 400mg/kg, the value become significant from 30 min and remain significant till 120 min, with the maximum significance at 90 min (P<0.01).

**Fig. 1**: show different types of Bell pepper (1. CAG, 2. CAR, 3. CAO, 4. CAY)

CAO 200 mg/kg, the extract showed significant result from 30 min to 150 min, with the maximum significance at 90 min (P<0.01). By inclining the dose up to 400 mg/kg the significance was observed at 60 min which remains till 150 min.

CAY 200 mg/kg showed positive results at 60 min and maximum significance at 90 seconds. Rest all values were non-significant as compare to the control. CAY 400 mg/kg, exhibited significant value from 30 to 90 min with the maximum significance at 30 min.

***Hot plate test***

Hot plate method determined that 95% ethanolic extracts of the varieties of *Capsicum annum* 200 and 400 mg/kg (bw) showed positive analgesic effect, except CAO which does not showed any effects (table 2).

For CAG 200 mg/kg, the value remains significant from 60 to 180 min, with the maximum effect at 150 min, which is high as compared to the standard p<0.01. In comparison with the control, the doses of 400 mg/kg showed dose dependent decrease in reaction time and the value remains significant from 30 min to 180 min, with the maximum significance at 30 min (18.33±3.23), which is higher as compare to positive control (8.66±0.33).

**Table 1**: Analgesic potential of *Capsicum annum* in mice by tail immersion test

| Group | Dose mg/kg | Time in seconds | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| Control | | 0.78±0.01 | 0.80±0.01 | 0.79±0.01 | 0.79±0.01 | 0.81±0.01 | 0.79±0.01 | 0.77±0.01 |
| CAG (*Capsicum annum* Green) | 200 | 0.92±0.04 | 2.11±0.21 | 2.82±0.44** | 2.53±0.31** | 2.09±0.07 | 3.15±0.41** | 1.91±0.20 |
| CAG (*Capsicum annum* Green) | 400 | 0.95±0.03 | 2.97±0.46** | 2.69±0.33** | 3.35±0.18** | 3.41±0.58** | 3.13±0.53** | 1.77±0.16 |
| CAR (*Capsicum annum* red) | 200 | 0.92±0.02 | 1.31±0.13 | 1.73±0.08 | 2.53±0.42** | 1.89±0.01 | 1.72±0.04 | 1.09±0.08 |
| CAR (*Capsicum annum* red) | 400 | 0.98±0.02 | 2.31±0.30* | 2.44±0.35* | 3.14±0.29** | 2.72±0.09* | 2.08±0.45 | 1.86±0.39 |
| CAO (*Capsicum annum* orange) | 200 | 1.02±0.02 | 2.94±0.02** | 3.50±0.17** | 3.62±0.16** | 3.02±0.24** | 2.42±0.20* | 1.85±0.27 |
| CAO (*Capsicum annum* orange) | 400 | 0.91±0.02 | 2.09±0.23 | 2.88±0.33** | 2.63±0.32** | 2.32±0.21* | 2.23±0.30* | 1.69±1.09 |
| CAY (*Capsicum annum* yellow) | 200 | 0.95±.02 | 1.30±0.09 | 2.27±0.23* | 3.66±0.17** | 2.17±0.24 | 1.63±0.12 | 1.13±0.12 |
| CAY (*Capsicum annum* yellow) | 400 | 1.00±0.02 | 3.40±0.53** | 2.45±0.17* | 3.23±0.21** | 1.67±0.13 | 1.23±0.09 | 0.99±0.08 |
| ASA (Positive control) | 100 | 0.82±0.02 | 1.33±0.07 | 3.28±0.15** | 4.79±0.16** | 5.11±0.18** | 3.21±0.03** | 2.03±0.03** |

**Table 2**: Analgesic potential of *Capsicum annum* in mice by hot plate test

| Group | Dose mg/kg | Time in seconds | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| Control | | 7.33 ±0.42 | 7.50 ±0.22 | 7.50 ±0.34 | 7.00 ±0.36 | 7.66 ±0.33 | 6.83 ±0.31 | 6.66 ±0.33 |
| CAG | 200 | 7.66 ±0.33 | 12.50 ±0.43 | 14.00 ±0.58** | 15.66 ±0.33** | 14.16 ±0.79** | 18.00 ±1.98** | 12.33 ±0.56* |
| CAG | 400 | 9.50 ±0.42 | 18.33 ±3.23** | 17.33 ±0.95** | 16.00 ±0.58** | 14.33 ±0.49* | 12.66 ±1.12* | 12.50 ±0.76* |
| CAR | 200 | 7.83 ±0.30 | 11.16 ±0.30 | 13.16 ±0.30* | 15.00 ±0.58** | 14.66 ±0.33* | 11.66 ±0.76 | 8.16 ±0.48 |
| CAR | 400 | 7.33 ±0.42 | 13.33 ±2.40 | 13.00 ±2.08* | 13.66 ±1.65* | 14.33 ±2.40* | 15.66 ±1.73** | 11.66 ±1.78 |
| CAO | 200 | 8.00 ±0.00 | 9.83 ±1.19 | 11.50 ±1.26 | 12.33 ±0.61 | 12.33 ±1.56 | 12.00 ±1.32 | 10.50 ±1.17 |
| CAO | 400 | 7.50 ±0.22 | 9.16 ±1.78 | 9.00 ±1.71 | 12.33 ±1.14 | 13.00 ±0.73 | 12.83 ±0.70 | 8.50 ±1.26 |
| CAY | 200 | 7.33 ±0.42 | 20.33 ±0.42** | 20.00 ±0.52** | 19.33 ±0.56** | 14.83 ±0.79* | 14.00 ±1.53* | 9.50 ±0.67 |
| CAY | 400 | 8.33 ±0.33 | 16.50 ±2.64** | 14.17 ±1.49** | 15.50 ±1.54** | 12.66 ±1.54 | 11.50 ±1.34 | 10.66 ±1.15 |
| ASA | 100 | 7.83 ±0.30 | 8.66 ±0.33 | 11.66 ±0.33 | 13.00 ±0.58** | 14.17 ±0.60** | 11.33 ±0.42** | 9.50 ±0.43** |

Observations were taken at 0, 30, 60, 90, 120, 150, 180 minutes, Values are mean ± SEM, N=7, *=p<0.05, **=p<0.01, *** = p<0.001

**Table 3**: Anti-inflammatory potential of *Capsicum annum* in the carrageenin-induced rats paw oedema model

| Group | Dose mg/kg | Percentage of inflammation at time (minutes) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 60 | 120 | 180 | 240 | 300 |
| Control | | 1.51± 0.04 | 2.90±0.03 | 3.75±0.06 | 4.55±0.05 | 5.43±0.07 | 5.70±0.07 |
| CAG | 200 | 1.94±0.02 | 3.10±0.06 | 3.52±0.04 | 3.72±0.03** | 3.82±0.03** | 3.71±0.42** |
| CAG | 400 | 1.73±0.05 | 2.70±0.07 | 2.90±0.04** | 2.94±0.04** | 2.48±0.04** | 2.12±0.03** |
| CAR | 200 | 1.91±0.11 | 3.04±0.02 | 3.34±0.04 | 3.55±0.05** | 3.63±0.04** | 3.39±0.04** |
| CAR | 400 | 1.92±0.05 | 2.99±0.13 | 3.30±0.18 | 3.12±0.08** | 2.90±0.03** | 2.62±0.03** |
| CAO | 200 | 1.98±0.03 | 3.10±0.03 | 3.68±0.03 | 3.66±0.06** | 3.64±0.05** | 3.44±0.04** |
| CAO | 400 | 1.71±0.07 | 3.13±0.13 | 3.35±0.11 | 3.33±0.13** | 3.29±0.17** | 2.62±0.09** |
| CAY | 200 | 1.99±0.02 | 3.17±0.07 | 3.47±0.05 | 3.75±0.03** | 3.93±0.01** | 3.68±0.06** |
| CAY | 400 | 1.65±0.04 | 2.92±0.05 | 3.27±0.03* | 3.43±0.05** | 2.70±0.02** | 2.28±0.12** |
| Diclofenac | 50 | 1.64±0.02 | 1.84±0.01 | 1.8±0.03** | 1.76±0.02** | 1.75±0.02** | 1.73±0.02** |

Observations were taken at 0, 60, 120, 180,240, 300 minutes, Values are mean ± SEM, N=7, *=p<0.05, **=p<0.01, *** = p<0.001
Values were considered significantly different when *=p<0.05 (significant), **=p<0.01 (more significant), *** = p<0.001 (highly significant)

**Table 4**: Anti-inflammatory potential of *Capsicum annum* in the carrageenin-induced rats paw oedema model expressed as: Percent of inhibition of oedema formation at time (minutes).

| Group | Dose mg/kg | Percentage of inhibition at time (minutes) | | | | |
|---|---|---|---|---|---|---|
| | | 60 | 120 | 180 | 240 | 300 |
| CAG | 200 | -6.8 | 6.2 | 18.7 | 29.7 | 35 |
| CAG | 400 | 6.8 | 23 | 35.4 | 54.4 | 63 |
| CAR | 200 | -4 | 11 | 22 | 34 | 41 |
| CAR | 400 | -3 | 12 | 31 | 47 | 55 |
| CAO | 200 | -6.8 | 2 | 19.6 | 33 | 40 |
| CAO | 400 | -7 | 14 | 27 | 39.5 | 54 |
| CAY | 200 | -9.31 | 8 | 18 | 27.7 | 36 |
| CAY | 400 | -0.6 | 12.8 | 25 | 50.3 | 60 |
| Diclofenac | 50 | 57.6 | 52 | 62 | 67.8 | 70 |

CAR 200 exhibit significant activity at 60 min and remains significant till 120 min with P<0.05, by increasing the dose to 400mg/kg, the value remains significant from 30 till 150 min.

CAO showed no significant analgesic effect at both 200 and 400mg/kg. CAY 200mg/kg, showed highly significant values from 30-90 min (P<0.01) and remain significant till 150 min with p<0.05 and by increasing the dose up to 400mg/kg, the significant value exhibited at 30 min to 90 min after that the values show no significant.

***Anti-Inflammatory activity***

*Carrageenan-induced paw edema test*

Control and the treatment with the extracts of bell pepper altogether diminishes paw edema at both the doses 200 and 400mg/kg (table 3). The ethanolic extracts of CAG, CAR, CAO, CAY at 200mg/kg, exhibit significant values at 180 min and remains significant till 300 min, with the maximum significance of CAY at 240 min with P<0.01.

By increasing the doses up to 400mg/kg, all the extracts of *Capsicum* i.e., CAG, CAR, CAO, CAY showed positive results from 180 to 300min with P<0.05, with exception of CAG and CAY, which also exhibit significance at 120 min. the maximum effect exhibited by CAY 400mg/kg at 180 min (3.43±0.05), that is highly significant as compared to positive control at 60 min (1.84±0.01).

Percentage inhibition is relatively high at high doses as compared to low doses of each test sample which indicate dose dependent response.

## DISCUSSION

*Capsicum annum* L. (Bell pepper) has traditionally been used for the treatment of dysmenorrhea (Valadeau *et al*., 2010) indicating that it may exhibit analgesic, as well as, anti-inflammatory activity. However, scientific validation for the same is missing. The current study was planned to assess the anti-inflammatory and analgesic potential of the four varieties of bell pepper using *in vivo* assays. For this purpose, we prepared ethanolic extracts of various species of *Capsicum annum* (bell pepper) including green (CAG), red (CAR), orange (CAO) and yellow (CAY) and investigated their analgesic and anti-inflammatory effect with possible mechanisms. Tail flick and hot plate methods were used to assess the analgesic effect, whereas, carrageenan-induced rat paw edema model was used to explore the anti-inflammatory potential.

Hot plate test as well as, tail flick (*in-vivo*) assays have become the standard methods for investigating the antinociceptive properties of compounds/extracts (Bianchi

and Franceschini, 1954, Ben-Bassat *et al*., 1959, Abdala *et al*., 2014). Numerous plants pigment's extracts have been reported to possess analgesic effect (Sengupta *et al*., 2012)

The oral administration of all the varieties of bell pepper extract exhibited significant analgesic effect as compared to the negative control when tested in tail flick assay. The onset of analgesic effect in all the bell peppers' species was 30 seconds and 60 seconds at 200 and 400mg/kg doses respectively, except CAR which exhibited a delayed onset of 90 seconds at the tested dose of 200 mg/kg. Amongst the 4 varieties studied, i.e., CAG, CAR, CAO and CAY, the analgesic activity was superior in CAG and CAO as the latency at 400mg/kg tested dose was maintained up till 150 seconds, as compared to CAR and CAY whose duration at the same dose was maintained up till 120 and 90 seconds respectively (CAG 400mg/kg = CAO 400mg/kg > CAR 400mg/kg > CAY 400mg/kg). This difference in effect could be attributed to the difference in phenolic contents in different species of bell pepper which are known to exhibit analgesic activity. Since HPLC analysis of green pepper showed higher amounts of phenolics as compared to red and yellow peppers (Zhang and Hamauzu, 2003), therefore we suggest that the predominant difference in analgesic activity in bell pepper varieties may be the reflection of different phenolic compounds present in each variety of bell pepper.

The current findings suggest that Bell pepper may be mediating its effect via spinal mediated anti-nociceptive effect, as this test determines animals' latencies in nociceptive response to thermal stimulus, a response characteristic of narcotic drugs such as morphine (YAKSH *et al*., 1977, Smith *et al*., 1982, Smith *et al*., 1985)

To further get an insight into possible mechanism of action we studied the different varieties of bell pepper extracts by using hot plate test. Oral administration of CAG, CAR, CAY and CAO reduced thermal sensation at the tested doses of 200mg/kg and 400 mg/kg, with maximal effect observed for CAG and minimal effect for CAO. CAG retained its analgesic activity till 180 seconds at both the tested doses. However, both the tested doses differed in onset of action, with CAG 200 mg/kg onset at 60 seconds, whereas CAG 400 dose exhibited its analgesic effect at 30 seconds. The least effective in terms of duration of action was CAO, whose effect only retained till 30 and 60 seconds at 200 and 400 mg/kg doses respectively. (CAG 400>CAG200>CAR400> CAR200>CAY400>CAY 200 >CAO 400> CAO 200) so, we can conclude that extracts of bell pepper can also exhibit centrally acting analgesic activity,

This analgesic activity via hot plate test depicts the possibility for bell pepper to be used as centrally acting analgesic agent and in this test animal nociceptive response latencies are measured by thermal stimulus which predominantly involves supraspinal pathway and is used to differentiate between central and peripheral analgesic effects. Thus, bell peppers mediate their analgesic activity by both the central and peripheral pathways (Bannon and Malmberg, 2007).

The analgesic effect produced by *Capsicum annum* may be attributed to the presence of Capsaicin (Alkaloid). Capsaicin and di-hydrocapsaicin constitute 90% of these compounds in pepper (Hamed *et al*., 2019), a known active compound of this plant, which has been employed as a topical analgesic against arthritis pain and inflammation since many decades(Sanatombi and Sharma, 2008). Powerful analgesic activity is observed by systemic capsaicin in animal models of chronic neuropathic pain. Capsaicin is known to mediate its antinociceptive effect via central pathways by desensitizing the primary sensory neurons mediated by inhibition of "substance P". (Szallasi *et al*., 2007, Liu and Nair, 2010)

Further to this we demonstrate for the first time the anti-inflammatory effects of four varieties of bell pepper by using paw edema method. The carrageenan- induced paw edema test is suitable for evaluation of anti-inflammatory drugs and assessment of the effect of natural products as anti-edematous (Hernández-Ortega *et al*., 2012). Development of edema induced by carrageenan has been described biphasic (Vinegar *et al*., 1969). After administration of carrageenan in the plantar tissues of rat's right-hand paw, there was sudden elevation of paw volume compared to histamine and serotonin injection(Lo *et al*., 1982). The anti-inflammatory effect of carrageenan induced paw edema is shown in table 3 and the percentage inhibition of inflammation is demonstrated in table 4. Both the positive control group (given diclofenac sodium) and treatment groups (administered different varieties of bell pepper, i.e., CAG, CAR, CAO and CAY) decrease in paw volume indicates the reduction of carrageenan induced inflammation and percentage inhibition is found to significantly high at 400mg/kg for all varieties as compared to control indicating the anti-inflammatory activity of all the varieties of bell pepper. There are two phases in these edema models, with first phase which is regarded as early phase (1-2h) is characterized by release of histamine, serotonin and increase of PG synthesis, while the late phase (2-5h) is initiated by bradykinin, leukotrienes, polymorphonuclear cell and Prostaglandins produced by tissue macrophages and also the induction of COX II. The pretreatment of rats with four varieties of bell pepper extracts produced no significant effect in early phase, but exhibited predominant effects in phase II. Thus, it is probable that all the varieties CAG, CAR, CAO and CAY mediated anti-inflammatory effect by inhibiting the neutrophil

migration and reducing the levels of pro-inflammatory cytokines.

The anti-inflammatory potential of bell pepper may be credited to the presence of Capsaicin, a major ingredient of pepper, which is known to exhibit an anti-inflammatory mechanism by the inhibition of PGE2 and NO production and also may be a consequence of antioxidants compounds present in bell peppers(Kim *et al*., 2003). The study on leaves of capsicum also confirms the immunosuppressive activity against T-cell activation *in vitro* responsible for anti-inflammatory activity (Hazekawa *et al*., 2017).

However, our study justifies the anti-inflammatory property of bell pepper fruits which were not studied before. There are no epidemiologic studies showing whether bell pepper intake may be beneficial in human inflammatory diseases despite these data showing the anti-inflammatory effects of bell pepper and capsaicin intake may be beneficial in human inflammatory diseases

## CONCLUSION

The present experimental study demonstrates for the very first time the potential analgesic and anti-inflammatory activities of native Pakistani bell pepper extracts.

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