

Pharmacological assessment of the aqueous extract of rose oil waste from *Rosa x damascena* Herrm cultivated in Georgia

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Abstract

Among Rosaceae family's most popular and important plants *Rosa x damascena* Herrm. holds one of the top places due to its centuries-long application in perfumery, cosmetics, aromatherapy and medicine. Despite this, the chemical and pharmacological study of different products obtained from *R. damascena* still does not lose its relevance. Currently, considerable attention is paid to the valorization of the waste from rose oil production in order to fully utilize valuable physiologically active constituents. The present study provides data on some pharmacological properties of the aqueous extract of rose oil waste from the *R. damascena* cultivated in Georgia. In particular, evaluation of gastro- and hepatoprotective activity of the aforesaid extract has been carried out in animal models and revealed its efficacy. The extract appeared to dose dependently stimulate leucopoiesis (up to 75%) and prevent ethanol-induced gastric injury (20-80%). As well, the extract ameliorated hepatotoxic effects of carbon tetrachloride by 63%.

Keywords: *Rosa x damascena* Herrm; Aqueous extract; Flavonoids; Gastroprotective; hepatoprotective

1. Introduction

Rosa x damascena Herrm - one of the most famous representatives of the genus *Rosa* L., comprising more than 400 species [1] [2] has been introduced and grown in different countries, owing to its excellent decorative features and the presence of valuable product - essential oil in the flower petals. Since ancient times, rose oil and rose water have been used as raw material for cosmetics, perfumery, aromatherapy, and diverse medical purposes [3].

The yield of the rose oil from flower petals is quite low (0.030–0.045%), and oil production process generates a significant amount of waste (solid residues and waste water) that still contains biologically active compounds. Polyphenols, flavonoids and polysaccharides were found in wastes from the rose oil industry [4-6]. On the other hand, the same constituents determine various pharmacological activities of *R. damascena* including but not limited to anti-inflammatory [7] [8], antioxidant [7] [9] [10] [11], and analgesic [12].

Recently, industrial production of rose oil from local cultivar of *R. damascena* have been established in Kakheti region of Georgia, in present study, we attempted to evaluate some pharmacological properties of the aqueous extract (RDE) of rose oil waste from the *R. damascena* Georgian cultivar. It is established, that the predominant constituents of RDE

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are represented by flavonoids [13] [14]. Flavonoids are of the most diverse and widespread groups of plant secondary metabolites well known for having a broad spectrum of biological activity, e.g., anti-inflammatory, anticarcinogenic, antidepressant, antibacterial, antimutagenic, anti-HIV, etc. [8] [15]. Due to generally known ability of flavonoids to scavenge reactive oxygen species and suppress the production of pro-inflammatory factors [16] [17] we did not repeat these assays and focused on in vivo experiments. In particular, the evaluation of gastro- and hepatoprotective activity of the aforesaid extract, as well as its leucopoietic properties, was carried out in corresponding animal models.

2. Material and methods

2.1. Drugs and chemicals

Carbon Tetrachloride (CAS Number: 56-23-5), Absolute Ethanol (CAS Number: 64-17-5), Pentobarbital sodium salt (CAS Number: 57-33-0) used in the experiments were obtained from Sigma Aldrich (USA). Cyclophosphamide and Sodium Chloride 0.9% were purchased from LLC "Aversi", Georgia. Plant raw was supplied by Pirose LTD, Georgia.

2.2. Aqueous extract of *R. damascena* flower petals (RDE)

R. damascena flower petals were processed by hydro distillation according to the standard procedure described in the European Pharmacopoeia (Ph. Eur. 2008). Waste water was concentrated using rotary vacuum evaporator below 40°C, frozen in a layer of 10 mm in the Petri dishes at -20 °C for 12 h, and vacuum dried to constant weight at -90 °C under 3.33 Pa. Finally, the dried material was powder-grinded and stored in vacuum desiccator until further use.

2.3. Experimental

2.3.1. Animals

Inbred white mice weighing 28 ± 2 g ($n = 40$) were obtained from the animal house of Tbilisi State Medical University I. Kutateladze Institute of Pharmacochimistry and quarantined for 1 week in the Department of Preclinical Pharmacological Research of above Institute. Animals were kept under standard conditions (temperature $20 \pm 2^\circ\text{C}$, humidity 55-65%, 12/12-hour light/darkness cycle, granulated food - 4 g/animal/day, water ad libitum). All experiments were carried out in accordance with the requirements of the EU Directive 2010/63. Research protocol was authorized by the Tbilisi State Medical University Ethics Committee on Animal Research (approval # AP-52-2021).

2.3.2. Determination of LD_{50}

The study was conducted using Lorke method [18] modified by Akhila et al. [19]. In brief, ten groups of three animals each were used. A range of doses of the RDE was tested, starting from the lowest dose (10 mg/kg, intraperitoneally), with increments of 2. The treated animals were monitored for 24 h for mortality after the administration of each dose. The geometric mean of the highest dose which did not killed any mice and the lowest dose which led to death of all animals these two doses was taken as the median lethal dose (LD_{50}).

2.3.3. Hepatoprotective activity (modification of CCl_4 -induced prolongation of pentobarbital sleeping time in mice)

The hepatoprotective effect of RDE was evaluated in a modified model of CCl_4 -induced liver damage (potentiation of pentobarbital sleeping time). Experiments were performed on 30 mice of both sexes weighing 26–32 g (10 animals in a group). Group II (experimental) - RDE was administered intraperitoneally in a dose 50 mg/kg for 3 days. Within 1 hour after the third injection and also on the next day, CCl_4 (diluted 1:1 with olive oil) in a dose of 1 ml/kg was injected subcutaneously. Then the same dose of RDE was given for another 6 days (9 in total). Group II (negative control) received saline (0.1 ml, i.p.) and the same dose of CCl_4 . Group I (intact control) - was given only saline (0.1 ml, i.p.) for 9 days and sham injected on day 3-4. On day 10 pentobarbital (45 mg/kg i.p.) was injected to mice of all groups and duration of pentobarbital sleeping time, defined as a time between loss and recovery of righting reflex, was recorded. Hepatoprotective efficacy was calculated by the following formula:

$$E = \frac{\Delta T_{\text{con}} - \Delta T_{\text{exp}}}{\Delta T_{\text{con}}} \times 100$$

where ΔT_{con} and ΔT_{exp} are the mean differences between sleeping time in groups II and III and II and I, correspondingly.

2.3.4. Gastroprotective activity (Ethanol induced ulcer model)

The experiment was carried out in accordance with the method described by Adinortey et al. [20]. In brief, 24 outbred mice were randomly distributed in three groups of animals, each consisting of eight mice. 24 hours prior to the experiment the access to food was restricted, and animals were relocated in cages with raised floors of wide wire mesh to prevent coprophagy. During the fasting period, all mice received a nutritive solution of 8% sucrose in 0.2% NaCl to avoid excessive dehydration. On day 2 absolute ethanol was given orally (1 ml/100 g) to all animals. RDE in a dose of 50 mg/kg, i.p. (Group III) or 100 mg/kg, i.p. (Group II) was given 1 hour prior the ethanol administration. Mice of control group (Group I) got 0.2 ml of saline. Animals were euthanized by CO₂ inhalation 1 hour after the ethanol administration. The stomachs were immediately removed, opened along the great curvature, rinsed consecutively with water and 10% formalin solution which contains about 4% formaldehyde w/v, fixed on white EPS foam board, and digitally photographed. Ulcerative lesions were measured using Image-J software and macroscopic ulcer index (MUI) was calculated for each stomach according to the following scale: 0 - no lesions; 1 - single petechial lesions (n <10); 2 - multiple (n ≥10) petechial or short linear (≤ 2 mm) haemorrhagic lesions; 3 - long (> 2 mm) linear haemorrhagic lesions; 4 - continuous linear haemorrhagic lesions along the entire length of the glandular part of stomach. The efficacy of RDE expressed as percentage of ulcer inhibition (% I) was estimated on the basis of the MUI and calculated using the formula:

$$\%I = \frac{MUI_c - MUI_T}{MUI_T} \times 100$$

where MUI_c and MUI_T are macroscopic ulcer indexes in control and test groups, respectively.

2.3.5. Leucopoietic activity (cyclophosphamide-induced leukopenia in mice)

32 mice were randomly divided in three groups (8 animals in each). Group I – intact control; group II – negative control (only cyclophosphamide); group III (cyclophosphamide + RDE 20 mg/kg); group IV (cyclophosphamide + RDE 50 mg/kg). Acute leukopenia was induced in mice of groups II-IV by a single intraperitoneal injection of cyclophosphamide at a dose of 350 mg/kg. Starting from day 2 after the administration of cyclophosphamide groups II and III were given RDE orally for 5 days. Blood sampling was performed on day 1 (basal level), day 2 (to estimate the rate of leukopenia) and after the completion of treatment (day 8 from the beginning of the experiment). Blood samples were collected under anaesthesia with pentobarbital sodium (45 mg/kg, i.p.) from abdominal vena cava in accordance with sampling protocol [21], and then mice were sacrificed by decapitation. Total white blood cell (WBC) counts were performed manually for each sample using a Neubauer chamber and microscopic examination of Romanowsky-stained smears with 70X objective [22].

2.3.6. Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis of the experimental data was performed using Student's t-test [23]. Differences were considered significant at p < 0.05.

3. Results and discussion

3.1. Acute toxicity studies

In the first phase, mice were divided into three groups of 3 mice each and treated with the RDE in a dose of 10, 500 and 5000 mg/kg body weight intraperitoneally. Animals were observed for 24 h for signs of toxicity, mortality and general behaviors. In the second phase, twenty-one mice were divided into 7 groups of three animals each that were administered with the RDE at doses interval between 10 and 650 mg/kg i.p. and median lethal dose (LD₅₀) was calculated. LD₅₀ of RDE appeared to be 350 mg/kg.

3.2. Ethanol induced ulcer model

In untreated animals, administration of absolute ethanol induced marked gross mucosal lesions, including full length haemorrhagic streaks along the longitudinal axis of the glandular part of stomach and petechial lesions. (Fig.1, a I). In mice given 100 mg/kg i.p. RDE none or only single petechial lesions were present (Fig.1, a II), whereas in animals pretreated with 50 mg/kg RDE mainly partial length longitudinal hemorrhagic streaks were observed (Fig.1, a III). Correspondingly, the MUI was significantly reduced in animals pretreated with RDE 100mg/kg (MUI=0.38±0.31; %I=83) compared to RDE 50 mg/kg (MUI=2.00±1.1; %I=22) and untreated mice (MUI=2.57±1.62, Fig. 1, b, c).

Since ethanol induced hemorrhagic damage on the gastric mucosa is commonly associated with oxidative stress [24] [25], it is natural to assume that natural compounds with antioxidant activity can prevent or reverse such damage. Among plant secondary metabolites, flavonoids are claimed to have various pharmacological activities in the gastroprotective domain, including anti-secretory, cytoprotective, antihistaminic and antioxidant characteristics [26–30]. On the other hand, it should be taken into consideration that the predominant constituents of the RDE are flavonoids, mainly quercetin glycosides. Hence it is likelihood that the observed gastroprotective effect of RDE may be attributed to antioxidative activity of its flavonoid content [24] [26] [31].

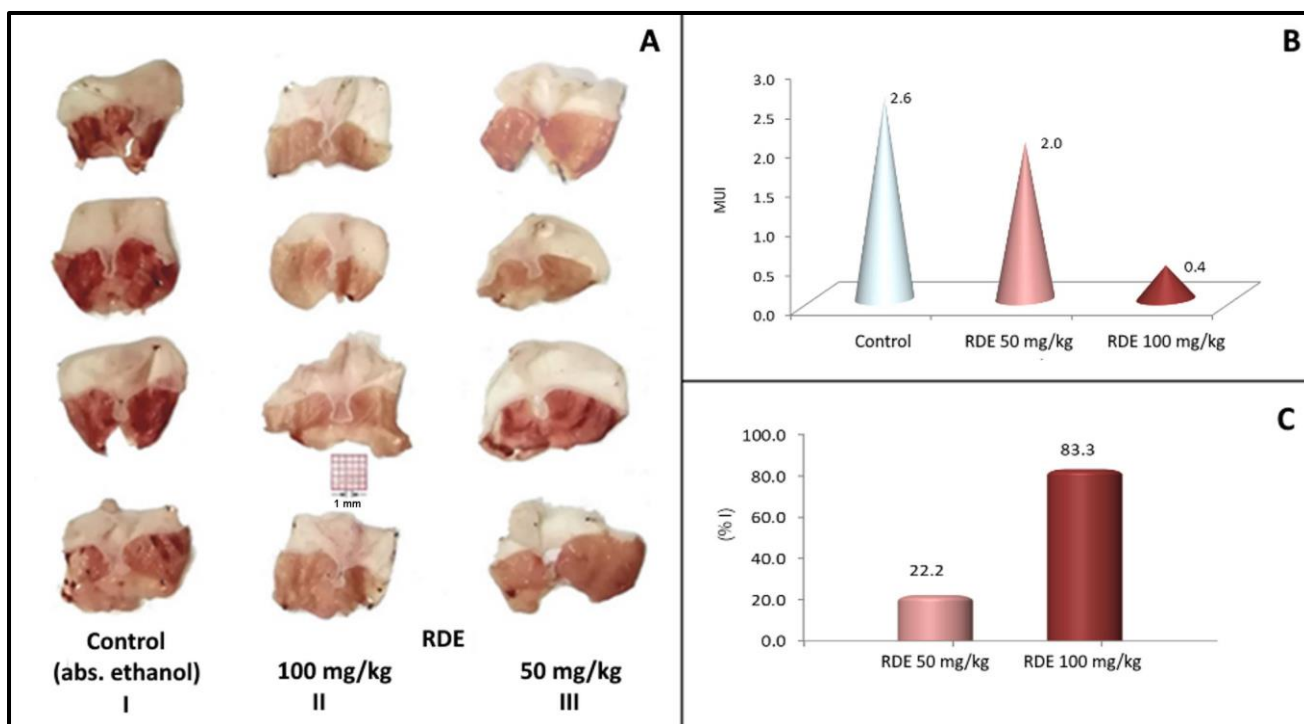


Figure 1 Gastroprotective effect of RDE. A – Macroscopic view of ethanol induced ulcer lesions in control (I) and RDE treated (II and III) mice; B – Macroscopic ulcer index (MUI); C - Efficacy of RDE (%I). Each value represents mean \pm SEM of 6 animals; * - $p < 0.05$ vs negative control

3.3. CCl₄-induced prolongation of pentobarbital sleeping time

In *in vivo* investigations, liver injury caused by CCl₄, the most well-studied system of xenobiotic-induced hepatotoxicity [32] [33] [34], is a common model for evaluating pharmacological anti-hepatotoxic/hepatoprotective activities [35] [36] [37].

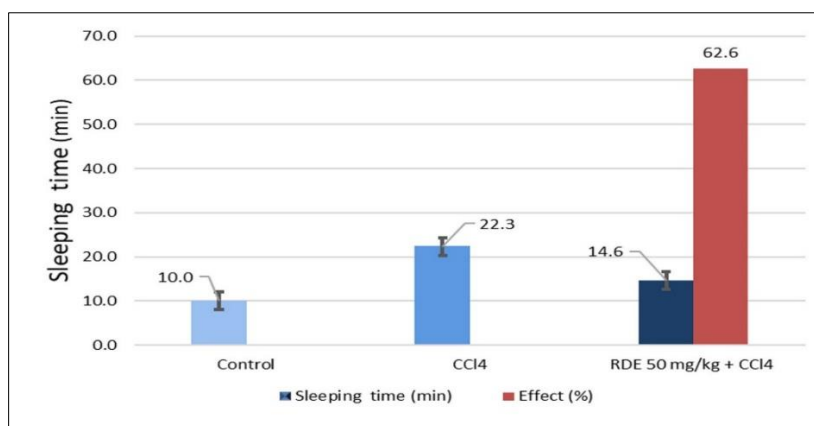


Figure 2 RDE hepatoprotective effect in CCl₄ treated mice. Each value represents mean \pm SEM of 6 animals. # - $p < 0.05$ vs CCl₄ group; * $p < 0.01$ vs control group

In particular, liver damage causes an increase in pentobarbital induced sleeping time after carbon tetrachloride poisoning. As pentobarbital is metabolized solely in the liver [38] sleep duration indicates the intensity of hepatic metabolism. Sleeping time is entirely or partially restored in the presence of a hepatoprotective medication [39] [40] [41] [42]. In our experiment CCl₄, expectedly, more than doubled the duration of pentobarbital-induced sleeping time, whereas the RDE treatment at a dose of 50 mg/kg reduced the hepatotoxic impact of CCl₄ resulting in a 63% reduction in sleep duration (Fig.2).

Most likely, the observed hepatoprotective effects of PDE may be related to the flavonoids present in the extract, since these phytochemicals, are claimed to have been implicated as hepatoprotectors on CCl₄ induced toxicity [43] [44] [45].

3.4. Leucopoietic activity (cyclophosphamide-induced leukopenia in mice)

The basal count of total WBC was within the normal range for mice in all groups. On day 2 after the cyclophosphamide administration approximately 80% reduction in total WBC was observed in groups II-IV (Table 1). 5 day RDE treatment led to the recovery of total WBC up to 64% and 75% of basal level in RDE treated groups III and IV, respectively (Fig.3).

Table 1 Ameliorative effect of RDE against cyclophosphamide (CP) induced leukopenia in mice

Group	Total WBC (*10 ⁹ /l)		
	day 1	day 2	day 8
I	5.34±0.84	5.27±1.42	5.10±1.20
II	5.98±1.12	1.08±0.85	2.20±0.65
III	6.18±1.46	1.24±0.38	3.95±1.02*
IV	5.47±1.34	1.15±0.44	4.10±0.86*

I - intact control; II - negative control (CP 350 mg/kg); III - CP+RDE 20 mg/kg; IV - CP+RDE 50 mg/kg; * - p<0.05 vs negative control

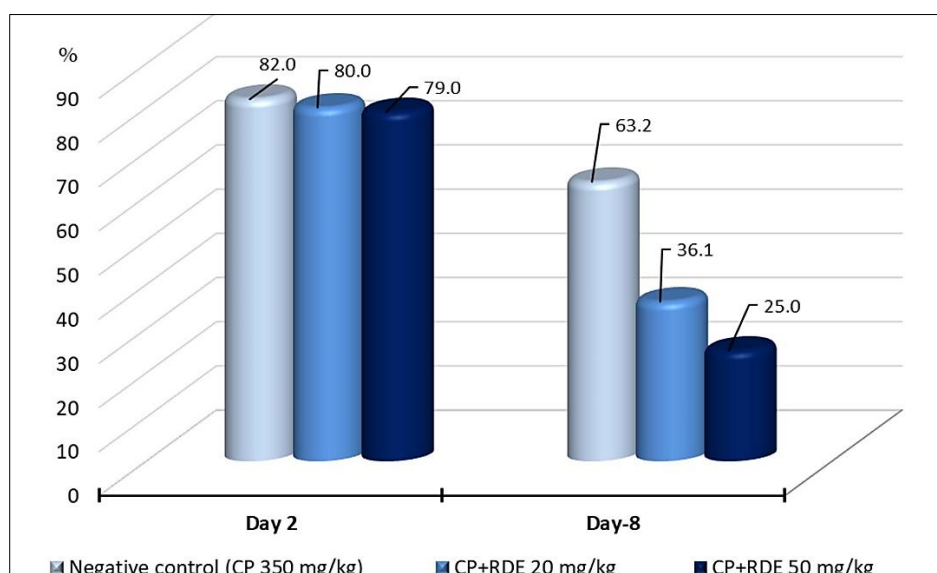


Figure 3 Rate of leucopenia (% reduction of total WBC) in CP and RDE treated animals. Each value represents mean ± SEM of 6 animals

Presumably, the observed effect is associated with the presence of flavonoids in RDE as complex flavonoid-containing preparations are said to reverse leucopenia caused by cyclophosphamide when given in combination with it [47] [48] [49] [50]. On the other hand, limitation of the study of such preparations is a lack of relation of the effect to the particular molecule in the complex compound. Therefore, further study is needed to determine the constituents of the RDE responsible for the leukopoietic activity.

4. Conclusion

The data of presented study indicate some beneficial effects of the RDE. In particular, it significantly protected against mucosal damage induced by absolute ethanol, alleviated carbon tetrachloride hepatotoxicity and contributed the recovery of white blood cells after cyclophosphamide treatment. It is notable that all of aforesaid disorders are closely linked with oxidative damage, and the observed effects may be attributed to predominant flavonoidal constituents of the RDE, including, but not limited, to quercetin glycosides. These results clearly indicate that further experimentation is needed to determine the active principles of RDE responsible for the observed pharmacological activity, and to elucidate the exact mechanisms of their action.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no financial or any other conflicts of interest in this work.

Statement of ethical approval

The animal research protocol was approved by the Tbilisi State Medical University Ethics Committee on Animal Research (registration #AP-52-2021).

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