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Rumex nervosus nanoparticles versus aqueous extract: introduction of novel immunomodulatory anti-inflammatory nano-preparation for management of paw oedema and gastritis in rats

Bassant M. M. Ibrahim[1*], Hanem M. Awad[2], Nermeen M. Shaffie[3], Eman R. Youness[4] and Ahlam H. Mahmoud[5]

Abstract

Background *Rumex nervosus* is abundant in East African and Arabian countries, and is used in curing gastrointestinal diseases as well as in wound healing. The current study intends to assess *Rumex nervosus* aqueous extract's anti-inflammatory benefits compared to its nanoparticles on rat paw edema and its gastric mucosal protection against ulceration.

Materials and methods In-vitro cytotoxicity effects and antioxidant activity of *Rumex nervosus* nanoparticles versus aqueous extract were studied, followed by a pilot in vivo pharmacological study to determine the suitable dose of nanoparticles that would be used in the safety and efficacy studies in comparison with the aqueous extract. Its protective effects on arthritis and soft tissue inflammation were studied in rat paw edema and gastric ulcer models. *Rumex nervosus* extract (250 and 500 mg/kg) and nanoparticles (3.3 and 6.6 mg/kg) were given to four groups of rats orally before induction of paw oedema with subplantar 0.2 ml (1% w/v) formaldehyde or gastritis with oral ethanol 1 ml (70%), besides negative, positive control and reference groups.

Results Paw volumes and gastric ulcer indices, as well as the anti-inflammatory and antioxidant parameters (kappa β, Paraoxonase1, and Malondialdehyde) that were measured in sera showed a marked reduction in groups treated with high doses of *Rumex nervosus* extract, and nanoparticles. Histopathologic and histochemical assessment of the stomachs confirmed the other investigations. All results were significant compared to positive control untreated groups.

Conclusions Most studies demonstrated *Rumex nervosus's* protective anti-inflammatory benefits with the superiority of large doses of nanoparticles, offering a promising natural solution for low cost against inflammation.

Keywords *Rumex nervosus*, Anti-inflammatory, Paw edema, Gastric ulcer, Nanoparticles

*Correspondence:
Bassant M. M. Ibrahim
bmmih1974@gmail.com; bm.ibrahim@nrc.sci.eg
Full list of author information is available at the end of the article

Background

Inflammation is tissue response at the site of chemical infectious or mechanical injuries. It is a defense mechanism against injurious agents (https://www.britannica.com/science/inflammation). It may show up as edema, which results from a drop in interstitial fluid pressure and, as a result, from increased filtration and edema (Ibrahim et al. 2016).

Inflammation can be dangerous when it affects internal organs such as the digestive tract organs to which the stomach belongs. Non-steroidal anti-inflammatory medications (NSAIDs) are increasingly used, either to stop pain or combat inflammatory conditions as well as unhealthy lifestyles including excessive alcohol intake, therefore there is an increase in gastric inflammation that subsequently may progress to gastric ulcers which has a serious impact on general health condition of suffering patients to the extent that it became considered a plague. Stress that occurs due to excessive alcohol intake is associated with serious disease development, for instance, excessive alcohol drinking causes deformation of the mucosa and decreases the blood flood flow which leads to necrosis and ulceration of the mucosa. Moreover, ethanol intake leads to the occurrence of gastric cancer (Saleh and Mutlag 2022).

Other factors that contribute to the incidence of gastric ulcers include severe stressful life conditions, smoking, and *helicobacter pylori* infection (Levenstein et al. 2015).

Treatment of gastric ulcers can be achieved by histamine (H_2) antagonist drugs such as ranitidine which competitively blocks H_2 receptors and reduces acid secretion. Yet H_2 antagonistic drugs may lead to high rate of ulcer relapse due to a rise in acidity secondary to drug use also tolerance to the medication occurs after repeated use for a long period (Aboul Naser et al. 2020).

Since ancient times up till now, plants have been used as a cure for various altered health conditions, because they are easily accessible and affordable, moreover, their constituents have negligible side effects when compared to synthetic drugs. One of the widely used herbs in Arabian and East African countries is *Rumex nervosus* (AlMousa et al. 2022). It is used in curing gastrointestinal diseases such as helminths, diarrhea, and aches (Alharbi 2017), it is also used for inhibition of bleeding (Qaid et al. 2022), and promotion of wound healing (Gemechu et al. 2021).

New drug delivery systems provide a more beneficial effect than conventional therapies, as the use of nanoparticles in delivery systems which offers more stability, higher specificity, increased capacity for drug carriage, possibility for controlled release, moreover they can be administered through different routes and have the ability to deliver polar and non-polar drug molecules.

The use of nanoparticle drug delivery system can be considered as a reliable mean of delivering drugs to their target diseased site with high specificity, at a suitable maintained dose concentration with minimum side effects (Adepu and Ramakrishna 2021).

Therefore, the goal of the current study is to determine if *Rumex nervos*us aqueous extract and nanoparticles have anti-inflammatory effects on gastrointestinal and peripheral tissues in rat models of gastric ulcer and paw edema.

Materials and methods

*Guideline ethics for plant usage in the phytochemical stu*dy. As authorization was acquired for the gathering of plant material, the current study complies with local and national regulations.

Guideline ethics for experimental animal handling in the in vivo *pharmacological study.* The Institutional Animal Ethical Committee (IAEC) and the National Regulations of Animal Welfare in Egypt were followed when conducting the experiments, and the results were reported in line with Animal Research: Guidelines for the Reporting of In Vivo Experiments (ARRIVE), sample size, inclusion and exclusion criteria, randomization, blinding, outcome measures, statistical methods, the ethical approval as well as gender and number of animals in each experiment were mentioned, the experimental procedures study is reported in accordance with ARRIVE guidelines. The results obtained were released in the present manuscript with transparency. The National Research Centre ethics committee granted its clearance under reference number 19021.

Preparation of Rumex nervosus extract and nanoparticles

Rumex nervosus aqueous extract

Whole *Rumex nervosus* herb was obtained from Tabuk in the Kingdom Saudi Arabia. Samples were cleaned with water, and dried in the shade. The dried aerial parts were ground into a fine powder using a grinder. For 48 h, the powder was submerged in 70% ethanol. Rotating evaporator was used to remove the solvent at low pressure and temperatures below 45 °C. A part of the crude extract was kept for the biological tests and the other part was used for HPLC analysis and nanoparticle preparation.

Proximate analysis was conducted to determine the nutritional value of *RN* as described by Melesse et al. (2018). High-performance liquid chromatography (HPLC) analysis was used for the identification, separation, and dosing of chemical compounds in the *RN* extract mixture.

A gas chromatography-mass spectrometry (GC–MS) test was used for detecting the chemical composition as

described by Adaszynska-Skwirzynska and Szczerbinska (2018).

Rumex nervosus nanoparticles

Nanoparticles of compound: Nanoparticles were created using the nano-precipitation method (Anwar et al. 2019).

In vitro cytotoxicity effects and antioxidant activity of *Rumex nervosus* nanoparticles versus aqueous extract

Sigma-Aldrich was used to purchase rutin, ascorbic acid, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH).

Cell culture of HepG-2 (Human liver carcinoma), MCF-7 (human breast adenocarcinoma) and RPE-1 (human normal Retina pigmented epithelium) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in DMEM medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100 U/ml penicillin and 100 U/ml streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

The cytotoxicity activities against HepG-2, MCF-7 and RPE-1 human cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells (Mohamed et al. 2017; Felafel et al. 2017; Hassan et al. 2017). Cells were dispensed in a 96 well sterile microplate (5 × 104 cells/well), and incubated at 37 oC with series of different concentrations, in DMSO, of each tested compound for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 µL of MTT (2.5 mg/ml) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 570 nm using a SpectraMax® Paradigm® Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were conducted in triplicate and repeated on three different days. All the values were represented as mean ± SD. IC50s were determined by probit analysis using the SPSS software program (SPSS Inc., Chicago, IL).

According to the stabilized DPPH free radical's ability to scavenge free radicals, the antioxidant activity of the extract, nanoparticles and the standards (rutin and ascorbic acid) were evaluated (Hassan et al. 2017; Hamdy et al. 2013). 90 µl of a 100 M methanol solution of DPPH were added to 10 µl of either the tested extract, nanoparticles or standard (series of various concentrations) in a 96-well microtiter plate. After incubation in dark at 37 °C for 30 min, the decrease in absorbance of each solution was measured at 520 nm using a SpectraMax® Paradigm® multi-Mode microplate reader. The absorbance of the blank sample containing the same amount of DMSO and DPPH solution was also prepared and measured. All experiments were carried out in triplicate. The scavenging potential was compared with solvent control (0% radical scavenging) and the standard compound. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Reduction of absorbance} = [(AB - AA)/AB] \times 100,$$

where AB—absorbance of blank sample and AA—absorbance of tested compound ($t = 30$ min). The concentration of the extract or nanoparticles required to scavenge 50% of DPPH (IC50) was determined as well (Hamdy et al. 2013; Soliman et al. 2014).

In vivo pharmacological study of the effects of Rumex nervosus nanoparticles versus aqueous extract

Materials

Animals

The National Research Centre's animal house colony in Dokki, Giza, Egypt provided the female Albino Wistar rats (160 and 175 g b.w.) that were used in the experiment. All animals were kept in stainless steel cages in a room that was kept at a constant temperature of 23 °C and had a 12-h light/dark cycle. They were also fed a normal laboratory meal and were given access to unlimited amounts of water. Rats were adapted to laboratory conditions for one week before starting the experiments. The Institutional Animal Ethical Committee (IAEC) and the National Regulations for Animal Welfare were also followed during the trials. All animal treatments were carried out in compliance with the National Research Center's Ethics Committee's guidelines and were given approval under certificate number 19021.

Drugs and chemicals

Under license from Merck Co. Inc.-Rahaway, indomethacin was purchased from Epico, Egypt International Pharmaceutical Industries Co., ARE. The ranitidine was made as a suspension in 1% tween 80 after being bought from Pharco, Egypt. We bought formaldehyde and diethyl ether from Sigma Chemical Co. in St. Louis, Missouri, in the United States.

Rumex nervosus herb purchased from Tabuk in the Kingdom of Saudi Arabia.

Diagnostic tools

For inflammatory marker identification: Santa Cruze (Santa Cruze Canada, USA) and Abcom (Cambridge, UK) provided the nuclear factor kappa B (NF-B) and

paraoxonase 1 enzyme (PON1) and oxidative stress marker lipid peroxidase (MDA) purchased from Bio diagnostic company, Egypt.

Methods

Pilot study for selecting the suitable Rumex nervosus nanoparticles dose

The dose of *Rumex nervosus* herbal extract (250 and 500 mg/kg) for performing the in vivo studies was selected. Accordingly, the dose of *Rumex nervosus* nanoparticles was subjected to be one hundredth the selected herbal extract dose. A pilot study for the expected nanoparticles anti-inflammatory effect on paw oedema was performed, it started by using 2.5 mg/kg nanoparticles given orally (Po) to five rats followed by induction of paw oedema, a parallel group acted as positive control for which paw edema was induced and didn't receive any treatment.

There was no difference at all between the volumes of paws of rats in both groups, which denoted that the 2.5 mg/kg of nanoparticles wasn't efficient as an anti-inflammatory. The study was repeated using 2.7, then 2.9, then 3.1, then 3.3 mg/kg of nanoparticles.

Since the best effect was obtained with the 3.3 mg/kg dose as it produced significant inhibition in paw edema; therefore, the dose 3.3 and its duplicate 6.6 mg/kg of nanoparticles were chosen for performing the sub-chronic safety study, and anti-inflammatory efficacy studies on soft tissue (paw edema) and on stomach (gastritis).

Safety assessment of the Rumex nervosus herbal extract and nanoparticles for use as an anti-inflammatory supplement

Thirty female Albino Wistar rats (170–180g b.w.), were used for the sub-chronic study.

Six rats were used as the negative control group, and only distilled water was administered orally (Po). The remaining 24 rats were divided evenly into 4 groups and administered *Rumex nervosus* aqueous extract Po (250 and 500 mg/kg), and nanoparticles Po (3.3 and 6.6 mg/kg) for two successive weeks.

All rats were weighed at zero time before giving either distilled water or *Rumex nervosus* and after two weeks after terminating the sub-chronic toxicity study. They were observed for any changes in bowel habits as signs of diarrhea manifested by soft or loose stools or signs of constipation manifested by hard stools, or flatulence. They were also observed for any change in behavior as lethargy or hyperactivity. Changes in fur color or its loss, abnormal nasal or lacrimal secretions or change in color of nostrils or signs of their irritation, as well as salivation or change in color of lips were observed. In addition, any mortality was considered.

At the end of the experiment, the rats were sacrificed and the stomachs were dissected for macroscopic examination of any signs of inflammation.

Efficacy study of Rumex nervosus aqueous extract and nanoparticles

The efficacy tests were carried out in accordance with the findings of the sub-chronic toxicity research, which demonstrated the safety of using *Rumex nervosus* aqueous extract and nanoparticles as prophylactic anti-gastritis and anti-inflammatory natural supplements (Table 2) as follows:

Study of the soft tissue anti-inflammatory effects of Rumex nervosus aqueous extract And nanoparticles.

Study design: Forty-two Female Wistar Albino rats (160–175 g b.w.), were classified into seven groups each consisting of six rats as follows:

Control groups: Negative group: Rats were given (1 ml) of saline and positive control group where paw edema was induced by 0.2 ml (1%, w/v) of formaldehyde injected in the sub-plantar area of the right hind paw of the rat (Ibrahim et al. 2016).

Prophylactic groups: *Rumex nervosus* extract and nanoparticles and Indomethacin were given one hour before induction of paw edema, and the groups were as follows:

Indomethacin was given orally (Po) in dose of 25 mg/kg (Moharram et al. 2018) and served as a reference group. *Rumex nervosus* aqueous extracts were given Po to two groups of rats in doses of 250 and 500 mg/kg, respectively. *Rumex nervosus* nanoparticles were given Po to two groups of rats in doses of 3.3 and 6.6 mg/kg, respectively.

Evaluation of the effect of treatment on tissue edema All left hind footpad thickness were measured at zero time (basal reading) immediately before, then every one hour for three consecutive hours, after induction of paw edema. The paw thickness was measured by using Vernier caliper (Hisamuddin et al. 2019).

The difference between initial and subsequent readings gave the change in edema thickness for the corresponding time. Edema volume of the paw of a positive control group (Vc) and the volume of the treated group (Vt) was used to calculate percentage (%) inhibition and (%) edema volume by using the following formula:

$$\begin{aligned}\%\ \text{Edema volume} &= (\text{edema volume after drug treatment}\\ &\quad /\text{base time volume})\\ &\quad \times 100 - 100\%\ \text{Inhibition}\\ &= (\text{Vt}/\text{Vc}) \times 100 - 100\ [2].\end{aligned}$$

Assessing Rumex nervosus's anti-inflammatory and antioxidant properties Twenty-four hours after the last dose of treatment, blood samples were collected from all groups of animals after they had been anesthetized with diethyl ether, the retro-orbital plexus of veins was punctured by sterile heparinized capillary tubes, the obtained blood was allowed to stand in clean dry centrifuge tubes for 30 min, then it was centrifuged for 15 min at 2500 rpm. The clear supernatant serum was collected by Pasteur pipette into a dry clean tube to be used for measurement of serum levels of nuclear factor kappa B (NF-κB), Paraoxonase1enzyme (PON1) according to manufacturer's guidelines, and oxidative stress marker lipid peroxide (malondialdehyde: MDA) according to Tang et al. (2019)

Studying the anti-gastro-ulcerogenic effect of Rumex nervosus nanoparticles and aqueous extract.

Study design: In seven groups of six, 42 female Wistar albino rats (160 and 175 g) were divided.

First group served as the negative control group and received a daily oral dose of 1 ml of distilled water.

The second group, which acted as the positive control group, received a single oral dose of 1 ml of 70% ethanol (Moharram et al. 2018).

treated populations:

Ranitidine was administered orally twice weekly to the third group at a dose of 50 mg/kg body weight (b. wt.) (Moharram et al. 2018).

For a week, *Rumex nervosus* aqueous extract (250 and 500 mg/kg b.wt.) was administered orally to the 4th and 5th groups. *Rumex nervosus* nanoparticles (3.3 and 6.6 mg/kg b.wt.) were administered orally daily for one week to the sixth and seventh groups.

The third through eighth groups received a single oral dosage of 1 ml of 70% ethanol for the induction of stomach ulcers, one hour following the last treatment dose (Moharram et al. 2018).

Macroscopic examination: One hour after induction of gastric ulcer, the rats were sacrificed by cervical dislocation after being lightly anaesthetized. The stomachs were excised, opened along the greater curvature, washed with saline and examined thoroughly for mucosal lesions. The number and severity of mucosal lesions were observed and lesions were scaled as follows; petechial lesions = 1, lesions < 1 mm = 2, lesions between 1 and 2 mm = 3, lesions between 2 and 4 mm = 4, lesions more than 4 mm = 5. A total lesion score for each animal was calculated by multiplying the total number of lesions by the respective severity scores. Results were expressed as the severity of lesions per rat (Mózsik Gy and Javor 1982).

Studies on histological evaluation: All animals' stomachs were dissected as soon as they died. The specimens were then opened, thoroughly cleansed of their contents, rinsed, and preserved for at least 72 h in 10% neutral-buffered formalin saline. After being rinsed in tap water for 30 min, all of the specimens were dehydrated in varying degrees of alcohol, cleaned in xylene, and then embedded in paraffin. For histological analysis, serial slices of 6 m thick were cut and stained with hematoxylin and eosin (Duraker et al. 2003).

Histochemical assessment study: To show the presence of mucopolysaccharides, additional sections were cut and stained with Periodic Acid Schiff (PAS) reagent (Yamabayashi 1987).

Statistical analysis

For the paw edema anti-inflammatory investigation, the Tukey Kramer multiple comparisons test was used to compare means after a two-way analysis of variance (ANOVA). It was agreed that p0.0001 was substantial. One-way analysis of variance and the Tukey Kramer multiple comparisons test was used to compare means for biochemical testing and the macroscopic assessment of stomach ulcers. P 0.05 was adopted as the threshold for significance. Values were reported as means S.E. All statistical analyses and tests were performed using the Graph Pad Prism software (version 7) (Fig. 1).

Results

In-vitro studies

The cytotoxicity activities of both the aqueous extract of *Rumex nervosus* (R) and nanoparticles of *Rumex* (*R*") were investigated against HepG-2, MCF-7, and RPE-1 human cell lines using MTT assay. Results of the percentage of the intact cells measured and compared to the control are expressed in Figs. 2, 3 and 4. These figures clearly demonstrate that neither extract had a harmful effect on any of the three types of human cells. On the contrary, both extracts showed dose-dependent cytoprotective effects and they caused enhancements in the cell growth percentage. However, the nanoparticles of *Rumex* showed much higher cell growth enhancement compared to the aqueous extract of *Rumex nervosus*.

Additionally, the antioxidant activity of extract and nanoparticles has been studied using the DPPH assay. According to the findings, both exhibited dose-dependent action (Fig. 5). Table 1 displays the associated IC50s for each of them. From these results, we conclude that both possess strong antioxidant activity compared to rutin as standard control. *Rumex* nanoparticles demonstrated extremely potent antioxidant activity 14.5 times more than the rutin control. In contrast, the whole *Rumex nervosus* extract displayed 1.6 times less activity than the standard control(rutin).

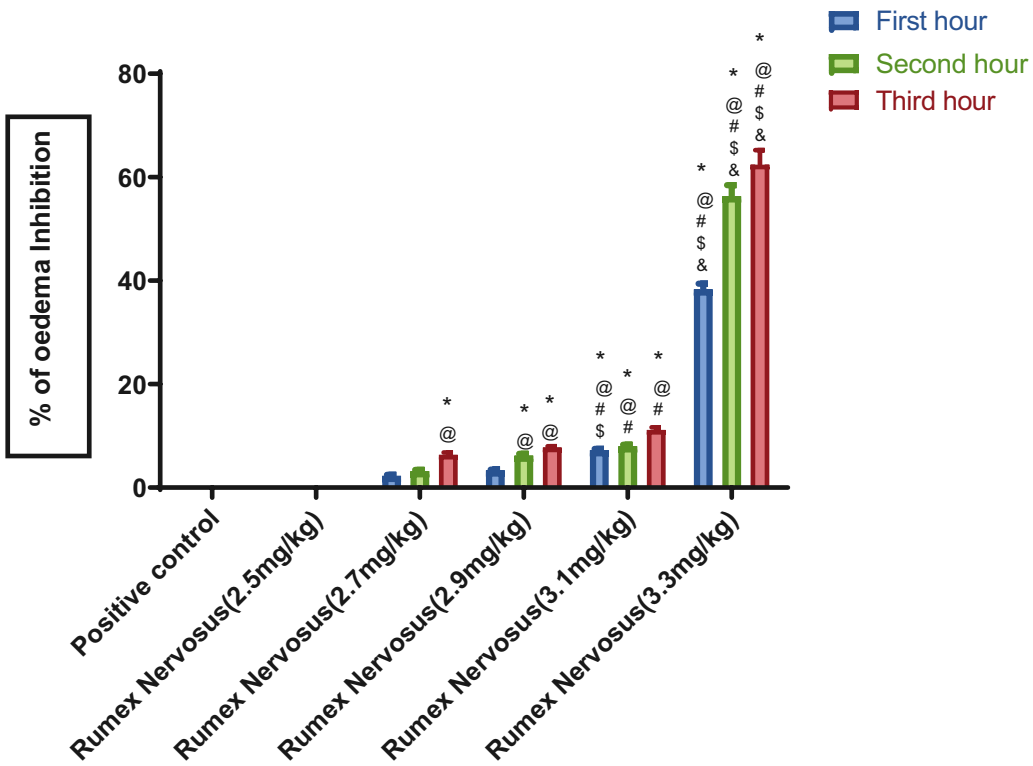

Fig. 1 Pilot study for selecting the suitable *Rumex nervosus* nanoparticles dose. Results of anti-inflammatory effect on paw oedema are expressed as means of % of inhibition of paw oedema ± SE. $N = 5$. *Significantly different from untreated positive control, @ significantly different from *Rumex nervosus nanoparticles* (2.5 mg/kg), # significantly different from *Rumex nervosus* nanoparticles (2.7 mg/kg), $significantly different from *Rumex nervosus* nanoparticles (2.9 mg/kg), and significantly different from *Rumex nervosus* nanoparticles (3.1 mg/kg). Two-way analysis of variance (ANOVA) was used for the statistical analysis, followed by the Tukey–Kramer multiple comparisons test between groups during the same time interval, $p \leq 0.0001$ was considered significant

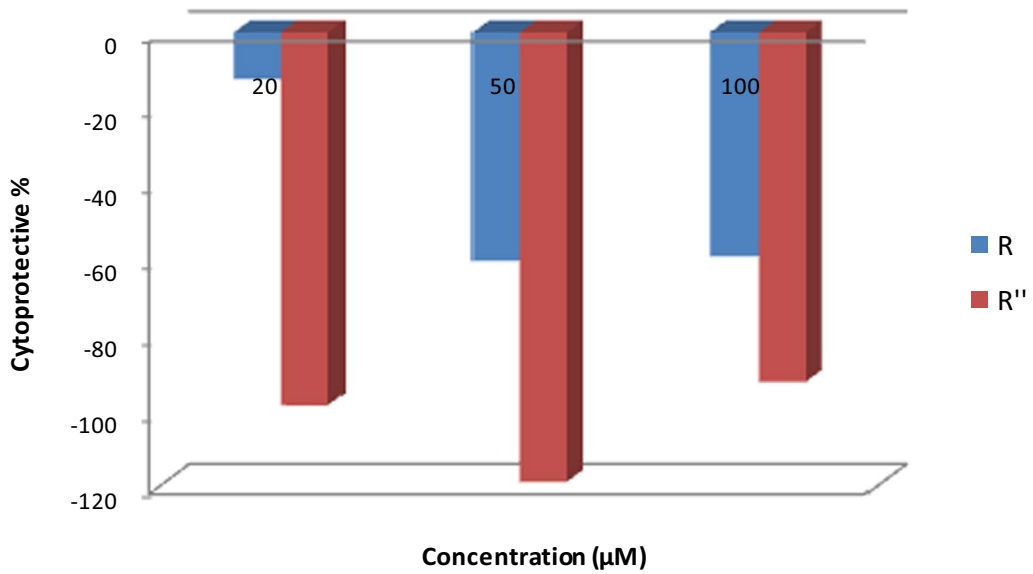

Fig. 2 Dose dependent cytotoxicity curves of *Rumex nervosus* aqueous extract and its nanoparticles against HepG-2 human cancer type, using MTT assay

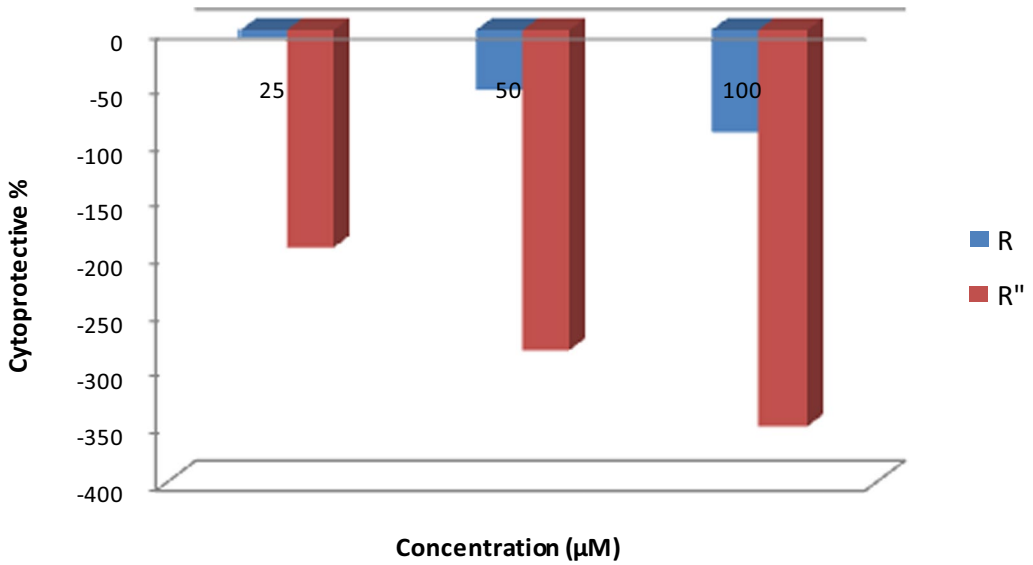

Fig. 3 Dose dependent cytotoxicity curves of the *Rumex nervosus* aqueous extract and its nanoparticles against MCF-7 human cancer type, using MTT assay

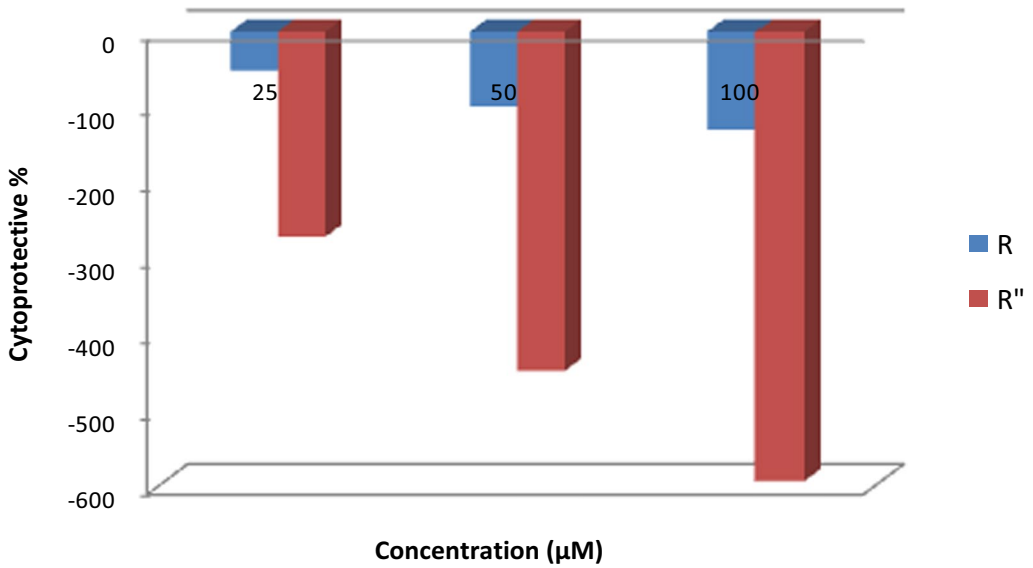

Fig. 4 Dose dependent cytotoxicity curves of the *Rumex nervosus* aqueous extract and its nanoparticles against RPE-1 human normal type, using MTT assay

In vivo studies

The pilot study performed before the sub-chronic toxicity study; in order to select the dose of nanoparticles with the best anti-inflammatory effects, revealed that the 3.3 mg/kg of nanoparticles given orally to rats produced the highest % of inhibition of paw edema during the first, second and third hours post-inflammation induction, and its effects were significantly higher when compared to all other groups (untreated positive control, groups that received 2.5, 2.7, 2.9 and 3.1 mg/kg of *RN* nanoparticles) during the same time intervals. At the 3rd hour measurement, the 3.3 mg/kg exhibited % of edema inhibition 62.41% ± 2.8 compared to those exhibited by the doses 2.7, 2.9, and 3.1 mg/kg which produced inhibition by 6.32% ± 0.48, 7.72% ± 0.26 and 11.17% ± 0.47, respectively (Fig. 1).

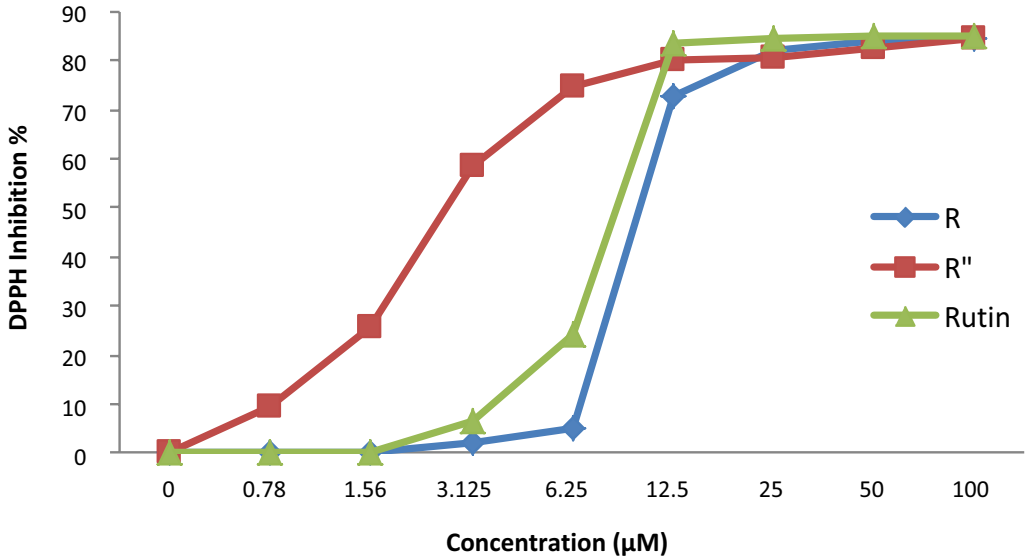

Fig. 5 Dose dependent antioxidant curves of the Rumex aqueous extract and its nanoparticles using DPPH assay

Table 1 The antioxidant IC_{50} values of the *Rumex nervosus* aqueous extract and its nanoparticles using DPPH assay

Compound	IC_{50} (µM) ± SD
Rumex nervosus aqueous extract (*R*)	34.1 ± 7.8
Rumex nervosus nanoparticles (*R*")	1.5 ± 3.5
Rutin	21.7 ± 3.9

According to the pilot study results, the *RN* nanoparticle doses selected for the safety and efficacy studies were 3.3 and 6.6 mg/kg given orally to rats.

Results of the safety study done for *RN* extract (250 and 500 mg/kg) and nanoparticles (3.3 and 6.6 mg/kg) are expressed in Table 2. There were no abnormal signs or any signs of toxicity, even the mortalities detected at the 6.6 mg/kg nano-dose were the same as those detected for the negative control group; moreover, they occurred later near the termination of the study, which arises the possibility to be for any other reason rather than the nanoparticles.

In the in vivo efficacy study, inflammation of peripheral tissue was induced by using formaldehyde injected in the rats' paws to induce paw edema. While gastritis and gastric ulcers were induced by ethanol. Both systemic and local inflammation were manifested by gastric ulceration and increased volume of rat paws. The severity of inflammation and effects of treatment on these findings were confirmed by measuring paw volumes, and estimation of the severity of gastric ulcer lesions.

When compared to the negative control group, subplantar injection of 0.2 ml 1% formaldehyde caused a considerable increase in the volume of all rat paws in all groups, however, treatment with indomethacin and *RN* extracts (250 and 500 mg/kg) and nanoparticles (3.3 and 6.6 mg/kg) significantly reduced the volumes of paws, which was time course dependent for *RN* and the low dose of nanoparticles only, and was dose-dependent for both the extract and nanoparticles (Table 3).

Anti-inflammatory and oxidative stress biomarkers result in serum (kappa β (NF-κB), Paraoxonase1enzyme (PON1), and oxidative stress marker Malondialdehyde (MDA)) are expressed in Table 4. Induction of inflammation elevated both NF-κB and MDA and reduced PON1 significantly relative to negative control. In contrast to the positive control group, treatment of all groups decreased NF-κB and elevated PON1 but non-significantly and reduced MDA but non-significantly.

Regarding the effect of *Rumex nervosus* aqueous extract (250 and 500 mg/kg) and nanoparticles (3.3&6.6 mg/kg); on gastric ulcer induced in rats by using ethanol (70%), the effects of ranitidine and both the high dose of extract and nanoparticles on the severity of lesions were markedly significantly better than the positive control, and the groups treated with low doses moreover the nanoparticles exhibited the highest protective effects (Fig. 6).

The results of the macroscopic examination were confirmed by histopathologic and histochemical assessment of the stomach lesions that are shown in Figs. 7, 8 and 9.

Table 2 Sub chronic safety study of *Rumex nervosus* aqueous extract (250–500 mg/kg) and its nanoparticles (3.3–6.6 mg/kg) given Po for two successive weeks

Signs	Group				
	Negative control	***Rumex nervosus* extract (250 mg/kg)**	***Rumex nervosus* extract (500 mg/kg)**	***Rumex nervosus* nanoparticles (3.3 mg/kg)**	***Rumex nervosus* nanoparticles (6.6 mg/kg)**
Body weight (g)					
Basal	176 ± 2.85	176 ± 2.85	176 ± 2.85	176 ± 2.85	176 ± 2.85
After 2weeks	187.7 ± 2.74	184.7 ± 2.21	186.8 ± 2.7	183.3 ± 2.53	179.7 ± 3.5
Bowel habits	NA	NA	NA	NA	NA
Behavior	NA	NA	NA	NA	NA
Fur changes	NA	NA	NA	NA	NA
Secretions and salivation	NA	NA	NA	NA	NA
Lips color	NA	NA	NA	NA	NA
% of Mortality	16.66 ± 0.01$^{@*\#}$ (after 10days)	0	0	0	16.66 ± 0.01$^{@*\#}$ (after 13days)

Results of weight are expressed as means of weight (g) ± SE, $n = 6$, non-significant change from base line weight in all groups or between groups after 2weeks of administration of *Rumex nervosus* extract or nanoparticles

Results of mortality are expressed as means of percent of deaths ± SE

NA, No abnormality detected

@significantly different from *Rumex nervosus extract* (250 mg/kg)

*Significantly different from *Rumex nervosus extract* (500 mg/kg)

$^{\#}$ Significantly different from *Rumex nervosus* nanoparticles (3.3 mg/kg)

Statistical analysis was done using two-way analysis of variance ANOVA followed by Tukey Kramer multiple comparisons test, $p \leq 0.0001$

Table 3 Oral administration time course effect of *Rumex nervosus* aqueous extract (250, 500 mg/kg), *Rumex nervosus* nanoparticles (3.3, 6.6 mg/kg) and Indomethacin (25 mg/kg) on rat's paw oedema formation induced by sub-plantar injection of 0.2 ml (1%, w/v) of Formaldehyde

Effect group	Time course					
	First hour		**Second hour**		**Third hour**	
	% of edema	**% of inhibition**	**% of edema**	**% of inhibition**	**% of edema**	**% of inhibition**
Negative control (1 ml saline)	Nil	–	Nil	–	Nil	–
Positive control Formaldehyde 0.2 ml (1%w/v)	109.6 ± 4.5	Nil	108.7 ± 8.3	Nil	98.6 ± 7	Nil
Indomethacin Reference drug (25 mg /kg)	30.1 ± 3.2*	72.09 ± 4.3	26.85 ± 3.2*	74.78 ± 3.8	26.34 ± 1.5*	72.5 ± 3.6
Rumex aqueous extract (250 g/kg)	65.03 ± 3.7$^{*@}$	40.47 ± 3.5$^{@}$	62.75 ± 4.1$^{*@}$	40.47 ± 8$^{@}$	32.15 ± 2*	66.7 ± 3.5
Rumex aqueous extract (500 g/kg)	64.12 ± 1.6$^{*@}$	41.18 ± 3$^{@}$	33.65 ± 2.2$^{*\#}$	68.23 ± 3.9$^{\#}$	29.72 ± 1.2*	69.07 ± 3.6
Rumex nanoparticles (3.3 g/kg)	61.68 ± 4.2$^{*@}$	40.69 ± 6.1$^{@}$	33.27 ± 2.5$^{*\#}$	67.98 ± 3.3$^{\#}$	28.24 ± 1.4*	69.79 ± 2.9
Rumex nano- particles (6.6 mg/kg)	30.14 ± 2$^{*\#\$\&}$	72.37 ± 2.2$^{\#\$\&}$	13.81 ± 3.7$^{*\#\$\&}$	87.37 ± 3$^{@\#\$\&}$	18.68 ± 2.3*	80.49 ± 3.2$^{\#}$

Results are expressed as means (a) % of edema ± SE and (b) % of inhibition ± SE, $n = 6$

Statistical analysis was done using a two-way analysis of variance ANOVA followed by Tukey Kramer multiple comparisons test, $p \leq 0.0001$, comparison is measured for the same time interval for all groups

*Significant difference from the positive control group at $p \leq 0.0001$at same time interval

$^{@}$ Significant difference from the Indomethacin (25 mg/kg) group at $p \leq 0.0001$ at same time interval

$^{\#}$ Significant difference from *Rumex* aqueous extract (250 mg/kg) at $p \leq 0.0001$ at the same time interval

$^{\$}$ Significant difference from *Rumex* aqueous extract (500 mg/kg) at $p \leq 0.0001$ at the same time interval

$^{\&}$ Significant difference from *Rumex* nanoparticles (3.3 mg/kg) at $p \leq 0.0001$ at the same time interval

The upper right portion of the stomach removed from negative control rats showed a larger magnification of gastric pits and superficial epithelium during histopathological analysis (Fig. 7A), Treating animals with ethanol 70%, caused severe damage in gastric mucosa, as about ¾ of mucosa layer were completely

Table 4 *Rumex nervosus* aqueous extract oral administration effect on serum levels of nuclear factor kappa B (NF-κB), Paraoxonase1enzyme (PON1), and Malondialdehyde (MDA)

Group	Parameter		
	NF-κB ng/ml	PON1 U/L	MDA nM/L
Negative control	0.9 ± 0.1	245 ± 3.67	14.18 ± 0.35
F. Positive control: formaldehyde 0.2 ml (1%w/v)	9.68 ± 0.74[@]	200.9 ± 9.8[@]	25.66 ± 2.98[@]
Ranitidine: reference drug (50 mg/kg)	2.35 ± 0.3[*]	188.1 ± 16.5[@#]	16.28 ± 0.69
Rumex aqueous extract (250 mg/kg)	3.63 ± 0.38[@*]	232.3 ± 7.43	22.02 ± 1.75
Rumex aqueous extract (500 mg/kg)	2.53 ± 0.85[*]	218.8 ± 3.33	18.45 ± 1.05
Rumex nanoparticles (3.3 mg/kg)	4.16 ± 0.6[@*]	223.2 ± 4.83	23.91 ± 4.6
Rumex nanoparticles (6.6 mg/kg)	0.88 ± 0.07[*#$]	183.7 ± 3.4[@#]	23 ± 1.1

Results are expressed as means of serum levels of markers ± SE. n = 6. Statistical analysis was done using one-way analysis of variance ANOVA followed by Tukey Kramer multiple comparisons test, $p \leq 0.05$

[@] Significant difference from the negative control group

*Significant difference from the positive control group

[#] Significant difference from *Rumex* aqueous extract (250 mg/kg)

[$] Significant difference from *Rumex* nanoparticles (3.3 mg/kg)

damaged in many areas leaving large gaps filled with degenerated cells and necrotic materials (Fig. 7B). It also caused the blood capillaries to be dilated and congested, the muscularis mucosa layer was markedly thickened to protect the underlying tissues (Fig. 7C).

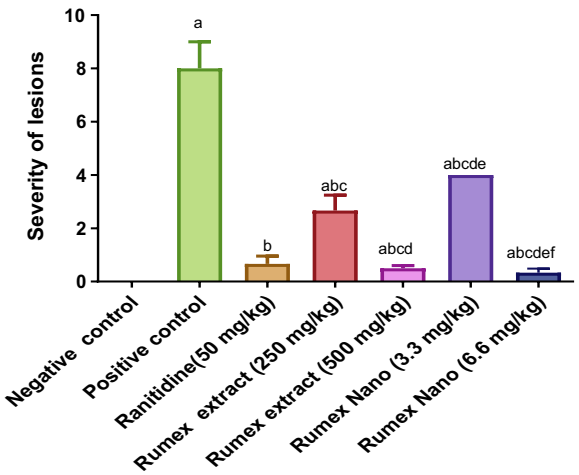

Fig. 6 Study of the effect of *Rumex nervosus* aqueous extract (250, 500 mg/kg) and nanoparticles (3.3, 6.6 mg/kg); on gastric ulcer induced in rats by using ethanol (70%). Results are expressed as means of score of lesions ± SE. *N* = 6. Statistical analysis was done using one way analysis of variance ANOVA followed by Tukey Kramer multiple comparisons test, $p \leq 0.05$: **a** Significantly different from Negative control group, **b** Significantly different from Positive control group **c** Significantly different from Ranitidine (Reference group), **d** Significantly different from *Rumex* extract (250 mg/kg), **e** Significantly different from *Rumex* extract (500 mg/kg), (f)Significantly different from *Rumex* nano (3.3 mg/kg)

Treating ulcer model animals with Ranitidine ameliorated the ethanol effect in an incomplete way. The fundamental framework of tubular glands was regained, although the different types of glandular cells were not well defined and the mucus-secreting superficial cells were atrophied (Fig. 7D).

The best results were obtained by using *Rumex* nanoparticles, especially with the high dose where the normal structure of gastric mucosa was completely restored (Fig. 8A, B). A less influential effect was observed by using *Rumex* extract as inflammatory cell infiltration and the absence of superficial epithelium was noticed with a low dose (Fig. 8C), while the high dose restored the normal structure of tissue but with slight inflammatory cell infiltrates (Fig. 8D).

The histochemical results confirmed those obtained from the histopathological investigation as ethanol caused depletion of mucus material from gastric mucosa (Fig. 9B) if compared to normal tissue (Fig. 9A).

Using reference drug led to weak restoration of mucus in some lesions or no regeneration in others, there was a weak positive reaction to the stain on both the surface and neck of glands. Another section showed no positive reaction all over the gastric mucosa (Fig. 9C, D).

Treating animals with *Rumex* nanoparticles (low dose) gave the least results, as mucus was noticed only in the neck region of gastric glands (Fig. 9E). The best results were obtained from groups treated with *Rumex* nanoparticles (high dose), where complete regeneration of gastric mucosa content of mucous material occurred (Fig. 9F). Results obtained from groups treated with *Rumex* extract were better than those of nanoparticles (low dose) but

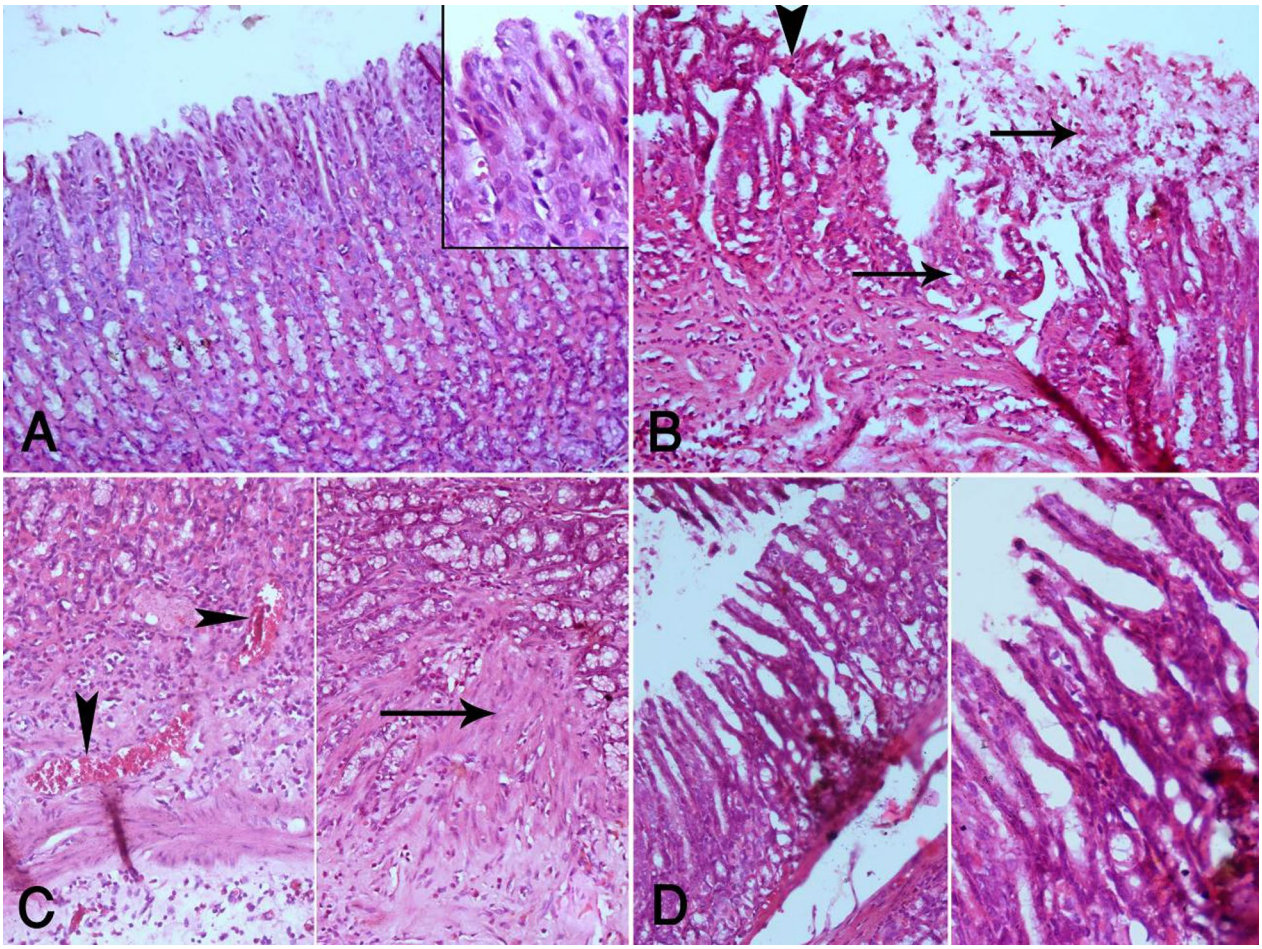

Fig. 7 A stomach tissue sections photomicrograph from: **A** control-ve rat shows the normal structure of gastric mucosa, the upper right part shows a higher magnification of gastric pits and superficial epithelium. **B** ethanol treated rat shows complete loss of gastric mucosa forming a large gap filled with degenerated cells & necrotic materials (arrow). **C** another figure for the same group shows the lower part of gastric mucosa with marked dilatation & congestion of blood capillaries (arrowhead) and thickening of muscularis mucosa layer (arrow). **D** ulcer model rat treated with Rantidine shows incomplete regeneration of gastric mucosa at the ulcer site. The right part of the figure shows in higher magnification large gastric pits due to atrophy of superficial mucus-secreting cells. The tubular glands below are deformed containing small mal-differentiated cells, (Hx & E X 200, 400)

less than those of nanoparticles (high dose), they were dose-dependent (Fig. 9G, F).

Discussion

In the present study, the in-vitro cytotoxicity study results revealed that both nanoparticles of *Rumex nervosus* and the aqueous extract may be used, in some treatments, to increase the cell growth in some diseases which cause cell growth inhibition, to compensate for that effect. Moreover, the *Rumex* nanoparticles showed very strong antioxidant activity. The cytoprotective and antioxidant activities of *RN* nanoparticles are stronger than those of the normal extract.

The effects of both the extract and nanoparticles were dose-dependent.

Rumex nervosus is a plant that grows in Yemen and Saudi Arabia Kingdom, and prior research on it found that its ethanolic extract varied in its cytotoxic effect against human cancer cell lines and non-toxic up to a level of 7.1 g/kg b.wt. (Qaid et al. 2021), also, *Rumex nervosus* was used for the management of wounds, diarrhea, typhus, rabies, and skin disorders (Al-Quraishy et al. 2020). Phytochemical anlysis in the present study revealed that *RN* extract contained quercetin "luteolin" acacetin, hesperetin, naringenin and liquiritin, which was consistent with the study done by Gemechu et al. (2021). This may aid in the interpretation of the results of most investigations in the present in vivo study.

Induction of systemic and local inflammation in the present study was manifested by macroscopic and

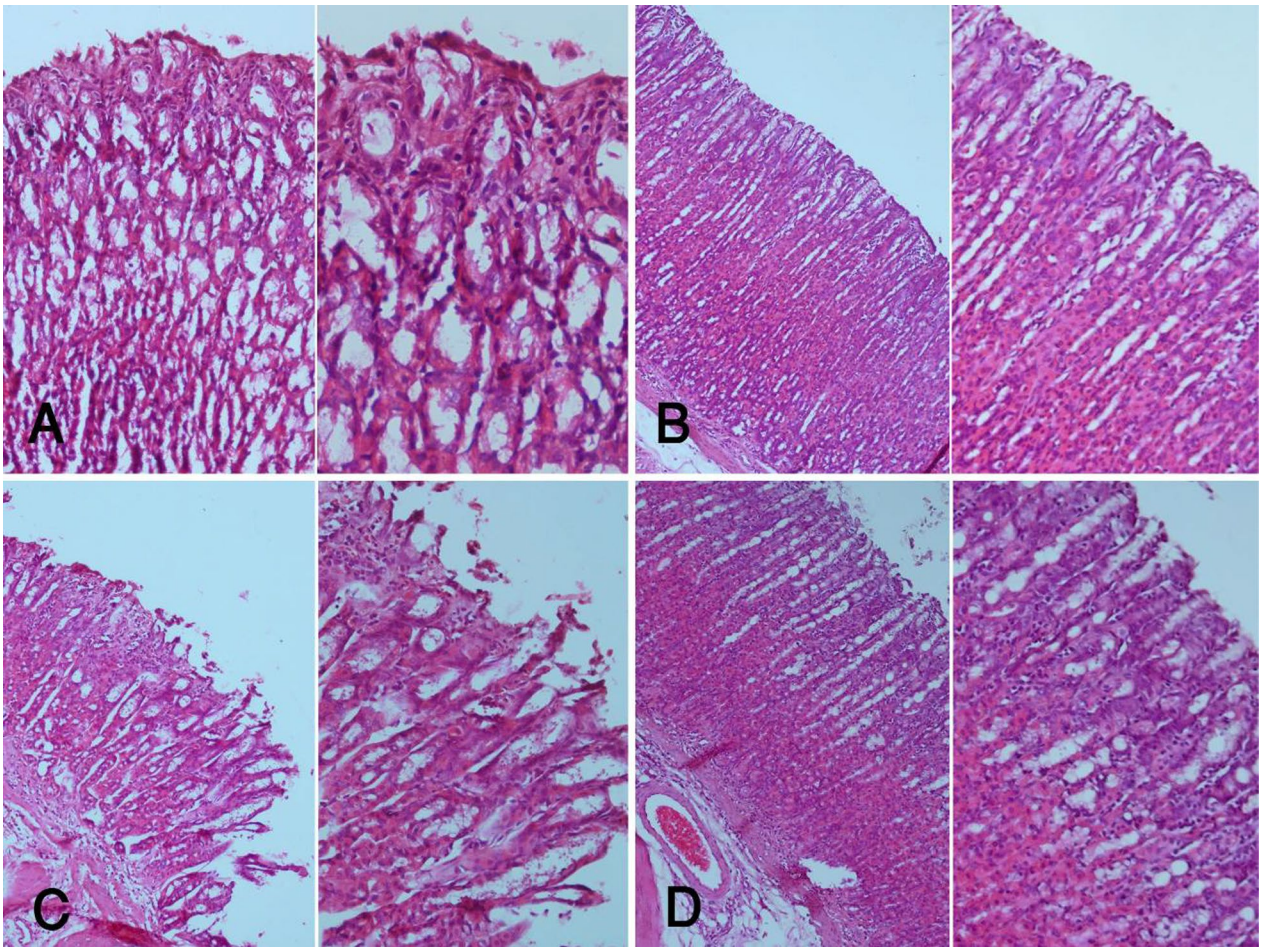

Fig. 8 A photomicrograph of gastric mucosa sections from ulcer model rats treated with: **A** *Rumex* nanoparticles low dose shows loss and/or abnormal superficial epithelium with deformed tubular glands below. **B** *Rumex* nano-high dose shows gastric mucosa close to normal. **C** *Rumex* extract low dose shows regeneration of the lower part only of gastric mucosa, while the upper part shows inflammatory cells infiltration (arrow) and absence of superficial epithelium. **D** *Rumex* extract high dose shows marked amelioration of gastric mucosa, however slight thickening of muscularis mucosa & dilatation of blood capillaries are still observed. The higher magnification part of the figure shows restoration of the normal structure but with slight inflammatory cell infiltration. (Hx & E X 200, 400)

microscopic gastritis and gastric ulceration as well as gross paw edema.

The percentage of paw edema inhibition in the treated groups was compared to the indomethacin group, which served as the study's reference medication. The peak effect of indomethacin was after the second hour of administration, after that, its effect started to decrease, however, the inhibitory effect of the *RN* extract (250 and 500 mg/kg) increased over time, reaching its peak after the third hour, additionally, this effect was dose-dependent. This may be an indication that it has a sustained slow effect which is suitable for chronic cases. The effect of

(See figure on next page.)

Fig. 9 A photomicrogrph of gastric mucosa- stained with PeriodicAcid Schiff reagent- from rats treated with: **a** Saline (control –ve) shows mucous material normal content– stained in magenta red- on the surface of tissue and in gastric glands neck region of. **b** Ethanol shows complete loss of the positive reaction to the stain on the glands surface and in neck. **c** Ethanol and Rantidin shows weak positive reaction to the stain on both the surface and neck of glands. **d** Other section from the previous group shows no positive reaction all over the gastric mucosa. **e** Ethanol and *Rumex* nano (low dose) show weak positive reactions to the stain in the neck region only. **f** Ethanol and *Rumex* nano (high dose) show strong positive reactions to normal- on both the surface and neck glands. **g** Ethanol and *Rumex* extract (low dose) show mild positive reactions in neck glands only. **h** Ethanol and *Rumex* extract (high dose) show a strong positive reaction in the neck glands but not on the surface. (PAS X 200)

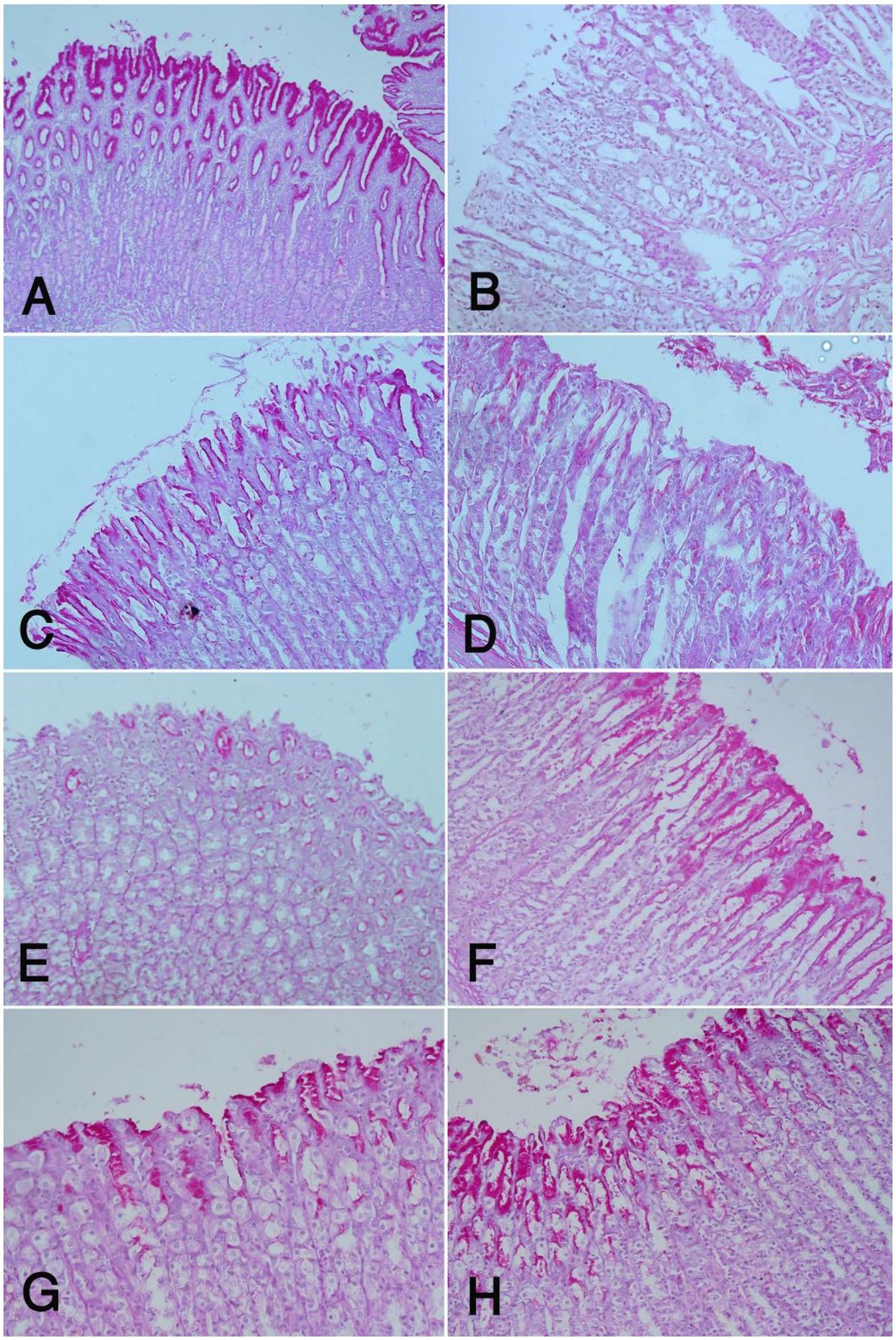

Fig. 9 (See legend on previous page.)

nanoparticles (3.3 and 6.6 mg/kg), was dose-dependent and the effect of the low dose was more or less like the effect of the high dose of the extract, moreover, this effect increased gradually. It is worth noting that the high dose of nanoparticles exhibited the most significant oedema inhibitory effect among all *RN* preparations, however its peak effect was as the reference drug during the second hour after that it decreased which may be due to clearance of high dose. This may be an indication for giving the low dose of nanoparticles twice daily to avoid the effect of a single loading dose and achieve the highest anti-inflammatory effect.

Systemic and local inflammations were accompanied by elevated inflammatory and oxidative stress markers. This picture, on the other hand, was dramatically improved in groups treated with *Rumex nervosus* extract and nanoparticles, with the high dose of nanoparticles effect superior in most evaluated parameters except MDA. MDA is an oxidative stress biomarker that is elevated in cases of alcoholism or drug abuse, to that result in lipid peroxidation, and subsequently, tissue inflammation manifested by serious illness such as ulceration and/or edema. Moreover, oxidative stress due to alcoholism increases the incidence of apoptosis, as a result of mitochondrial dysfunction, which reduces the synthesis of ATP, with an augmented generation of free radicals associated with depolarization of cell membrane as well as loss of cell membrane potential together with the production of Cytochrome C that augments apoptosis (Taler-Verčič et al. 2020). This explains why the anti-apoptotic marker nuclear factor kappa (NF-B) was significantly higher in the positive control group compared to the negative control group in the current study, emphasizing the prophylactic effects of both *Rumex nervosus* extracts and nanoparticles, as NF-B levels in treated groups were significantly lower than in the positive control groups. The fact that NF-B is chronically active in inflammatory illnesses such as rheumatoid arthritis and inflammatory bowel disease explains our findings (Park and Hong 2016).

A glycoprotein paraoxonase-1 (PON1) that is produced in the liver, has hydrolytic activities such as paraoxonase, arylesterase, and lactonase (Taler-Verčič et al. 2020). It modulates the immune system by hydrolyzing homocysteine thiolactone (Bacchetti et al. 2019). It attaches to cell membranes, protecting lipids from peroxidation (Sarandol et al. 2023), which is critical in inflammation and the onset of inflammatory disorders. However, during systemic inflammation its aryl esterase activity may be disrupted leading to loss of its protective effect (Meisinger et al. 2021), which was observed in the present study and manifested by impaired levels of PON1 in the groups when compared to the negative control group.

During re-programming of cell function during inflammation, cells change their phenotype and eventually express combinations of genes that prepare the tissue for regeneration when the inflammatory factor is removed (Kourtzelis et al. 2019). This may explain the mechanism of ulcer healing of the groups that were treated with *Rumex* in gastric insult models in our study, which was confirmed by the histopathologic study.

The effects of *Rumex* in the current study are most probably due to the abundance of gallic acid (700 _g/g) in leaf extracts and essential phytochemical ingredients like carbohydrates, vitamins, sterols, glycosides, triterpenes, saponins, tannins, and flavonoids in the root, stem, leaf, and metabolites of *Rumex nervosus* (Shaaban et al. 2016).

Conclusions

1. The in vitro study of cytotoxicity and antioxidant activities reveals that *Rumex* nanoparticles are much better than the total extract of *Rumex nervosus* as an antioxidant and cytoprotective agent. Additionally, the in vivo studies show that *RN* is a promising herb that can be used as an anti-inflammatory supplement for protection against tissue inflammation and ulceration and promotion of wound healing. The *RN* nanoparticles can be considered for formulation in novel drug delivery systems for attaining a better efficacy of that promising herb, as they exhibited significant improvement in inflammatory conditions compared to the extract when given in much lower doses.
2. Nanoparticles are used to achieve better compliance as the same efficacy can be reached with lower doses, less frequency of administration, and lower treatment costs.

Abbreviations

ATP	Adenosine triphosphate
b.wt	Body weight
DPPH	2,2-Diphenyl-1-picrylhydrazyl
g	Gram
HepG-2	Human liver carcinoma
Kg	Kilogram
MCF-7	Human breast adenocarcinoma
MDA	Malondialdehyde
Mg	Milligram
ml	Milli liter
MTT	3-[4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide
NF-κB	Nuclear factor kappa *β*
Pas	Periodic acid-Schiff stain
PON1	Paraoxonase-1
RN	*Rumex nervosus*
RPE-1	Human normal retina pigmented epithelium

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Un-applicable.

Author contributions
AHM and HMA prepared the extract and nanoparticles performed the in vitro cytotoxicity and antioxidant activity as well as shared in writing, analyzing data, and revising data of this section. BMMI performed, wrote, analyzed data, and revised the in vivo pharmacological study including the pilot study, safety and efficacy studies done on intact living animals as well as macroscopic evaluation of anti-ulcerogenic effects. ERY performed, wrote, analyzed data, and revised the in vivo biochemical parameters study. NMS performed, wrote, and revised the immune-histochemical and histopathological examination study.
All authors have read and approved the manuscript.

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The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Un-applicable.

Consent for publication
Un-applicable.

Competing interests
The authors confirm that there is no conflict of interest of any type regarding the present study.

Author details
[1]Department of Pharmacology, National Research Centre, Dokki, Giza 12622, Egypt. [2]Department of Tanning Materials and Leather Technology, National Research Centre, Dokki, Giza 12622, Egypt. [3]Department of Pathology, National Research Centre, Dokki, Giza 12622, Egypt. [4]Department of Medical Biochemistry, National Research Centre, Dokki, Giza 12622, Egypt. [5]Department of Therapeutic Chemistry, National Research Centre, Dokki, Giza 12622, Egypt.

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