Comparison of Antiulcer Activity of Orange and Purple Carrot Juices in Rats Gastric

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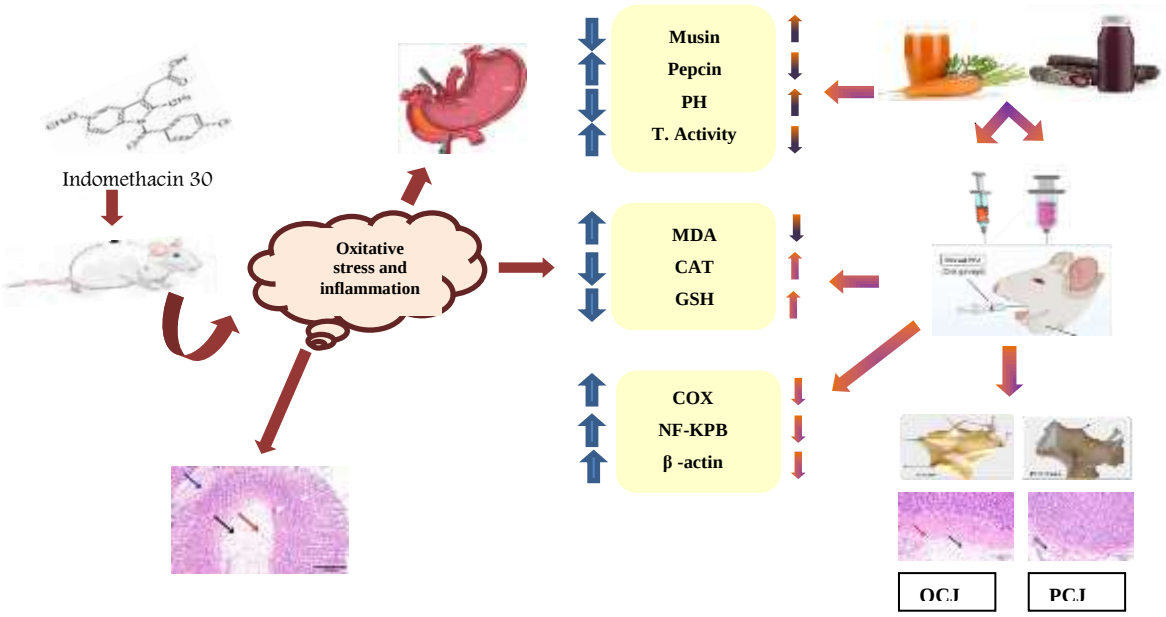
Abstract:

Carrot is a nutrient-rich root vegetable grown, consumed globally and contains anti-inflammatory substances as pigments and polyphenol compounds which can be an alternative to prevent peptic ulcers. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (INDO) drug used to manage acute pain, rheumatoid arthritis and osteoarthritis, but they cause adverse effects on the gastrointestinal tract, including ulcers and inflammation. Therefore, the current study aimed to investigate and compare the effects of orange and purple carrot juice (*Daucus carota L.*) on indomethacin-induced gastrointestinal damage in rats. To test these effects of orang carrot juice (OCJ) and purple carrot juice (PCJ) in gastric ulcer; biochemical and histopathological markers were examined in the stomach tissues of 36 rats were divided in to six groups. The results indicated that INDO caused significant decreases ($P < 0.05$) in gastric pH and mucin; also, it caused an increase in total acidity and pepsin compared to the control group; while, rats treated with OCJ and PCJ showed a significant increase in gastric pH and mucin with a significant decrease in total acidity and pepsin activity. The ulcerated gastric mucosa of rats by indomethacin had lower GSH (0.613 mg/g) and CAT (0.57 U/g protein) activity, and higher MDA (0.22 nmole/g) content compared to control rats (1.70613 mg/g, 0.81 U/g protein and 0.14 nmole/g) respectively. INDO caused significant increases ($P < 0.05$) in the value of COX, NF-kB and β -actin for the INDO group than the control; while, groups treated with OCJ and PCJ, groups have a near value of control. The results show that PCJ improved the biochemical and histopathology parameters caused by indomethacin. The study revealed that purple carrot juice has antiulcer activity and can help strengthen necrosis of gastric mucosa caused by indomethacin and the potentiality of using purple carrot juice to prevent gastric ulcers.



Key words: carrot phytochemical, indomethacin, macroscopical examination

1. Introduction:

Peptic ulcer is one of the major gastrointestinal disorders that occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors **(Chandra *et al.*, 2015).** It affects 10-15% of the population, is a disease characterized by the disruption of mucosal integrity of the esophagus, stomach and duodenum **(Agbaje *et al.*, 2017).** It is divided into two types: duodenal ulcers and stomach ulcers **(Zhang, 2022).** Gastric ulcer is one of the most common problems found in almost all adults around the world particularly those who accustomed to consuming coffee, tea and spicy foods **(Ajaib *et al.*, 2022)**. Gastric ulcers of various forms have been related to stress, smoking, the overuse of non-steroid -inflammatory drugs **(El-shafey *et al.*, 2022).**

The prevalence of peptic ulcer in the world population, affects 4 million people around the world. 2%-14% of the ulcers will perforate and it can happen at any age between 30 and 60 years **(Farghali *et al.*, 2014; Vomero and Colpo, 2014).** Its occurrence depends to a large extent on the nature of the diet and others **(Abed and Sameen, 2023).** The gastric ulcer is one of the common causes of eutrophic infiltration, blood flow reduction, increased oxidative stress and inflammation **(Xue *et al.*, 2019).**

In recent years, there has also been growing interest in alternative therapies and the use of natural products, especially those derived from plants; possibly due to availability, fewer adverse effects and lower costs for treatment ulcer **(Revathi *et al.*, 2017).** Nutritional therapy's recommendations define aspects of a healthy diet as a way to promote health and prevent of gastritis **(Vomero and Colpo, 2014; Sozen *et al.*, 2023).** Natural fruit and vegetable beverages are tasty, nourishing, and rich in vitamins, minerals, and phytonutrients, more precisely natural

bioactive compounds that interact positively with food fibers and other substances taken from food **(Butu and Rodino, 2019).** Carrots one of these natural vegetable beverages are being increasingly consumed, mainly due to their abundant health-promoting phytochemicals and high level of antioxidant capacity **(Ma *et al.*, 2020).**

Carrot (*Daucus carrota L.)* is one of the main vegetable crops grown and consumed globally. A biennial plant (life cycle 12-24 months), and belong to the Apiaceae family; carrot plant morphology grows up to 30-100 cm or more and it is considered nutrient-rich root vegetable **(Gajewskil *et al.*, 2007; Engla, 2021; Kalia *et al.*, 2023).** It is divided into two major types; the eastern carrot (black, purple, red or rarely yellowish) or the western carrot (orange, yellow or white) **(Cebeci and Hanci, 2019).** The first types of carrots were (yellow and purple as fleshed cultivars); while today they will become more popular with orange carrot **(Singh *et al.*, 2021).**

Carrot is a highly nutritious vegetable and has been ranked 10^{th} in nutritional value; which contains a high value of carotenes, ascorbic acid, thiamin, riboflavin, niacin, pyridoxine and folic acid (**Malik and Garg, 2011; El-Abasy *et al.*, 2012)**.Carrot rich in dietary fiber, several polyphenols. As well as, good vegetable source of potassium, sodium, phosphorus and the trace mineral as molybdenum, magnesium and manganese **(Dias, 2014; Soleti *et al.*, 2020);** which are very important for human body function **(Haq and Prasad, 2018).**

The nutrient contents of carrots changed depending on colors, purple colour is the result of anthocyanin; while orange and yellow colors are used by carotenoids **(Yusuf *et al.*, 2021).** Purple carrot extracts was used as a healthy alternative to synthetic colorants in food products **(Demircan and Velioglu, 2023).** Many studies confirmed that carrot nutrients can play an important role for prevent neurological diseases

that are result from oxidative stress, cancer and cardio-vascular diseases **(Kiraci and Padem, 2016).** Also, the availability anti-inflammatory substances as phenolic in carrots can be used as an alternative to prevent peptic ulcers **(Susanto *et al.*, 2022).** So, the current study aimed to investigate and compare the effects of orange and purple carrot as a natural alternative to prevent gastric ulcer in rats.

2. Materials and Methods

2.1 Materials

- Source of plant:

Orange and purple carrots were obtained from a local market in Minia Governorate, Egypt.

- Reagents and chemicals

All solvents and chemicals were obtained from El-Gomhoryia Company for chemicals, medical instruments and trading drugs in Cairo, Egypt. Indomethacin was purchased from SIGMA-ALDRICH (22 Abo zar El-Ghafary St. from El-Tayaran St, Nasr City -Cairo). Kits that were used to determine serum PH, T-ACID, pepsin, mucin, reduced glutathione, catalase, MDA, COX, NF-kB and β-ACTIN were obtained from a bio-diagnostic Chemical Company.

2.2 Methods

- Preparation of Juice Sample:

Fresh carrots roots juice (orange and purple) were prepared according to **Revathi *et al.*, (2017)** method as follow:

Step 1: Check for any damage or injury; broken or damaged carrots were manually sorted and removed.

Step 2: Carrots were cleaned by removing foreign matter and then washed with cold water several times to ensure cleanliness.

Step 3: Cut the carrots into suitable pieces, juice the carrots by using a fruit juicer (Sokany fruit juicer 800 watts).

Step 4: Filtrate orange carrot juice (OCJ) and purple carrot juice (PCJ) through muslin cloth. And after squeezing, pack them properly in airtight glass containers until use.

-Determination of Chemical Composition:

At the Food Technology Research Institute, we assessed moisture, protein, ash, fat and fiber using the methods described in **A.O.A.C. (2012).**

-Determination of Phytochemical Composition:

Determination of total flavonoids content (TFC), total phenols content (TPC), and The 2, 2- diphenyl -1 picrylhydrazyl (DPPH) radical scavenging ability according to **(Abu Bakar *et al*., 2009; Musa *et al*., 2011; Oms-Oliu *et al*., 2009)** respectively.

-Determination of Anthocyanin and β-carotene Content:

According to the method by **Ranganna, (1977),** total anthocyanin content was measured colorimetrically. β-carotene content was estimated by **Singh *et al*., (2015)** method, the optical density(OD) of the solution was estimated using the following equation:

$$\textbf{β-carotene} = \frac{OD \times 13{:}9 \times 10.000 \times 100}{\text{Weight of sample (g)} \times 560 \times 1000}$$

- Experimental design:

- Animals:

36 male albino rats of the Sprague-Dawley strain, weighting 160.9 ± 2 g acclimatized for two weeks in the Chemistry Department's Biological Laboratory at the Faculty of Agriculture, Minia University, Egypt. The rats were housed in plastic cages in air condition room at 25 ± 2^{o}c (with a 12 h light/ dark cycle). The animal were fed on a commercial balanced diet and supplied with tap water.

- Ethical approval:

All experimental procedures for this study were conducted according to the ethical standards approved by Scientific Research Ethics Committee (SREC) Faculty of Specific Education, Minia University, Egypt.

-Evaluation of Anti-gastric Ulcer Activity of OCJ and PCJ:

After an acclimatization period of one week, rats were randomly divided into 6 groups (n=6 for each) and assigned daily food continued for 21 days according to **Çalışkan *et al.*, (2016)** method with a few adjustments as follows:

Control: 1.5 mL/day distilled water was given orally for 21 days.

OCJ: 1.5 mL/day OCJ was given by gavage orally for 21 days.

PCJ: 1.5 mL/day PCJ was given by gavage orally for 21 days.

INDO: 1.5 mL/day distilled water was given orally for 21 days. Indomethacin was given orally at a dose of 30 mg/kg on the 23th day

OCJ + INDO: 1.5 mL/day OCJ was given by gavage orally for 21 days. Indomethacin was given orally at a dose of 30 mg/kg on the 23th day

PCJ + INDO: 1.5 mL/day PCJ was given by gavage orally for 21 days. Indomethacin was given orally at a dose of 30 mg/kg on the 23th day

At the end of the experiment, all positive groups (INDO, OCJ + INDO and PCJ + INDO) were fasted for 24 hours according to **Balaha *et al.*, (2022),** then gastric hemorrhagic lesions in rats were induced by intragastric administration of 30 mg/kg of indomethacin according to the procedure described by **Gohar and Zaki, (2014)**; and killed 24 hours later under deep ether anesthesia **(Mizoguchi *et al.*, 2001).**

- Collection of Gastric Samples:

To prevent accidental losses of gastric secretion, the cardiac end of the stomach was clamped before removal of the organ. After removal and washing with distilled water, the stomach was gently dried. The fundus was then punctured and the gastric contents were collected in a graduated centrifuge tube. The samples were centrifuged at 3500 rpm

for 15 minutes and those with more than 0.5 ml of solids were discarded **(Dai and Ogle, 1972).**

- Preparation of Animal Tissue Homogenate:

Animals were dissected and stomach was excised, wiped with filter paper and weight. The stomach parts were homogenized in ice –cold 100mM phosphate buffer, pH 7.4. Homogenates were centrifuged at 7000 r.p.m for 20 minutes and the resulting supernatants were immediately used for the assessment of lipid peroxidation (LPO) as malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), cyclooxygenase (COX), β-actin and nuclear factor kappa of B cells (NF-kB).

-Determination of pH, Total-Acidity, Pepsin and Mucin Activity:-

According to **Dashputre and Naikwade, (2011)** method, pH was measured by using a pH meter**.** Total acidity was determined by **Misher, (1969)** method. Pepsin was measured according to **Clokey, (1981).** And mucin was measured according to **Bancroft and Gamble, (2008).**

- Determination of MDA, GSH and CAT:-

Malondialdehyde, glutathione and catalase were determined enzymatically and reduced by the method introduced by **Ohkawa *et al.*, (1979); Beutler *et al.*, (1963) and Aebi, (1984).**

-Determination of COX; NF-κB and β-actin content:

Gastric tissue COX levels were measured according to **Kulmacz and Lands, (1983)**; determination of NF-κB content was according to **Salama *et al.*, (2023)** method and determination of the β-actin content by **Balakrishnan *et al.*, (2021)** method.

-Histopathological examination of stomach:

Autopsy samples were taken from the stomach of rats in different groups and fixed in 10% formal saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohols (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin and examination was done through the light electric microscope **(Banchroft *et al*., 1996).**

Histopathological alterations in the stomach were graded and scored from (0-3) through the determination of the percentage of the lesions in five randomly examined microscopic fields per animal as follows: (0) indicated no changes, (1), (2) and (3) indicated mild, moderate and severe changes, respectively, while the grading was determined by percentage as follows: (<30%) showed mild changes, (<30% – 50%) indicated moderate changes and changes greater than 50% (>50%) indicated severe changes **(Abd-Alla *et al*., 2022).**

-Statistical analysis:

Experimental results were expressed as means ± SD of three parallel measurements. Analysis of variance was performed by ANOVA procedures. Graph Pad Prism® was used for statistical calculations Graph Pad Software, San Diego, CA, USA **(Motulsky, 1999)**.

3- Results and Discussion:

3.1. Proximate Composition

The chemical composition of OCJ and PCJ were presented in Table (1). The results clarified that OCJ had the largest content of moisture, protein and fat (88.95, 0.406 and 0.212%) respectively, compared to PCJ which recorded (87.45, 0.334 and 0.204%) respectively; while PCJ had the highest content of ash, fiber and total carbohydrates (0.823,

2.117 and 9.067%) respectively, compared to OCJ which recorded the lowest value (0.0665, 1.289 and 8.841%) respectively. Our results were in agreement with **Sharma and Sharma,(2020)** confirmed that orange carrot and purple carrot recorded 83.35% and 91.50% respectively at moisture. And in agreement with **Gaikwad *et al.*, (2019)** showed that the moisture content was 86%, fat 0.27%, carbohydrate 10.3 %, protein 1.0%, ash 1.20% and fiber 1.23% respectively.

Such data are in partially accordance with **Al Tamim, (2014)** finding that the moisture, protein, fat, ash, fiber and carbohydrate values of carrot were (85.98, 0.78, 0.31, 1.05, 1.65 and 10.23%) respectively, and our results were nearly identical to those presented by **Hassan *et al.*, (2021)** showed that carrot contain 87.0% moisture and 9.81% carbohydrate. As well as, with data obtained by **Suhail and Dilshad, (2022)** reported that nutritional composition of carrot value for fiber, fat and carbohydrates were (2.8, 2.4 and 9.58%) respectively.

Sarker *et al.*, (2022) observed that fresh carrot juice contains 8.17% carbohydrate, 0.57% fat, 0.77% protein and 0.89% ash. **Hussein *et al.*, (2022)** recorded that carrot content was 86% moisture, 0.20% fat, 1.10% ash, 1.20% fiber; but were differ in protein and carbohydrate content were 0.90 and10.60 % respectively.

Table (1): Proximate composition of OCJ and PCJ

Chemical Composition (g.100g)	OCJ	PCJ
Moisture	**88.95 ± 0.83**	**87.46 ± 0.02**
Crude Protein	**0.406±0.01**	**0.334±0.01**
Crude Fat	**0.212 ± 0.003**	**0.204 ± 0.002**
Ash	**0.665 ± 0.004**	**0.824 ± 0.034**
Fiber	**1.289 ±0.15**	**2.117 ± 0.04**
Total Carbohydrates	**8.841±0.08**	**9.067±0.036**

*Every value represents the mean value. Mean ± SD of three determinations

On the other hand, our results are disagree with data obtain by **Abdel-Hady *et al.*, (2014**) reported that purple carrot had 93.4% moisture. And with **Boadi *et al.*, (2021)** reported that fibre content in three types of carrot extract ranged from 7.18% to 8.87%, protein content was 6.46 to 10.73% and carbohydrate content was 6.25 to 8.39%.

3.2. Phytochemicals Content:

Natural juice of carrot is a good source of antioxidant activity, flavonoids and total phenolic contents, which have some beneficial physiological effects that include reduced oxidative damage **(Owolade *et al.*, 2017; Elwakeel, 2020).** Data in Table (2) present the content of TPC, TFC, DPPH, total anthocyanin and β-carotene and in OCJ and PCJ. The results showed that PCJ has the high value of TPC and TFC 265.9 GAE/100g, 59.22 mg /100g compared to OCJ recorded 97.48 GAE/100g, 41.85 mg/100g. While OCJ has higher content of β-carotene was 11.51 µg /g than PCJ was 3.08 µg /g. Also, OCJ has total anthocyanin content was 0.013 mg /100g less than PCJ was 20.89 mg /100g. Our results indicated that PCJ has a higher value than OCJ in DPPH was 68.18 and 36.18 % respectively.

Table (2): Phytochemicals composition content of OCJ and PCJ

Phytochemicals Composition	OCJ	PCJ
Total Polyphenols (TPC) (mg GAE/100g)	**97.47±2.7**	**265.9±25.47**
Total Flavonoids (TFC) (mg quercetin/100g)	**41.85± 4.6**	**59.22±5.1**
Total anthocyanin (mg /100g)	**0.013 ± 0.006**	**20.88 ± 0.87**
β-carotene (µg /g)	**11.51±0.23**	**3.08±0.26**
DPPH (%)	**36.18±1.02**	**68.18±2.56**

*Every value represents the mean value. Mean ± SD of three determinations

Our results agreed with **Aderinola and Abaire,(2019)** confirmed that carrot juice contains a higher TPC as well as a higher TFC content**.**

And with data obtain by **Azizuddin and Aneela Qadeer, (2017)** reported that juice of whole carrot has high total phenolic content, total flavonoid and total antioxidant activities. **Sharma and Sharma, (2020)** revealed that the addition of carrot juice concentrate had improved the nutritional quality of cookies as evident from its higher total phenolic content (15.85 mg/100g), carotenoids (8.37 mg/100g) and antioxidant potential (35.69 % free radical scavenging activity).**Demircan and Velioglu, (2023)** reported that purple carrot puree processed under different drying conditions exhibited variable ranges for TPC (2777.58 to 5929.09 ppm GAE).

Moreover, all varieties of carrots contain antioxidant nutrients, included traditional antioxidants as vitamin C and phytonutrient antioxidants as β-carotene **(Versha *et al.*, 2019).** Total content of carotenoids is responsible for the orange color of carrots, and the intensity of the color is considered an indicator of higher nutritional value **(Pavlović1 *et al.*, 2024).** Data of the present study are in accordance with that obtained by **Mendelova *et al.*, (2016) and Amuna, (2023)** found that different varieties of carrots juice contain a high concentration of beta carotene. Also, our results were agree with **Abdel-Hady *et al.*, (2014)** revealed that value of total anthocyanin and total phenol contents were increased gradually by increasing the amount of purple carrot.

3.3. Biological Experimental:

- Effect of OCJ and PCJ on pH and total acidity of gastric ulcer rats

Results in Table (3) reveal that INDO caused significant decreases ($P < 0.05$) in gastric pH and increase in total acidity, that change rate reached (-20.1 and +26.64%) respectively compared to the control group. Our results were in agreement with **Sabiu *et al*., (2015)** reported that indomethacin administration caused significant ($p<0.05$) decrease in pH value. **Singaravelu *et al*., (2018)** reported that the total acidity of gastric juice was significantly increased in control positive group (indomethacin group) at $p<0.001$ when compared to normal control group.

Data in Table (3) confirmed that pre-treatment with (purple and orang) carrots showed a significantly ($P <0.05$) increased gastric pH, that change rate reached (+7.65 and +29.66%) respectively, with significant decrease in total acidity level, that change rate reached (-12.88 and - 20.39 %) respectively versus the control group. Our result were in agreement with **Chandra *et al*., (2015)** confirmed that rats were administered by 50% ethanol extract from carrota roots extract (100 and 200 mg/kg) decreased the total acidity and increased the pH when evaluated the gastric juice pH and total acidity for 4 hours after pyloric ligation.

Table (3): Total acidity and pH of gastric ulcer rats treated with OCJ and PCJ

Groups	pH	% of change	Total Acidity (mEq/L)	% of change
Control	2.090±0.070	0.00	87.24 ±1.135	0.00
OCJ	3.577± 0.065 [a]	+71.1	45.28 ±1.13 [a]	- 48.10
PCJ	3.837±0.075 [a]	+83.59	50.3 ±17.66 [a]	- 42.34
INDO	1.690±0.280 [a]	-20.1	110.48±1.59[a]	+26.64
OCJ + INDO	2.250±0.33 [b]	+7.65	76.00 ±18.74 [b]	- 12.88
PCJ + INDO	2.707±0.525 [ab]	+29.66	69.45±1.085 [ab]	- 20.39

Data represent the mean± S.D. of observation from six rats
[a] Significant difference at P < 0.05 relative to the control group
[b] Significant difference at P < 0.05 relative to the INDO group

Results are in agreement with **Asdaq *et al.*, (2020)** reported that the gastric anti-ulcer effect of the carrot dose (200 mg/kg) reduced total acidity of the gastric juice; while a high dose of carrot (500 mg/kg) showed excellent effects on gastric ulcers induced by indomethacin. Pretreatment with OCJ and PCJ (1.5 ml/ daily) had a gastroprotective effect and showed improved in pH value compared to the indomethacin group.

- Effect of OCJ and PCJ on pepsin activity and mucin content of gastric ulcer rats:-

The stomach play a critical role in food digestion and it is secretes a "gastric juice comprises water, mucus, hydrochloric acid and pepsin. Pepsin is considering the principal enzyme protein digestion and hydrochloric acid (HCl) is playing for pepsin activity (Low pH) **(Heda *et al.*, 2019). Allen and Flemström, (2005)** explained that the stomach creating an adherent layer of bicarbonate-rich mucus lining for protects itself from the digestive properties of pepsin. Pepsin has the potential to

be a biomarker of diseases associated with reflux of gastric content **(Stanforth *et al.*, 2022).**

Results in Table (4) showed the effect of treatment by (purple and orang) carrot juice on mucin content. Whereas the mean value of mucin content for control group was shown higher value, and significant decreases (P<0.05) of mucin content in INDO group; that change rate reached – 46.5% compared to control group. Our result were agreement with **AlKreathy *et al.*, (2020)** reported that mucin content from indomethacin-exposed group showed significant reduction of mucus production compared to the control group.

Table (4): Pepsin activity and mucin content of gastric ulcer rats treated with OCJ and PCJ

Groups	Mucin (g/mL)	% of change	Pepsin (g/mL)	% of change
Control	0.86 ± 0.02	0.00	3.42±0.135	0.00
OCJ	0.79 ± 0.10	- 8.13	2.28±0.625 [a]	- 33.33
PCJ	0.82 ± 0.015	- 4.7	2.60±0.480 [a]	- 23.98
INDO	0.46 ± 0.02[a]	- 46.5	4.33±0.135 [a]	+ 26.61
OCJ + INDO	0.56 ± 0.12[ab]	- 34.9	3.74±0.025 [b]	+ 9.36
PCJ + INDO	0.62 ± 0.015[ab]	-27.90	3.59±0.014[b]	+4.97

Data represent the mean± S.D. of observation from six rats
[a] Significant difference at P < 0.05 relative to the control group
[b] Significant difference at P < 0.05 relative to the INDO group

On the other hand pretreatment with OCJ and PCJ (1.5 ml/ day) with INDO showed improved mucus production compared to the indomethacin group and that treatment with OCJ and PCJ had a gastroprotective effect compared to INDO group; which that change rate reached only (-34.9 and -27.90% respectively) compared to control group. Also, Table (4) showed the results of that mean value of pepsin content for INDO group was shown high value; and significant increases (P<0.05) of pepsin activity in INDO group; that change rate

reached (+26.61%) compared to control group. INDO caused decreased pH; therefore it makes the pH required for pepsin activity **(Bardhan *et al.*, 2012)**. In the same Table results showed that the rest treated groups (OCJ + INDO and PCJ+ INDO) have increase changed by (+9.36 and +4.97 % only) respectively.

- Effect of OCJ and PCJ on MDA content, CAT and GSH of gastric ulcer rats:-

As shown results in Table (5) INDO caused an increase (1.6 folds) in gastric tissues MDA contents compared to values in tissues from the control ; where's the change rate reached (+57.14%). Our results were in agreement with **AlKreathy *et al.*, (2020)** confirmed that indomethacin caused significant increase (2.5 folds) of MAD in experimental rats group as compared to the control group. In the same Table (5), groups (OCJ, PCJ and PCJ + INDO) which treatment by (orange and purple) carrot juice had significant decreased effect on MDA levels compared to control group; that change rate reached (-14.3, -28.75 and -7.14%) respectively; that because drank carrot juice may protect by increasing total antioxidant status and by decreasing lipid peroxidation (**Potter *et al.*, 2011).**

Our results in same Table show the assessment the antioxidant effect OCJ and PCJ in gastric homogenate on CAT and GSH. Indomethacin group had reduced CAT and GSH content. Regarding CAT activity, as shown in Table (5), INDO caused a significant reduction in CAT activity by -66.67 % compared to the control group. Our findings were in agreement with **Khan *et al.*, (2019)** reported that INDO caused significant decrease of CAT activities in tissues. Moreover, groups (OCJ, PCJ, OCJ + INDO and PCJ + INDO) which

treated by (orange and purple) carrot juice caused improvement in CAT activity with change percent (+6.17, +8.64, -4.94 and -2.47%) respectively compared to control group.

Table (5): MDA, CAT and GSH of gastric ulcer rats treated with OCJ and PCJ

Groups	MDA (nmole/g)	% of change	CAT (U/g protein)	% of change	GSH (mg/g)	% of change
Control	0.14±0.01	0.00	0.81±0.03	0.00	1.47±0.11	0.00
OCJ	0.12±0.02	-14.3	0.86±0.01	+6.17	1.60±0.10 [a]	+8.84
PCJ	0.10±0.05	-28.57	0.88±0.04	+8.64	1.70±0.01 [a]	+15.64
INDO	0.22±0.01[a]	+57.14	0.57±0.04 [a]	- 66.67	0.613±0.015 [a]	-58.3
OCJ + INDO	0.17±0.01 [ab]	+21.43	0.77±0.02[b]	-4.94	1.10±0.10 [ab]	-25.17
PCJ + INDO	0.13±0.02[b]	-7.14	0.79±0.02[b]	-2.47	1.2±0.02 [ab]	-18.37

Data represent the mean± S.D. of observation from six rats
[a] Significant difference at P < 0.05 relative to the control group
[b] Significant difference at P < 0.05 relative to the INDO group

Data in Table 5 are showed significantly reduced GSH content in group exposure to indomethacin by about -58.3% as matched to the control group. Our results were in agreement with **AlKreathy *et al.*, (2020)** confirmed that indomethacin caused significant decrease in GSH content in indomethacin group compared to the control group. **Turkyilmaz and Yanardağ, (2023)** reported the same alterations in GSH and LPO levels in their study, which confirm the harmful effects of INDO in the tissue. The present results are in accordance with report by **(Khan *et al.*, 2019**).

In the same Table, groups (OCJ and PCJ) whose treatments by (orange and purple) carrot juice caused an improvement in GSH activity with change percent (+8.84 and +15.64%) respectively compared to control group. In addition, markedly demonstrated

increase GSH activity in OCJ+INDO and PCJ+ INDO groups with change percent (-25.17 and -18.37%) respectively compared to control group; it led to that carrot has a stronger anti-oxidative effect **(Rasheed *et al.*, 2022)**. Also, groups pretreatment by carrot juice (OCJ+INDO and PCJ+ INDO) caused reversed the levels of these parameters and significant increase (1.80 and 1.96 folds approximately) respectively in GSH content compared to INDO group.

It is not easy to interpret the meaning of the increase or decrease of antioxidant enzymes; enzymes are used up or increase the generation when the oxidizing stress is high, because the human body works to maintain the physiological homeostasis **(Lee *et al.*, 2011).** In this study, carrots are rich in vitamin C and carotene **(Teshome *et al.*, 2024**); vitamin C protects GSH from further oxidation, and helps in protects the membrane structure from unwanted; also carotene supports lipid protection **(Ali *et al.*, 2020; Turkyilmaz and Yanardağ, 2023).**

- Effect of OCJ and PCJ on Cyclooxygenase, Nuclear factor kappa of B cells and β-ACTIN of gastric ulcerated rats:

Results in Table (6) show the effect of feeding on OCJ and PCJ on cyclooxygenase, nuclear factor kappa of beta-cells and β-actin of indomethacin ulcerated rats. Groups were treated with OCJ and PCJ have a near COX value of control group were (2.673, 2.603 and 2.51 u/mg) respectively. In the same Table results showed that group treatment with INDO caused significant increases ($P < 0.05$) in gastric COX compared to control group and the rest of treated groups. On other hand value of COX for INDO group was showing higher value than control group were (5.63 and 2.51 u/mg) respectively as change percent was (+ 124.42%). Also, the values of OCJ + INDO group and

PCJ + INDO group showed improved and decreased value of COX being (3.827 and 3.393 u/mg) respectively; where's the percent of change was (+52.6 and +35.6%) respectively compared to control group.

Table (6): COX, NF-kB and β- actin of gastric ulcer rats treated with OCJ and PCJ

Groups	COX (u/mg)	% of change	NF-kB (g/ml)	% of change	β -ACTIN (g/ml)	% of change
Control	**2.51± 0.03**	**0.00**	**1.52±0.02**	**0.00**	**3.23±0.017**	**0.00**
OCJ	**2.673±0.02**	**+6.37**	**1.71±0.09**	**+12.5**	**3.50±0.26**	**+8.36**
PCJ	**2.603±0.021**	**+3.7**	**1.60±0.02**	**+5.26**	**3.37±0.06**	**+4.33**
INDO	**5.633±0.025[a]**	**+ 124.42**	**3.61±0.01 [a]**	**+137.5**	**8.38±0.45 [a]**	**+159.44**
OCJ + INDO	**3.827±0.252 [ab]**	**+52.6**	**2.14±0.02 [ab]**	**+40.79**	**5.18±0.14 [ab]**	**+60.37**
PCJ + INDO	**3.393±0.531 [ab]**	**+35.6**	**2.01±0.015 [ab]**	**+32.24**	**5.10±0.03[ab]**	**+57.89**

Data represent the mean± S.D. of observation from six rats
[a] Significant difference at P < 0.05 relative to the control group
[b] Significant difference at P < 0.05 relative to the INDO group

As the result of pretreatment groups (OCJ, PCJ, OCJ + INDO and PCJ + INDO) have significantly inhibited the over expression of these inflammatory response COX cause of the anti-inflammatory. **Dias, (2014) and De Araújo *et al.*, (2018)** reported that carotenoids, polyphenols, vitamins and flavonoids such as anthocyanosides which were present in carrot act as antioxidants, anti-carcinogens, immune enhancers and anti-ulcerogenic properties. In addition, flavonoids possessed both cytoprotective and anti-secretion properties, may protect gastric epithelial cells from oxidative damage by decreasing

ROS production in gastric mucous and inhibiting COX-2 levels. Groups were treated with OCJ and PCJ, and the near values of control group were (1.71, 1.60 and 1.52 g/ml) respectively. In the same Table results were shown that group treatment with INDO caused significant increases ($P < 0.05$) in NF-kB value compared to control group and the rest of treated groups. Mean value of NF-kB for INDO group was shown higher value than control group being (3.61and 1.52 g/ml) respectively as change percent was (+ 137.5 %) compare to control group. Our results were agreement with **Lin *et al*., (2022) and Song *et al*., (2022)** reported that treatment with INDO caused increases in NF-kB.

On other hand, OCJ + INDO and PCJ + INDO groups have significantly decreased ($P<0.05$) of NF-kB content than the INDO group being (2.14, 2.01 and 1.52 g/ml). And have improved and decreased the change percent being (+ 40.79 and +32.24 %) respectively compare to control group. That as the result of pretreatment for rats with white OCJ and PCJ have significantly inhibited the over expression of this inflammatory parameter and attenuated inflammatory response NF-kB led to the anti-inflammatory activities of flavonoids, phenolic acids **(Zhao *et al*., 2018)** and carotenoids (**Kujawska *et al*., 2018).**

In addition, in the same Table results were shown that group treatment with INDO only caused significant increases ($P < 0.05$) in gastric β- actin compared to the control group and the rest of treated groups. Mean value of β- actin for INDO group was shown to be higher than control group were (8.38 and 3.23 g/ml) respectively as change percent was (+ 159.44 %) compared to control group.

On the other hand, OCJ + INDO and PCJ + INDO groups have significantly decreased (P<0.05) of β –actin value than the INDO group being (5.18, 5.10 and 8.38 g/ml). And have improved and decreased the change percent being (+60.37and +57.89%) respectively compared to control group. While, groups treatment with OCJ and PCJ have a near value of control were (3.50 and 3.37 g/ml) respectively. Our results were in agreement with data obtained by **Susanto *et al.*, (2022)** indicated that there was an effect of the carrot juice in preventing inflammation on the gastric histopathology ($p < 0.05$).

Carrot juice exert had a protective effect against gastric ulcer, shown by a decrease in the gastric ulcer index and score as well as the biochemical parameters in serum **(Sayed *et al.*, 2022).** And had possesses gastroprotective effect when consumed with aspirin and thus support the use of carrot as an alternative treatment **(Jiin *et al.*, 2014)**. Moreover, β- carotene in carrot has an anti-inflammatory and anti-pain **(Saputri and Yuliaswati, 2023).**

-Effect of OCJ and PCJ on histopathological examination on gastric ulcer rats:

A gastric ulcer was produced in the rat by a necrotizing substance such as indomethacin. As shown in Table (7) and figure (1) the histopathological examination of gastric tissue of rats from control group exhibited the normal histoarchitecture of gastric layers. In contrast, stomach of rats from INDO group showed histopathological damage characterized by focal necrosis of gastric mucosa (blue arrow), sub-mucosal inflammatory cells infiltration (red arrow) and edema (black arrow).

The development of gastric ulcer lesions in rats was prevented by pretreatment with orange and purple carrot juice respectively. Ulcer intensity was detected by ulcer area, as seen macroscopically in the

positive non-treated groups photo (1). Where's, gastric rats from OCJ and PCJ groups revealed histologically normal gastric tissue.

Gastric rats from OCJ + INDO group revealed from mild to moderate submucosal edema and from few to moderate inflammatory cells infiltration. Meanwhile, stomach of rats from PCJ + INDO group demonstrated normal histological structure of gastric tissue with mild submucosal edema. The effects ability of carrot juice (orang and purple) may refer to the enhancement activity of their phytochemical through increased secretion of mucus and formation a strength defensive cover on the mucosal surface of gastric.

Our findings were consistent with the results of many studies suggested that purple carrot bioactive compounds as carotenoids, which are inversely proportional to oxidative stress in the human body, as well as polyphenols which are effectively down regulated the secretion of certain pro-inflammatory markers **(Rasheed *et al.*, 2022);** decreased other pro-inflammatory factors, such as cyclooxygenase-2 (Cox-2), inducible nitric oxide synthase (iNos), and possess anthocyanins which able to cross the intestinal barrier and may potentially contribute to health effects in human body tissues **(Olejnik *et al.*, 2016).**

Poudyal *et al.*, (2010) explained that the anthocyanin is responsible for the antioxidant and anti-inflammatory properties of purple carrot juice to improve glucose tolerance as well as cardiovascular function. These phytochemical can play an important role in the treatment of inflammatory peptic diseases such as gastric ulcer because of their anti-inflammatory properties.

Table (7): Effect of OCJ and PCJ on histopathological examination of gastric ulcer rats

Histopathological lesion / Groups	Necrosis of gastric mucosa	Submucosal edema	Inflammatory cells infiltration
Control	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0
OCJ	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0
PCJ	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0
INDO	2,2,2,3,2	3,3,3,3,2	3,3,3,2,2
OCJ + INDO	0,0,0,0,0	2,2,1,1,1	1,1,1,1,2
PCJ + INDO	0,0,0,0,0	1,0,1,1,1	1,1,1,0,2

✳(0): indicated no changes; (1): indicated mild changes
(2): indicated moderate changes; (3): indicated severe changes

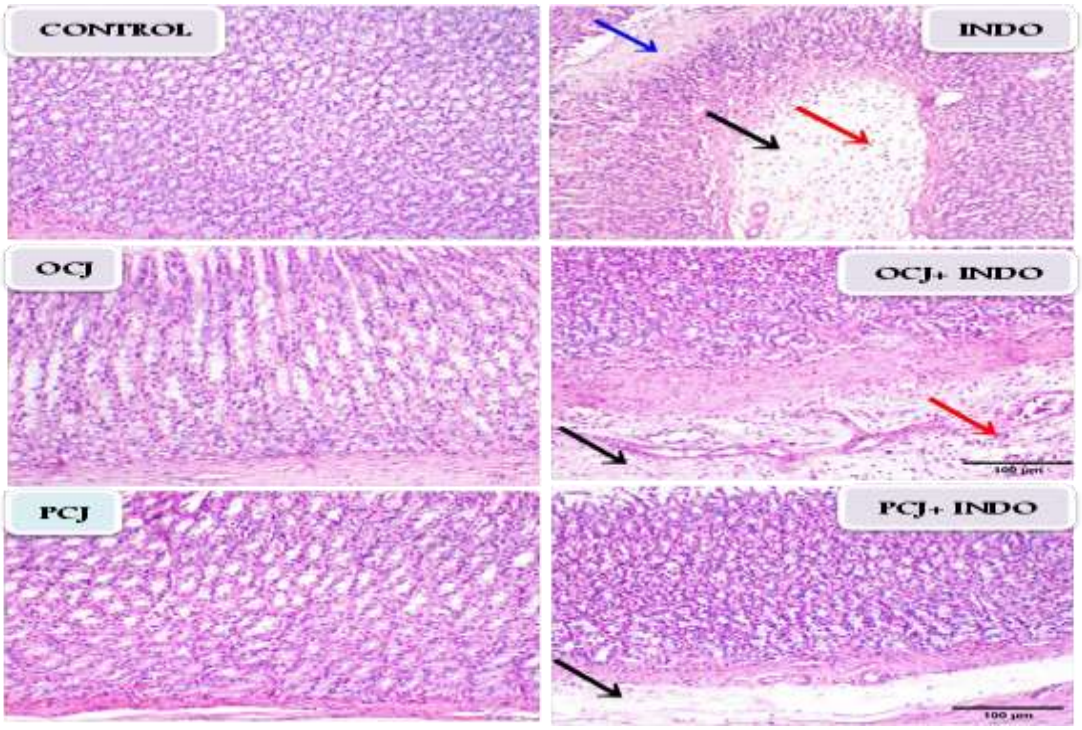


Figure (1): Photomicrograph of gastric rats

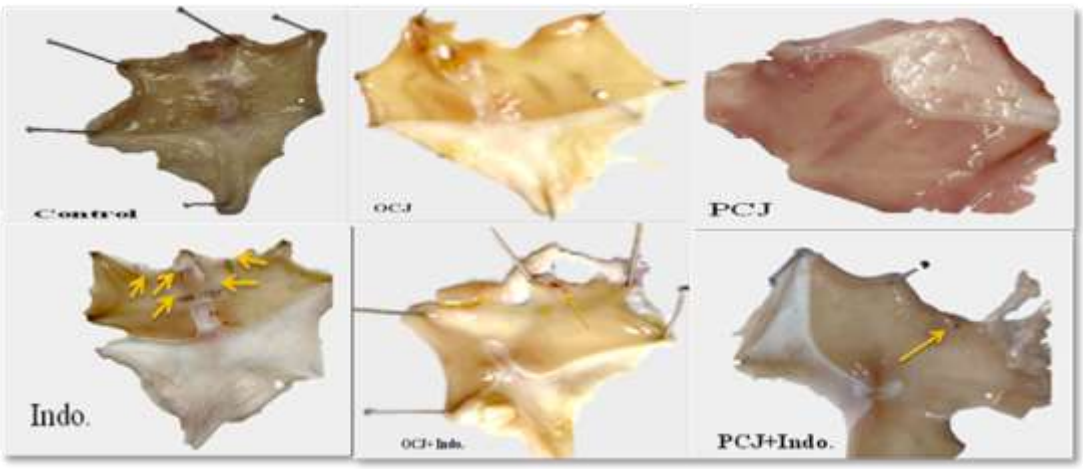


Photo (1): Macroscopical appearance of gastric ulcers in rats groups

Conclusion

This study demonstrates the potential of both carrot juices (orang and purple) in mitigating indomethacin-induced gastrointestinal damage in rats. The findings suggest that purple carrot juice has the ability to have protective effects against indomethacin complications, which are caused by their high content phyto- properties as high content of polyphenol, flavonoid, antioxidant activity and anthocyanin. The outcomes may contribute to the development of preventive strategies or complementary treatments for individuals using indomethacin for promoting gastrointestinal health and reducing the risk of adverse effects.

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مقارنة النشاط المضاد للقرحة لعصير الجزر البرتقالي والأرجواني في معدة الفئران

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المستخلص:

الجزر هو نبات جذري غني بالعناصر الغذائية يزرع ويستهلك عالميًا ويحتوي على مواد مضادة للالتهابات مثل الصبغات ومركبات البوليفينول الذي يمكن أن يكون بديلاً للوقاية من قرحة المعدة. والأدوية المضادة للالتهابات غير الستيرويدية مثل الإندوميثاسين يعالج الألم الحاد والتهاب المفاصل الروماتويدي وهشاشة العظام، ولكنها تسبب آثاراً ضارة على الجهاز الهضمي، بما في ذلك القرحة والالتهابات. لذلك، هدفت الدراسة الحالية إلى التحقيق ومقارنة تأثير عصير الجزر البرتقالي وعصير الجزر الأرجواني على تلف الجهاز الهضمي الناجم عن الإندوميثاسين في الفئران. لإختبار هذه التأثيرات لعصير الجزر البرتقالي وعصير الجزر الأرجواني في قرحة المعدة؛ تم فحص العلامات الكيميائية الحيوية والنسيجية لأنسجة المعدة في 36 فأراً تم تقسيمهم إلى ست مجموعات. أشارت النتائج إلى أن الإندوميثاسين تسبب في انخفاض كبير ($P < 0.05$) في درجة حموضة المعدة والميوسين. كما تسبب في زيادة في الحموضة الكلية والبيبسين مقارنة بمجموعة الكنترول بينما أظهر العلاج بعصير الجزر البرتقالي وعصير الجزر الأرجواني زيادة كبيرة في درجة حموضة المعدة والميوسين مع إنخفاض كبير في الحموضة الكلية ونشاط البيبسين. كان للغشاء المخاطي المعدي المتقرح للفئران بواسطة الإندوميثاسين نشاط منخفض في GSH(0,613 مجم / جم) و CAT (0.57 وحدة / جم بروتين) ومحتوى مرتفع من MDA (0.22 نانومول / جم) مقارنة بفئران التحكم (1.70613 مجم / جم و 0.81 وحدة / جم بروتين و 0.14 نانومول / جم) على التوالي. تسبب الإندوميثاسين في زيادات كبيرة ($P < 0.05$) في قيمة COX و NF-kB و β-actin مقارنة بالمجموعة الضابطة؛ بينما في المجموعات المعالجة بعصير الجزر البرتقالي وعصير الجزر الأرجواني كانت قيمة المجموعات قريبة من قيمة المجموعة الضابطة. تظهر النتائج أن عصير الجزر الأرجواني حسن المعايير الكيميائية الحيوية والهستوباثولوجية التي يسببها الإندوميثاسين. ونستنتج أن عصير الجزر الأرجواني له نشاط مضاد للقرحة ويمكن أن يساعد في تقوية نخر الغشاء المخاطي في المعدة الناجم عن الإندوميثاسين وإمكانية استخدامه لمنع قرحة المعدة.

الكلمات المفتاحيه: المركبات الحيوية للجزر، الإندوميثاسين، الفحص الميكروسكوبي والنسيجي لقرحة المعدة