Ameliorative effects of ribes rubrum oil against gastric ulcers caused by indomethacin in experimental models

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ABSTRACT

The objective of this study was to assess the anti-inflammatory effects of ribes rubrum oil at three different doses (5, 10 and 15 ml/kg b.w/day) in adult male albino rats with indomethacin-induced stomach ulcers (IND). Forty rats (135 ± 5 g) categorized into 5 groups (n = 8), for 45 days. Group (1) normal control, thirty-two rats were gavaged IND as single oral dose (30 mg/Kg b.w) resulted in gastric ulcer, then distributed to four groups, group (2) IND-intoxicated control, Groups 3, 4 and 5 were administrated ribes rubrum oil at levels of (5, 10 & 15 ml/kg b.w) respectively. Administrated levels of ribes rubrum oil found to have remarkable elevation in food conversion efficiency in experimental rats, gastric juice pH, in compared to the drunken control group, gastric prostaglandin E2 and gastric cytochrome P450 reductase levels were lower. The levels of pro-inflammatory cytokines NO, TNF-α, and IL-1 were dramatically reduced, which was related with an increase in blood hemoglobin (Hb), packed cell volume (PCV), and red blood cells (RBCs)in ulcerogenic rats compared to intoxicated control. Data showed that, the main components of ribes rubrum oil are α-Pinene, γ-linolenic and Linalool oxide levels (25.9%, 23.10% and 10.5%, respectively) for their antioxidant activity. Findings showed that administrate ribes rubrum oil at dose 15 ml/kg followed by 10 ml/kg had the best results against ulcerogenic rats. In conclusion, the outcomes are consistent with the concept that ribes rubrum oil had a gastroprotective and antisecretory effects against gastric ulcer that may be attributed to the antioxidant properties of the oil that ameliorates the damage occur in gastric of rats.

1. Introduction

One of the most common gastrointestinal tract pathogens is gastric ulcer that harmed humans for centuries. Exogenous factors that because peptic ulcers include the use of nonsteroidal anti-inflammatory medicines (NSAIDs), anxiety, nutritional inadequacies, smoking, and a hereditary propensity. In addition to intrinsic variables such as gastric acid, pepsin exudation, gastric microcirculation, prostaglandin E2 (PGE2) content, and interleukin (IL)-1 release, all of which play a significant role in the course of gastric mucosal injury (Wang et al., 2011).

Non-steroidal anti-inflammatory medicines are used as costly financial burdens throughout the world. Indomethacin is an anti-inflammatory non-steroid that reduces fever, pain and inflammation. It works by reducing the production of prostaglandins like ibuprofen and naproxen (Takeuchi et al., 1991). Indomethacin prevents and reduced prostaglandin levels in enzymes producing prostaglandins (COX1 and COX 2). The result is reduced fever, pain and swelling. Indomethacin in an extended release form is available (De La Lastra et al., 2002). Nowadays, gastric ulcer therapy is severely hampered because the vast majority of drugs on the market today have low efficiency against gastric disease and frequently have severe side effects.

Herbal medicines are in constant expansion globally for the prevention and treatment of diverse illnesses. In the Gooseberry family Grossulariaceae, Ribes rubrum (Ribes rubrum L) is a part of a genus of Ribes. Ribes rubrum is a good source of anthocyanins. A diverse range of phenolics also contains high antioxidant levels and major polyunsaturated acids with pungent constituents, such as α-linolenic acid and μ-linolenic acid, that are distinguishable among plants oils, in particular ascorbic acid and minerals, in particular potassium, calcium and magnesium. Polyunsaturated fatty acids:
acids are quickly affected to oxidation, but somehow, they are sta-
bilized in the intact seeds, suggesting existence of powerful natural
antiozonants that makes ribes rubrum an interesting target of
functional foods (Leskien et al., 2009; Nour et al., 2011). Further-
more, extracts of ribs seed exert health effects to many diseases,
with many biological actions that attributed to their antioxidant
activity (Godovac et al., 2012). As a source of gamma-linolins and
tocopherols, rib oils offer significant promise, and contain rela-
tively large concentrations of these chemicals. Gamma linolenic
acid is a vital human component since it intermediates chemicals
with many biological actions that attributed to their antioxidant
activity (Godevac et al., 2012). Numerous researches have shown that gamma linolenic acid acts as a strong scavenger and has several health benefits. The effects are anti-inflammatry and antiproliferative, decrease body fat and facilitate beta-oxidation of fatty acids in the liver (Aneta et al.,
2013; Takada et al., 1994), it works as a powerful cytotoxic agent
for superficial gallbladder cancer (Makarova and Eremeeva, 2015;
Solomon et al., 1998). Medicinal plant intervention entails financial
strain and has an uncertain reported incidence (Okewumi and
Oyeyemi, 2012). The present investigation was therefore conceived
to hypothesize the potential protective impact of rubber rib natural
oil on gastrical mucosal injury caused by indomethacin to rats.

2. Material and methods

Plant material: Agricultural Research Centre, Giza, Egypt, was
the source of ribes rubrum oil for conducting this study.

Experimental animals: Forty male albino rats of the Wistar
breed weighing 1255 g were acquired from Animal Colony in Hel-
wan, Egypt. The animals were fed a formulated food and water
ad libitum for seven days prior to the trial, according to NRC
(Council, 1995), and were monitored.

Drugs: Indomethacin (IND) was obtained from SEDICO Pharma-
ceutical Company, Giza, Egypt.

2.1. Methods

DPPH Radicals Scavenging activities: The ribes rubrum oil
antioxidant activity was measured using the DPPH assay
(Malenčič et al., 2007).

GC–MS (Gas Chromatography–Mass Spectrometry): Oil of
Ribes rubrum were analyzed according to Adams et al studies with
slight modification (Adams, 2001). The GC equipment was an Agi-
 lent technology (HP) 6890 system with an HP-5MS (60 m 0.25 mm,
film span 0.25 ml) capillary column. The program began with the
oven temperature at 40 °C, which was maintained for 1 min before
being increased to 230 °C at a rate of 3C/min and maintained for
10 min. Helium was used as a carrier gas at a flow rate of 1.0 ml/
min. The detector and injector had temperatures of 250 and
280 °C, respectively. The HP 6890 GC analysis was performed on
the same capillary column as previously on a 5973–network mass
selector detector with carrier gaseous helium at a rate of one mI/
min and a split rate of 1/50. The petroleum compounds were iden-
tified by comparing their retention indices (RI), mass spectrum
fragmentation to those found in the Wiley 7n.1 mass computer
library, NIST (National Institute of Standards and Technology).

Indomethacin-induced gastric ulcer: According to Sayanti
et al a single indomethacin dose (30 mg/kg body weight) was
given to rats to create stomach damage (Bhattacharya et al., 2007).

Experimental procedures: All rats were fed a baseline diet for
one week prior to the start of the experiment, then the forty rats
categorized into 5 groups (n = 8), for 45 days. Group (1) normal
control, the rest thirty-two rats were gavaged IND as single oral
dose (30 mg/kg b.w) resulted in gastric ulcer, then distributed to
four groups, group (2) IND-intoxicated control, Groups 3, 4 and
5 were administrated ribes rubrum oil at levels of (5, 10 &
15 ml/kg b.w) respectively, orally by stomach tube. Weekly food
intake and weight growth were calculated. The food efficiency
ratio (FER) was developed in accordance with the plan of
(Chapman et al., 1959). The experimental methodology was
followed in compliance with European Community Directive
2010/63/EU. After fasting overnight, after ether anesthesia, rats
were slaughtered at the end of the experiment. The blood samples
have been obtained from the vein of the hepatic portal, a little
portion of the tube has been extracted and the remaining parts left
at room temperature to clot for 15 min then centrifuged for 20 min
at 3000 rpm. Serum has been properly separated and transformed
into clean plastic pipes which have been fairly fit and frozen at
−20 °C until the analysis is complete.

Determination of Gastric Ulceration: With a minor nick, the
stomach fundus was drilled at a higher stomach curvature. The lar-
gest stomach curvature was opened and injected with the cardiac
and pyloric end ligation by distilling water (3 ml). It was collected
and measured the volume and pH of the accumulated stomach
fluid. In the ulcer index calculations, gastric mucosa was found
under the lupus glass (Kiliç et al., 2006). Ulcer index (UI) and cura-
tive ratio were recorded along with (Parmar and Desai, 1993) by
the formula:

\[
\text{UI} = 1 \times (\text{number of lesions of grade 1}) + 2 \times (\text{number of lesions of grade 2}) + 3 \times (\text{number of lesions of grade 3})
\]

Then a factor of 10 divided the overall result, which was identified as an index of the
ulcer. Also, the curative ratio was recorded for each group as
following:

“Curative ratio = (length of gastric ulcer in control positive group – length of gastric ulcer in treated group / length of gastric
ulcer in control positive group) × 100”.

2.2. Biochemical analysis

Analyses of blood parameters: Hemoglobin, packed cell vol-
ume and Red blood cells (RBCs) were estimated respectively
(Cynthia et al., 1993; Drabkin, 1949; MC-INORY, 1954). The serum
was collected from the left blood after coagulated and centrifuged
for 15 min at 3000 rpm. Nitric oxide (NO), interleukin 1 (IL-18),
and tumor necrosis factor alpha (TNF-α) levels were all measured
(Beutler et al., 1985; Grassi et al., 1991; Green et al., 1982).

Gastric mucosa determination: The gastric mucosa was deter-
mined in accordance with the criteria for cyclooxygenase gastric
(cox-2) cytochromes; prostaglandin E2 (PGE2), cytochrome P450
reductase (cytopatamina-450) (Hamberg and Samuelsson, 1973;
Hemler and Lands, 1976; McLean and Day, 1974).

Statistical analysis: The results were explored in one-way ana-
lyze of variance followed by the Multiple Range Test by Duncan
using version 11 of SPSS (SPSS, Chicago, IL) (DMRT) (Snedecor
and Cochran, 1969). P < 0.05 was chosen for statistical mean-
ing (Khan et al., 2019).

3. Results

GC–MS profiling of oil of ribes rubrum revealed the presence of
6 different phytoconstituents consisting of β-Pinene (25.9%), fol-
lowed by γ-linolenic (23.10%), Linalool oxide (10.5%), 1,8-Cineol
(4.6%), α–Epi bisabolol (3.55%) and Myristicin (2.17%) respectively
(Table 1). The statistical data in Table 3 predicted that, intoxicated
rat group showed decline in food conversion efficiency in IND
administrated intoxicated group, while treated with (5, 10 and
15 ml) ribes rubrum oil showed no significant difference in final
weight, weight gain, food intake and FER comparing with normal
control. The presented data in Table 4 indicated that indomethacin
administration effectively induced ulcer in rats which was confirmed by elevated volume levels of gastric juice allied with momentous reduce in intoxicated rat group PH. On the other hand, treating with ribes rubrum oil with different levels produced a momentous reduces in volume of gastric juice and a noteworthy elevation in PH compared to intoxicated control group. Significant decrease was witnessed on values of gastric ulcer index of all treated rat groups in contrast with intoxicated group. Treating with ribes rubrum oil at doses of (5, 10 and 15 ml) showed no significant difference in curative ratio percentage. On the other hand, the ribes rubrum oil at doses of (5, 10 and 15 ml) treated groups showed no significant difference of Hb, PCV and RBCs levels in comparison to normal control (Table 5).

Table 6 reported that the delivery of indomethacin to rats resulted in a significant (p < 0.05) decrease in rats’ levels of gastric COX-2 and PGE2 (prostaglandin E2) and momentous increase in ROS), commencement of lipid peroxidation and inhibition of prostaglandin synthesis may be ascribed to the ulceration generated by indomethacin. The decreased prostaglandin level undermines nearly every component of gastritis and increases acid discharges.

4. Discussion

The most abundant fatty acids in ribes rubrum oil were β-Pinene and γ-linolenic acids, comprising 49% of the total fatty acids. γ-linolenic is a vital fatty acid for human and an intermediary component in the formation of prostaglandins, tromboxanes and prostacycins (Paccalin et al., 1985). Crucial rib oil fatty acids have been shown to be anti-inflammatory and anticancer (Goffman and Galletti, 2001). Furthermore, several studies demonstrated that γ-Linoleic acid is a powerful antioxidant, having various health benefits, such as reducing body fat and reducing hepatic beta-oxidation in fatty acid, increase muscle and bone strength, boost and strengthen an immune system (Makarova and Eremeeva, 2015; Takada et al., 1994). These results were supported with measuring Antioxidant activity (as DPPH) for Ribes oil that presented in Table 2. DPPH radical scavenging activity of oil assorted from 5 to 20 (µg/ml) with high antioxidant activity for (15 µg/ml) with 97.3% followed by (20 µg/ml) with antioxidant activity 93.2%, (10 µg/ml) with 87.7% and (5 µg/ml with 75.4%). These results are in parallel with the finding of (Velioglu et al., 1998) as they reported that the antioxidant of ribes rubrum was 90.86% with total phenolics 1421(mg GAE/100 g).

This decrease in nutritional parameters may be due to decrease of appetite, disturbance of stomach enzymes’ secretions, with alterations in the PH of the gastric secretion addition to neuropeptide-Y hormone levels. These results are in correlation with the findings of previous studies (El-Metwally, 2014; Haithem et al., 2014; Helmy, 2011). Using ribes rubrum oil (RRO) in treatment of ulcer at doses of (5, 10 and 15 ml) resulted in significant elevation in food conversion efficiency compared to intoxicated group. These data may be based on the bioactive characteristics of oil with antioxidant and its pharmaceutical effects on the healing of gastric ulcers (Chiang et al., 2005; Makri and Kintzios, 2008).

Individual mechanisms, like production of reactive oxygen (ROS), commencement of lipid peroxidation and inhibition of prostaglandin synthesis may be ascribed to the ulceration generated by indomethacin. The decreased prostaglandin level undermines nearly every component of gastritis and increases acid discharges.

**Table 1**

| Component       | Content (%) |
|-----------------|-------------|
| 1,8-Cineole     | 4.6         |
| β-Pinene        | 25.9        |
| Linalool oxide  | 10.5        |
| Myristicin      | 2.17        |
| α-Epi bisabolol | 3.55        |
| γ-linolenic     | 23.10       |

**Table 2**

Antioxidant activity of ribes rubrum oil.

| Parameters | Scavenging activity of DPPH radicals (%) |
|------------|-----------------------------------------|
| 5 (µg/ml)  | 75.4 ± 1.6                              |
| 10 (µg/ml) | 87.7 ± 1.7                              |
| 15 (µg/ml) | 97.3 ± 2.2*                             |
| 20 (µg/ml) | 93.2 ± 1.5                              |

**Table 3**

Food conversion efficiency in normal and IND administrated groups.

| Variables       | Normal Control | Intoxicated Control | Treated with RRO |
|-----------------|----------------|---------------------|-----------------|
|                 | 5 ml/kg        | 10 ml/kg            | 15 ml/kg        |
| Initial weight  |                |                     |                 |
| (g)             | 120.31 ± 3.45  | 122.24 ± 4.55       | 123.21 ± 3.55   |
| Final weight    | 236.08 ± 27.17 | 189.13 ± 17.37      | 226.35 ± 22.53  |
| Weight gain     | 115.77 ± 8.11  | 66.89 ± 6.11        | 103.14 ± 9.13   |
| Food intake     | 15.32 ± 2.14   | 13.55 ± 2.55        | 15.45 ± 2.42    |
| FER             | 0.125 ± 0.01   | 0.082 ± 0.03         | 0.111 ± 0.02    |

**Table 4**

pH, volume of gastric secretion, ulcer index and Curative ratio% of normal and IND administrated groups.

| Variables       | Normal Control | Intoxicated Control | Treated with RRO |
|-----------------|----------------|---------------------|-----------------|
|                 | 5 ml/kg        | 10 ml/kg            | 15 ml/kg        |
| PH              | 4.17 ± 0.53    | 2.03 ± 0.41         | 3.34 ± 0.17     |
| Volume of gastric juice (ml) | 2.14 ± 0.08 | 5.05 ± 0.16         | 1.53 ± 0.16     |
| Ulcer index (mm) |                | 2.18 ± 0.14         | 2.37 ± 0.38     |
| Curative ratio% |                | 54.13 ± 4.16        | 68.44 ± 6.89    |

* Values are expressed as mean ± S.D. n = 8 rats/group.
**Values not sharing a common superscript differ significantly at p < 0.05 (DMRT).
Hemoglobin (HB), packed cell (PCV) and Red blood cells (RBCs) of normal and IND administrated groups. Antioxidant activities (GoIzzo, 2000). It revealed that flavonoids protect gastric mucosa are in harmony with those obtained by prior results (Borrelli and 2013). The production of free radicals is reported to have a crucial role in NSAID pathogenesis (Taye and Saad, 2009). The current work, hematological values (Hb, PCV and RBCs) results were significantly (p < 0.05) reduced in indomethacin administrated rats compared to normal control. On the other hand, the ribes rubrum oil at doses (5, 10 and 15 ml) treated groups showed no significant difference of Hb, PCV and RBCs levels in comparison to normal control (Table 5). These results agree with the findings of previous studies who reported that in the development of many illnesses reactive oxygen species production (ROS) plays an important role (Chakraborty et al., 2012; Uduak et al., 2012). Free radicals also cause damage to cellular antioxidant enzymes, which operate as the first line of cellular defense. During gastric ulceration this can lead to increased tissue damage (AlRashdi et al., 2012). Table 6 results are consistent with the results of previous study (Whittle, 2003). The defense and repair processes of the mucosa include the COX-2-induced ulcerated gastric mucosa, and that inhibition of the mucosa is delayed through a specific COX-2 inhibitor. COX-2 is only found in stomach mesenchymal cells such fibroblasts and ulcer border inflammatory cells, implying that COX-2 is an important part of the ulcer repair process expressed in mesenchymal cells along the ulcer’s edge (Miura et al., 2004). Treatment with oil of ribes rubrum significantly inversely all subjects when comparing with indomethacin treated rats. These findings are in harmony with those obtained by prior results (Borrelli and Izzo, 2000). It revealed that flavonoids protect gastric mucosa injury by decrease prostaglandin mucosa levels and through inhibition by histidine decarboxylase of histamine release from mast cells. Ribes rubrum seems to have anti-inflammatory properties through decreasing the activity of COX-2 (Anea et al., 2013). Ribes rubrum oil, substance, rich in phenolics, knowing to possess antioxidant activities (Godnev et al., 2012; Leskinen et al., 2009). From Tables 1 and 2, data in this investigation demonstrated that ribes oil contain major phytoconstituents with high antioxidant activity, that responsible for the gastroprotective effect against indomethacin in rats. The ribes rubrum oil administration in indomethacin rats offers a significant improvement on all parameters tested. In treated groups of rats, oils of 5 ml, 10 ml and 15 ml of the rubber were similar to normal levels of the levels of all parameters. The antioxidant characteristics of ribs rubrum oil can be ascribed to these outcomes, which rectify and remedy damaging rats’ gastricity.

### 5. Conclusion

Gastric ulcers have become a complex condition and a serious socio-economic burden and face a huge difficulty in their treatment. A number of barriers have been encountered, including low effectiveness, numerous side effects and costs for gastric ulcers, with drugs and drugs for the treatment of gastric ulcer. Medical plants that are more secure, cost-effective and with low adverse effects are a replacement for gastric ulcers. Ribes rubrum oil therefore has a significant potential in combination with or alone, as a gastro-protective medication. It protected against the mucosal damage caused by indomethacin via its antioxidant characteristics and the ability to retain tissue and cellular integrity.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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