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Studying Anti-inflammatory and Anti-Peptic Ulcer Effects of Aqueous Extract of Red Beetroots on Male Rats

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Abstract

The root vegetable *Beta vulgaris rubra* are known as red beetroot. Beetroot a powerful dietary source and has many health promoting agents that holds potential as therapeutic treatment for several pathological disorders. Beetroot is also being considered as a promising therapeutic treatment in a range of clinical pathologies associated with oxidative stress and inflammation. The present study was planned to find out the possibility anti-inflammatory and anti-peptic ulcer activity of water extract of beetroots in male albino rats. It was conducted on a total of 50 male albino rats of Sprague-Dawley strain weighing 175±5 g. Animals were maintained under standard condition and divided into two main groups (25 rats each) to investigate the anti-ulcer and anti-inflammatory effects of beetroot extract. The obtained results showed that aqueous extract of red beetroots extract had a significantly positive effect as anti-inflammatory and antiulcer effects which were more detectable with increasing doses of extract. Results concluded that regular intake of fresh or juice of beetroots had antiulcer and anti-inflammatory effects.

Keywords: Acute and chronic inflammation – Peptic Ulcer – Beetroots – Albino Rats.

Introduction

Inflammation is a complex immune process that can be defined by the sequential release of mediators such as pro-inflammatory cytokines (interleukin-1 (IL-1) and tumor necrosis factor (TNF)) and anti-inflammatory cytokines (IL-10, and reactive oxygen and nitrogen species). These mediators initiate the inflammatory response, recruit and activate other cells to the site of injury and subsequently resolve the inflammatory process. The inhibition of the overproduction of such mediators, especially pro-inflammatory cytokines, may prevent or suppress a variety of inflammatory diseases (Kim *et al.*, 2003). Multiple risk factors are known to exist that will attack cells at various parts of the body eliciting new inflammation sites hence new inflammatory diseases. Acute inflammation usually takes place first in response to the attack of the risk factor (s). As soon as the risk factor (s) is removed, the acute inflammatory response will stop. However, if the risk factor (s) continues to exist, the acute inflammation will progress to chronic inflammation.

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The chronic inflammation plays a major role in the pathogenesis of all inflammatory diseases (James and Lily, 2007).

Gastrointestinal tract is one of the largest organs in the body extends from the mouth to the anus and includes the pharyngeal structures, esophagus, stomach, liver and gallbladder, pancreas, and small and large intestine. It is extremely active in carrying out the physiologic and metabolic function of secretion, digestion, absorption and cellular replication (Kathleen, 2008). Peptic ulcer is a lesion in the mucosa of the stomach or duodenum in which acid and pepsin play a major role, the term is often used to encompass any gastric or duodenal ulceration (Debjit *et al.*, 2010). Peptic ulcer happens because of a discrepancy among the destructive factors (acid, pepsin, *Helicobacter pylori*) and protective factors (bicarbonate secretion, prostaglandins, gastric mucus, native confrontation of the mucosal cell (factors) (Kumar *et al.*, 2011). It is the ulceration of mucous covering piercing during the mucosa uncovered to most harmful agents like acid, pepsin, bile acids, food ingredients, bacterial products, certain drugs and pathological condition such as Zollinger – Ellison Syndrome, in stomach and duodenum. If the

ulceration occurs in stomach, it is called gastric ulcer and when it occurs in duodenum, it is called duodenal Ulcer (Emanuel and John, 1994). Peptic ulcer occurs frequently in men than in women. It is now well established that peptic ulcer can be prevented by amplification the self- protective mechanisms of gastric and duodenal mucosa rather than diminishing factors of assault causing ulceration (Hoogerwerf and Pasricha, 2011).

In recent years, focus on traditional plants research has increased all over the world and large evidence has been found to show immense potential of medicinal plants used in various traditional systems. Root vegetable *Beta vulgaris rubra*, are known as red beetroot has many attentions as a health promoting functional food (Ninfali and Angelino, 2013). Beetroot regularly consumed as part of the normal diet as salad or fresh juice, and commonly used in manufacturing as a food coloring agent known as E162 (Tom *et al.*, 2015). Also, beetroot have several beneficial physiological effects for several pathologies, such as hypertension, atherosclerosis, type2 diabetes and dementia (Gilchrist *et al.*, 2014). It is a rich source of phytochemical compounds includes ascorbic acid, carotenoids, phenolic acids and flavonoids (Wootton-Beard and Ryan, 2011) and betalains (Vulić *et al.*, 2014). Betalain family are categorized as betacyanin pigments (red-violet in color) and betaxanthin pigments (yellow-orange in color) (Ninfali and Angelino, 2013). A number of investigations have reported that betalains have high antioxidant and anti-inflammatory capabilities in vitro and a variety of in vivo animal models (Vidal *et al.*, 2014). Therefore, the present study was conducted to investigate the possibility of anti-inflammatory and anti-peptic ulcer activity of aqueous extract of beetroots in male albino rats.

Material and Methods

Plants

Fresh beetroots (*Portulaca Oleracea*) free from any physical defect were purchased from the local market in Makkah, KSA.

Animals and Diet

Fifty albino rats of Sprague-Dawley strain weighing 175 ± 5 g were obtained from the Laboratory Animal Colony, Medicine College, Umm Al Qura University, KSA. Basal diet constituents were purchased from Baghafar Company for Pharmaceutical and Chemical, Jeddah, KSA.

Drugs

Anti-inflammatory agent (Feldene (Piroxicam) and anti-ulcer agent (Zantac™ (Ranitidine) were

obtained in the form of ampoules from the local pharmacy in Makkah, KSA.

Preparation of extract

Fresh beetroots were washed with tap water to remove possible potential pathogenic microorganisms. Afterwards, the beetroots cut into small pieces of about 1 cm. Beetroots juice was prepared using juicer and then filtered through cloth for obtaining clear reed beetroot juice. Clear beetroots juice was concentrated at temperature of (50°C) using in an oven under vacuum until all the water had been removed to give an extract sample. Dried extract was dissolved in a mixture of carboxy methylcellulose and few drops of Tween 80 as a suspending agent to obtain 10%, 20 and 30% concentrated liquid extract.

Preparation of Basal diet

The basal diet (AIN-93M) was prepared according to Reeves *et al.*, (1993). It consists of casein 20%, soybean oil 5%, Choline chloride 0.20%, vitamin mixture 1.0%, mineral mixture 4.0%, fibers 5%, L-Cystine 0.18%, sucrose 10% and the reminder was corn starch.

Experimental Design

Animals were maintained under standard conditions of humidity, temperature, alternating 12-hour light-dark cycle, fed on the basal diet and water *ad libitum* for one week before starting the experimental for acclimatization. After acclimatization period (one week), all animals (n= 50) were randomly assigned to main groups (25 each) to study anti-inflammatory and effect of reed beetroot extract.

Anti-inflammatory Test

Twenty five rats were divided into five groups (5 rats each) as follows: Groups (1) and (2) were fed on the basal diet only, received orally 1 ml/100g of saline solution and kept as a, positive control and standard groups, respectively. Groups (3), (4) and (5) fed on the basal diet and given orally extract by tube feeding for two weeks at a dose of 100, 200 and 300 mg/kg of body weight, respectively.

Anti-inflammatory study was achieved as described by (Northover and Subramanian, 1962). It depends upon induction of pedal inflammation in rats paw by 0.1 ml of formalin 4%. At the end of experimental period (14 days), the second group was given (I/P) intraperitoneally Feldene (Piroxicam) as anti-inflammatory agent in a dose of 4 mg/kg of body weight. After one hour of the treatment, each rat in all groups was injected with 0.1 ml of formalin 4% in the plantar side of the right hind paw. The paw thickness caused by formalin was measured using skin caliber immediately and every two hours till 10

hours after injection. The difference between subsequent readings gave the actual edema volume. Anti-inflammatory effect was assessed by the reduction in the thickness of rat's paw.

Anti-peptic test

Twenty five rats were divided into five groups (5 rats each). Group (1) served as positive control group (untreated group), and group (2) served as standard group (treated with antiulcer agent). Both groups were fed on the basal diet and given orally saline solution at volume of 1.0 ml / 100 g of body weight. Groups (3), (4) and (5) served as tested groups. Groups (3), (4) and (5) were fed on the basal diet and given orally beetroot extract by tube feeding for two weeks at a dose of 100, 200 and 300 mg/kg of body weight, respectively.

At the last day of experimental period (14 days), all rats were starved of food but not of water for 18 hours. After fasting period, standard group (2) was given (I/P) intraperitoneally Zantac (ranitidine) at a dose 6 mg/100g body weight, 60 min prior administered ethanol. Groups (1 and 2) were given orally saline and the other three groups were given beetroot extract, 120 min prior administered ethanol (Jafri et al., 2001). Then ethanol was administered orally to all groups at 0.5ml/100g (Hollander et al., 1985).

Determination gastric juice volume

After four hours of ethyl alcohol administration, all animals were sacrificed using an overdose of diethyl ether and their stomachs were taken out and washed by normal saline. The gastric juice for each animal was collected in clear tubes and centrifuged at 3000 rpm for 10 min. The supernatant gastric juice was put in a measuring cylinder to measure the volume of the gastric contents. The measuring cylinder was a minimum graduation of 0.1ml.

Determination of gastric juice PH

The supernatant of stomach content was decanted off in a clean container. The pH was measured by using digital pH meter (pH scan-2, Eutech Cybernetics Pvt.Ltd., Singapore). The pH meter was measured the pH up to one decimal digit with ± 0.1 pH variation.

Gastric Ulcer Index

The method described by Agrawal et al., (2000) was employed in the present study. In brief, after 4 hours of administered ethanol, all rats were sacrificed after using an overdose of diethyl ether and their stomachs removed and washed by saline. The gastric juice was collected in test tube. Then stomachs opened along the greater curvature, washed with saline and examined under dissecting microscope for gastric ulcers. The sum of length for all lesions area for each

animal was measured and served as the ulcer index. The curative ratio was calculated for each group using following equation:

$$\text{Curative ratio (CR)} = (\text{LC-LT} / \text{LC}) \times 100$$

LC: The length of gastric ulcer in positive group.

LT: The length of gastric ulcer in treated group.

Statistical Analysis

The results were expressed as mean \pm SD and statistical significance was assessed using one-way analysis of variance (ANOVA) test. Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 16.00, Chicago, USA).

Results and Discussion

Anti-inflammatory Test

The effect of aqueous extract of beetroot at the three different doses (100, 200 and 300 mg/kg of body weight) on paw's thickness (edema) of rats are recorded in Table 1. The present results revealed that administration of beetroot extract at a dose of 100 mg/kg b. wt. caused no significant changes ($p < 0.05$) in paw's edema at the 2hrs, post administration, while, administration of extract at doses of 200 and 300 mg/kg b. wt. caused significant reduction in paw's thickness compared to that of treated rats with anti-inflammatory agent.

On the other hand, administration of beetroot extract at a dose of 200 and 300mg/kg b. wt. caused significant ($p < 0.05$) decrease in paw's thickness (mm) at the 4, 6, 8 and 10 hrs. post administration, while, administration of beetroot extract at a dose of 100mg/kg b. wt. caused no significant changes compared with that of treated rats with anti-inflammatory agent.

In an effort to identify the effects of beetroot on acute inflammation and peptic ulcer in male albino rats, we performed the presented study on 50 male albino rats. The inflammatory process is itself a pathological process, whereas the natural anti-inflammatory response that ensues after acute inflammation tends to reverse tissue homeostasis towards normality and should therefore be regarded as a true defensive reaction of the affected tissue (Srdan, 2012). Moreover, reactive oxygen species (ROS) are formed in both physiological and pathological conditions in mammalian tissues. The uncontrolled production of free radicals is considered to be an important factor in tissue damage which can induce pathophysiological changes. Free radicals also play an important role in inflammation that can mediate tissue destruction (Kottarapat et al., 2013). In the present study the determination of anti-inflammatory activity of beetroot was based on measurement of edema produced

by injection with formalin 0.1 ml (4%) in of right paw of rat by using caliper. Anti-inflammatory effect was assessed by the reduction in the thickness of rat's paw. The obtained results revealed that treated rats with aqueous extract of beetroots at 100, 200 and 300 mg/kg of b. wt had significant anti-inflammatory effects as showed by decreasing signs and paw's thickness (edema), compared with that of treated with anti-inflammatory agent at the 2, 4 and 6 and 8 hrs post administration.

The present results were agreed with (Chakole et al., 2011) who demonstrated that anti-inflammatory activity of beetroot extract may be related to its effect on inhibits the release of histamine, serotonin kinas and prostaglandin in the first hour post-administration of formalin In addition, several other reports reported that beetroot extract has betalains (E162) which is water-soluble, nitrogen-containing pigments .They are divided into two groups: the red violet in colour (betacyanins pigments) and the yellow-orange in colour (betaxanthins pigment) (Tom et al., 2015 and Fernando et al., 2016). Betalains have high antioxidant and anti-inflammatory capabilities in vitro and a variety of in vivo animal models (Tom et al., 2015). Also, beetroot contains carotenoids and flavanoids which have antioxidants properties and can help reduce the oxidation (Kumar, 2011).

Anti-Peptic Ulcer Test

pH of Gastric Juice

pH values of gastric juice of treated rats with antiulcer drug and orally administration of beetroots extract at the three different doses are presented in Table (2). The recorded results demonstrated that treated rats (standard group) with antiulcer drug and treated rats with the three different doses of beetroots extract (100, 200 and 300 mg/kg b. wt) had significant increase in pH values of gastric juice at $p < 0.05$ as compared to that of untreated rats (positive group). In contrast, administration of beetroots extract at the three different doses significantly increased pH values of gastric juice as compared to that of standard group. The increase in pH values of gastric juice was more detectable with increasing the dose of beetroots extract.

Volume of Gastric Juice

Volume of gastric juice (cm^3) in treated rats with antiulcer drug and different doses of beetroots extract recorded in Table (2). Data obvious that volume of gastric juice (cm^3) of treated rats (I/P) with antiulcer drug (standard group) and given rats orally different doses of beetroots extract had significant decrease in the volume of gastric juice at $p < 0.05$ as compared to that of the positive group. Administration of

beetroots extract at doses of 200 and 300 mg/kg of b. wt induced a significant decrease in the volume of gastric juice as compared to that of the standard rats. The decrease in volume of gastric juice was more detectable with increasing the dose of beetroots extract.

Length of Gastric Ulcer and Curative Ratio

The lengths of gastric ulcers (mm) in untreated and treated rats with aqueous extract of beetroots at the three different doses are illustrated in Table (3). Tabulated results revealed that the length of gastric ulcer (mm) as mean \pm SD of treated group with antiulcer drug (standard group) was significant decrease at $p < 0.05$ as compared to that of the positive group. Groups given orally beetroots extract at doses of (100, 200 and 300 g/kg of b. wt) had significant decrease in the length of gastric ulcer at $p < 0.05$ as compared to that of the positive groups. Rats given orally the beetroots extract at a dose of 200 and 300mg/kg of b. wt had significant decrease in the length of gastric ulcer at $p < 0.05$ as compared to that of the standard group.

Mean values of curative ratio of treated rats with aqueous extract at doses of 100, 200 and 300 mg/kg of b .wt were higher compared with that of treated rats with antiulcer drug. The decreasing in gastric lengths and curative ratio of treated rats with beetroots extract was more detectable with increasing the doses of beetroots extract.

A peptic ulcer is a sore on the inner lining of the stomach or duodenum—the first part of the small intestine. Gastric hyperacidity and ulcer are very common causing human suffering today. Therefore, the present study was done to investigate the antiulcer effect of oral administration of aqueous extract of red beetroots and compare it with antiulcer drug as a reference of antiulcer. The present results showed that oral administration of aqueous extract of red beetroots at the tested three different doses (100, 200 and 300mg/kg of b. wt) had significant increased in pH values and decreases in volume of gastric juice as well as decreases in the length of gastric ulcers compared to untreated group. The positive effect of of aqueous extract of red beetroots on pH values and volume of gastric juice, lower gastric ulcer length and curative ratio were more detectable with increasing the dose of plant juice. Our results was agreed with Karimi et al., (2004) who studied the aqueous and ethanolic of whole plant extracts in mice for their ability to inhibit gastric lesions induced by HCl, absolute ethanol and pylorus-ligation, and compare it with sucralfate. They demonstrated that both extracts

showed a dose-dependent reduction in severity of ulcers. The highest dose of extracts exerted similar activity to sucralfate. The oral administration of extracts reduced the gastric acidity in pylorus-ligated mice. On the other hand, Kumar et al., (2011) investigated gastro-protective effect of ethanolic extract of beet roots in different gastric ulcer models in rats. They showed that dose dependent inhibition of ulcer index with maximum index reduction in ethanol and minimum in aspirin induced ulcer. Sunil et al., (2014) founded that extract of beetroots induced noticeable protection of the gastric mucosa against the acid attack, protect the gastric mucosa significantly and cure the ulcerations. Gastroprotective activity of aqueous extract of red beetroots might be due to gastric defense factors. Extract also prevents the oxidative damage of gastric mucosa by blocking lipid peroxidation and by significant decrease in superoxide dismutase, and increase in catalase activity (Kumar et al., 2011). The antioxidant property of beetroots is attributed to its constituents, such as gallo-tannins, omega-3 fatty acids, ascorbic acid, α -tocopherols, kaempferol, quercetin, and apigenin (Zhu et al., 2010).

Conclusion

On the basis of above results, it could be concluded that beetroots has a potent anti-inflammatory and anti-ulcer effect in rats. It may be stated that beetroots extract contains the active anti-inflammatory and antiulcer agent. Hence, regular intake of beetroots as or using it for enriching food product may help functional foods to improve health status.

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Table 1: Effect of reed beetroots extract on the paws thickness (mm) after induction of pedal rat's inflammation

Time after inflammation induction	Paw's thickness (mm) as Mean ± SD				
	Positive group	Standard group	Treated group with 100 mg/kg b.wt.	Treated group with 200 mg/kg b.wt.	Treated group with 300 mg/kg b.wt.
At the 0 hrs	4.08 ± .8 ^a	4.08 ± 0.8 ^a	4.04 ± .05 ^a	4.04 ± .09 ^a	4.04 ± 0.05 ^a
At the 2 hrs	8.24 ± 0.82 ^a	7.28 ± 0.83 ^a	8.06 ± 0.13 ^a	6.14 ± 0.71 ^b	5.28 ± 0.16 ^c
At the 4 hrs	8.98 ± 0.57 ^a	8.70 ± 0.42 ^{ab}	8.06 ± 0.53 ^b	7.14 ± 0.65 ^c	6.82 ± 0.08 ^d
At the 6 hrs	9.50 ± 0.37 ^a	7.98 ± 0.44 ^b	7.64 ± 0.23 ^b	6.36 ± 0.87 ^c	5.26 ± 0.05 ^d
At the 8 hrs	9.32 ± 0.24 ^a	6.74 ± 0.39 ^b	6.98 ± 0.26 ^b	5.80 ± 0.76 ^c	4.92 ± 0.11 ^d
At the 10 hrs	9.32 ± 0.44 ^a	5.78 ± 0.38 ^b	6.02 ± 0.044 ^b	5.24 ± 0.54 ^c	4.70 ± 0.10 ^d

Different superscript letters in the same row denotes significant differences at P<0.05

Table 2: Effect of antiulcer drug and oral administration of beetroots extract on volume and pH value of gastric juice in rats

Groups	Parameter as Mean ± SD	
	Volume of gastric juice (ml)	pH of gastric juice
Positive group	3.34 ± 0.11 ^a	4.50 ± 0.21 ^d
Standard group (treated with drugs)	2.14 ± 0.09 ^b	5.68 ± 0.22 ^c

Treated groups with beetroots extract at a doses of:	100mg/kg of b.wt	2.22 ± 0.18 ^b	6.46 ± 0.23 ^b
	200mg/kg of b.wt	1.36 ± 0.17 ^c	7.80 ± 0.26 ^a
	300mg/kg of b.wt	1.26 ± 0.17 ^c	8.20±0.12 ^a

Different superscript letters in the same column denotes significant differences at p<0.05.

Table 3: Effect of antiulcer drug and oral administration of beetroots extract on the length of gastric ulcer (mm) and curative ratio in rats

Groups		Parameter as Mean ± SE	
		Length of gastric ulcer (mm)	Curative ratio (%)
Positive group		a 7.82 ± 0.81	0
Standard group (treated with drugs)		c 3.56 ± 0.27	54.47
Treated groups with beetroots extract at a doses of:	100mg/kg of b.wt	b 5.14 ± 0.13	34.27
	200mg/kg of b.wt	d 1.48 ± 0.22	81.01
	300mg/kg of b.wt	0.92±0.12 ^e	88.25

Different superscript letters in the same column denotes significant differences at p<0.05.

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