Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 3(6): 221-232
Received: 15-01-2015
Accepted: 02-02-2015

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Phytochemical screening and gastroprotective effect of the aerial parts of *Salasola terrandra* Forssk. Against aspirin induced gastric ulceration in rats

Fatma S. Elsharabasy, Amina A.M. AL-Mushhin, Sherifa Araffa, Abdel Razik H. Farrag

Abstract
Salsola plants are widely used in folk medicine**.** Our previous studies have showed that the ethanol extract of *S. inermis*, *S. volkensii*, *S. villosa* grown in Egyptian desert have antioxidant, hepatoprotective and anti-inflammatory activity. The present study deals with the identification of the phytochemical constituents of the extract from the aerial parts of *S. tetrandra* which grow in Saudi Arabia using GC-MS and GLC analysis. On the other hand, the effect of the alcoholic extract of the aerial parts of *Salasola terrandra* against Aspirin induced gastric ulceration in rats.
The aerial parts of *Salsola tetrandra* (forsskal) were collected from wild plants growing El Doubia at El Riyadh- El Dallamroad. Air-dried and powdered aerial parts of *Salsola tetrandra* were extracted with petroleum ether, the solvent was evaporated under reduced pressure then extracted with 70% alcohol in H_2O and the solvent was stripped off under reduced pressure gave 50 g. The petroleum ether extract 10 g was subjected to saponification for subsequent investigation. Identification of compounds was done using different chemical and spectroscopic methods. The 70% alcoholic extract was subjected to examine its antiulcer against Aspirin induced gastric ulceration in rats. Thirty five albino rats were divided into five groups: Group I: Control (untreated) group, Group II: Aspirin group (150 mg/kg), Group III: *Salsola* group (50 mg/kg), Group IV: *Salsola* group (100 mg/kg), Group V: Standard drug group (Ranitidine 20 mg/kg). Sections of stomach were prepared for histopathological and histochemical examinations. Phytochemical investigation of the alcoholic extract from the aerial parts of *S. tetrandra* revealed the presence of Coumarins, Saponins, Alkaloids, Terpenes and Steroids. GC-MS chromatograms of the unsaponifable matter from petroleum ether extract of *Salsola tetrandra* showed fourteen peaks indicating the presence of fourteen compounds, Ten major phytochemical constituents were identified and are represented as Tridecanamine (1), 2,7-Dimethyl-1-octanol (2); Isohexyl-2-pentylester (3); 3,9-Diethyl-6-tridecanol (4); Methyl palmitate (5);9-octadecynoic acid (6); Hexadecanoic acid, ethyl ester (7); 9,12-octadecadienoic (Z,Z), methyl ester (8); 2,3-Dihydroxypropyl octadecanoate (9), Tetradecanoic acid, methyl ester. GLC of total fatty acids revealed the presence of saturated and unsaturated fatty acids. The percentage of unsaturated fatty acids was 56.84% (polyunsaturated fatty acids was 48.59% and monounsaturated was 8.25%), while saturated fatty acids percentage was 43.16%. Histopathological and histochemical results indicated that ulcer index showed a significant decrease ($p< 0.05$) in the *Salsola* treated rats, whereas, there was increase in gastric mucus and reduction of the erosion of the mucosa. These changes were dose dependant. The reverse was seen in Aspirin group. The study showed that *Salsola tetrandra* extract has an ulcer protective effect similar to that of Ranitidine.

Keywords: *Salsola tetrandra*, Saudian desert, GC-Mass, aspirin, gastric ulcer.

1. Introduction
Gastric ulcers are a common disorder of the entire gastrointestinal tract that occurs mainly in the stomach and the proximal duodenum. This disease is multifactorial and its treatment faces great difficulties due to the limited effectiveness and severe side effects of the currently available drugs (Mota *et al*. 2009) [20].
Aspirin is a potent non-steroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Gastric ulcer associated with the use of aspirin is a major problem. Many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E_2 (PGE_2) content (Laine, *et al*. 2008) [20] and proinflammatory cytokines interleukin (IL-1β) and tumor necrosis factor (TNF) –α play important role in the genesis of gastric mucosal damage and its subsequent development (Wang, *et al*. 2007; Wallace 2008) [36]. Plant extracts are attractive sources of new drugs and have been shown to produce

promising results in the treatment of gastric ulcers (Al kathar and Munir, 1989) [3]. Numerous plants and herbs are used to treat gastrointestinal disorders in traditional medicine (Schmeda-Hirschmann and Yesilada, 2005; Mota *et al*. 2009) [20].

The genus *Salsola,* family Chenopodiaceae **(**Goosefoot family) consists of over100species found in the dried regions of Asia, Europe and Africa (El Hadidi& Fayed, 1994; Boulos, 1995) [10, 7]. Some *Salsola* plants are widely used as folk medicine for the treatment of hepatitis [Abegaz & Woldu, 1991] [1] or infections caused by tapeworm andparasites [Saratikov & Vengerovskil, 1995] [26] they also have pronounced vasoconstrictive, hypertensive, and cardiac stimulant action [Beyaoui, *et al*, 2012] [6] and can act as an allergenic substance [Nofal, 2004; Assarehzadegan, 2009] [21, 4]. *Salsola* species have antioxidant and anti-inflammatory properties [Ahlam & Fatma, 2007] [2]. Alkaloid extracts from *Salsola* species (Chenopodiaceae) have potential role in the treatment of Alzheimer's disease [Tundis R, 2009] [34]. Previous phytochemical investigation of the genus resulted in the isolation of alkaloids, saponins, sterols and their glucosides, comarinolignan, isoflavonoids, and flavonoids (Karawya *et al,* 1972; Wassell, *et al* 1979; Woldu and Abegaz, 1990; Syrchina *et al*, 1991; Oueslati *et al,* 2004; Oueslati et al, 2006; Xiang *et al,* 2007; Elsharabasy et al, 2013) [15, 39, 40, 31, 22, 23, 41, 11].

Therefore, the present study deals with the identification of the phytochemical constituents of the hydro alcoholic extract from the aerial parts of *S. tetrandra*. Besides, the antiulcer effect of the extract of *Salsola* was evaluated on asprin-induced ulcer in rats.

2. Materials and Methods

Collection and Identification of Plant Material:

The plant was collected in2013-2014 and taxonomically identified in March, 2013 by Sherifa Arafa, Assistant Professor of Taxonomy and Flora in Salman University.

Extraction of Plant Material

Air-dried and powdered aerial parts of *Salsola tetrandra* (500 g) were extracted with petroleum ether (40-60 ^{O}C) in continuous extraction apparatus. The solvent was stripped off under reduced pressure gave (10 g) residue. The above lipoidal matter was subjected to saponification for subsequent investigation of both unsaponifiable and saponifiable fractions.

Preparation of unsaponifiable matter and fatty acids

The above light petroleum extract was refluxed with alcoholic potassium hydroxide (10%) for 2 hrs, after stripping off ethanol and dilution with water, the unsaponifiable matter was extracted with chloroform. The residue left after evaporation of chloroform (U.S.M) was weighed (5.89 g) and kept for further investigation. Samples of the unsaponifiable fraction were subjected to GLC analysis.

Preparation of fatty acids methyl ester

The soapy aqueous layer in each case was acidified with 10% hydrochloric acid and the liberated fatty acids were extracted with ether. The residue left after evaporation of ether was weighed (0.64 g) and kept for further study. Total fatty acids (TFA) were subjected to methylation [Abegaz & Woldu, 1991] [1].

The aerial parts of *Salsola tetrandra* (500 g) were defatted with petroleum ether (40- 60 ^{O}C) and extracted three times with 70% aqueous EtOH, evaporation of the solvent under pressure from the combined extract afforded ethanolic extract (50 g).

Phytochemical Analysis

The following Phytochemicals of 70% alcoholic extract from the aerial parts of *Salsola tetrandra* which include Coumarins, Saponins, Alkaloids, Tannins, Steroids, Terpenes, Fatty acids and hydrocarbons were analyzed according the methods of Brain and Turner (1975), Trease and Evans (2005) and Harbone (1975)] [8, 33, 13].

Instruments and chromatographic conditions:

GC-MS analysis of the extracts was carried out using a Thermo Scientific, Trace GC, Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1 mL/min. The injector and MS transfer line temperature was set at 280 ^{O}C. The oven temperature was programmed at an initial temperature 50 ^{O}C (hold 2 min) to 150 ^{O}C at an increasing rate of 7 ^{O}C /min. then to 270 at an increasing rate 5 ^{O}C min (hold 2min) then to 310 as a final temperature atan increasing rate of 3.5 ^{O}C min (hold 10 min).

The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the compiled data for known compounds.

Biological study

Drugs and Chemicals:

Aspirin was obtained from Chemical Industries Development (CID) Giza (Egypt) as tablets each tablets contain 75 mg acetyl salycilic acid, and was suspended in 0.5% carboxymethylcelluose (El Nasr Chemicals Company, Egypt). Ranitidine was kindly provided by Glaxo SmithKline, Egypt.

Animals:

The present study was conducted on adult female albino rats (National Research Centre Animal House, Dokki, Cairo, Egypt). A total number of 30 female albino rats weighing between 120 -150 g were used. Rats were fed a standard diet of commercial rat chow and tap water and left to acclimatize to the environment for at least one week prior to inclusion in the experiments under standard conditions of humidity, temperature and light (12 hours light and 12 hour dark cycle). This study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee

Experimental design:

Rats were randomly divided into five groups with six animals in each group as follows:

Group I: Control (untreated) group

Group II: Aspirin ulcer induced (200 mg/kg)

Group III: hydro alcoholic extract of *Salsola* (50 mg/kg) in ulcer rats.

Group IV: hydro alcoholic extract of *Salsola* (100 mg/kg) in ulcer rats

Group V: Standard drug group (Ranitidine 20 mg/kg) in ulcer rats

All rats were fasted for 24 hours but excess water was allowed. *Salsola* extract (50 and 100 mg/kg) and the standard drug (Ranitidine 20 mg/kg) were administered orally to the

respective groups. One hour after their pretreatment, all animals were gavaged with aspirin (200 mg/kg).

Gross examination:
The animals were sacrificed 6 h later and their stomachs were removed and openedalong the greater curvatureto calculate the ulcer index by Kunchandy method [Kunchandy, *et al,* 1985] [16]. The numbers of ulcers were counted using magnifying lenses. Each ulcer was then measured with a verniercalliper to assess the diameter. The percent protection with each test drug dose was also calculated by the following formula (Suzuki *et al*. 1976) [30]:

$$\% \text{ Protection} = \frac{\text{UI control} - \text{UI treated}}{\text{UI control}} \times 100$$

Where, UI stands for ulcer index.

Histopathological studies
After recording the ulcers produced in the stomach, a longitudinal section of the gastric tissue was taken from the anterior part of the stomach and fixed in a 10% formalin solution. After 24 h of fixation followed by embedding in a paraffin block, it was cut into sections of 5 µm onto a glass slide and stained with hematoxylin-eosinfor histological assessment of the gastric mucosa according to **Bancroft *et al.* (1996)** [5].

Histochemical studies
The polysaccharides and total proteins
Periodic acid Schiff method (Suvarna *et al*, 2013) and Mercury bromophenol blue method (Mazia, 1953) were applied for visualization of the polysaccharide and total proteins materials in the mucosa of the stomach, respectively. These materials were demonstrated in sections with 5 µm thickness.

Statistical analysis
Data are expressed as Mean ± S.E. with a value of $P<0.05$ considered statistically significant. Statistical evaluation was performed by ANOVA followed by the Student's t-test. All analysis was made with the statistical software Microcal Origin (Version 6, Microcal Software Inc. Northampton, USA).

3. Results and Discussion
Phytochemical analysis of 70% alcoholic extractfrom theaerial parts of *Salsola tetrandra* revealed the presence of Saponins, Terpenes, Coumarins, Steroids and Alkaloids.**GC-MS** chromatograms of the unsaponifable matter from petroleum ether extract of *Salsola tetrandra* showed fourteen peaks indicating the presence of fourteen compounds and are presented in Fig.1. Identification of the compounds was carried out by comparison of their retention time with the available reference compounds (Table 1).
When the mass spectra of these fourteen peaks were compared with those of the compiled data for known compounds, nine major phytochemical constituents were identified and are represented in Table. 1. with their retention time (RT), molecular formula, molecular weight and peak area (%). The mass spectra of the nine major constituents are presented in Fig. 2 to Fig. 10.
GLC of total fatty acids revealed the presence of long chain fatty acids, the percentage of unsaturated fatty acids was 56.84% (polyunsaturated fatty acids was 48.59% and monounsaturated was 8.25%, and saturated fatty acids percentage was 43.16% (Table 2).

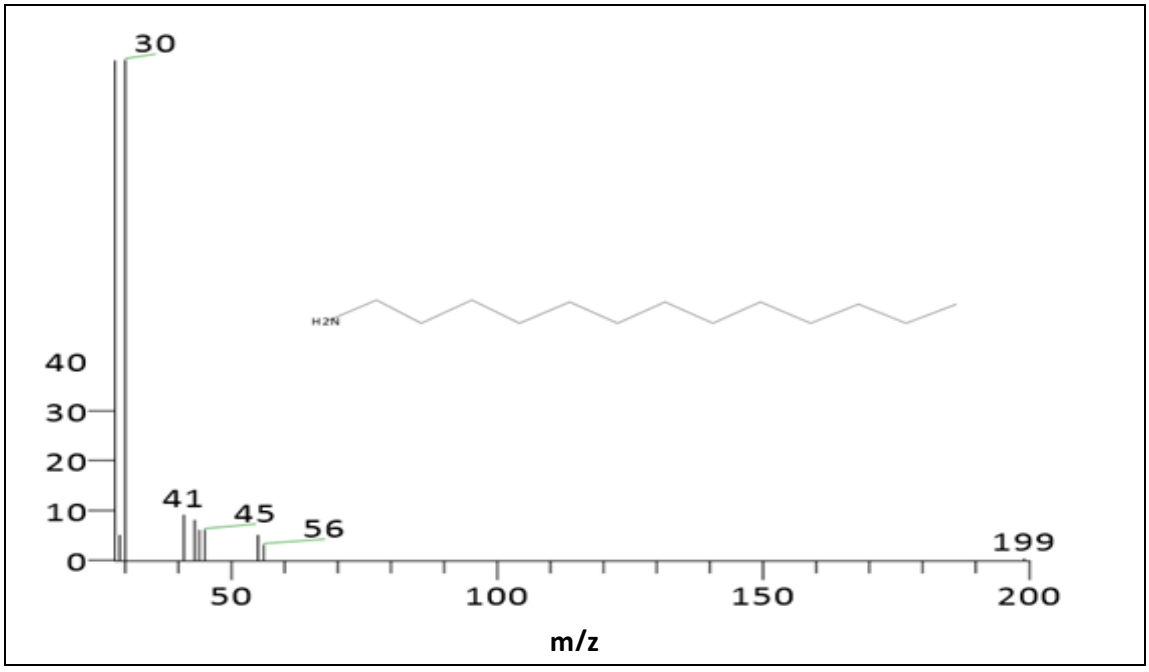


Fig 1: Tridecanamine

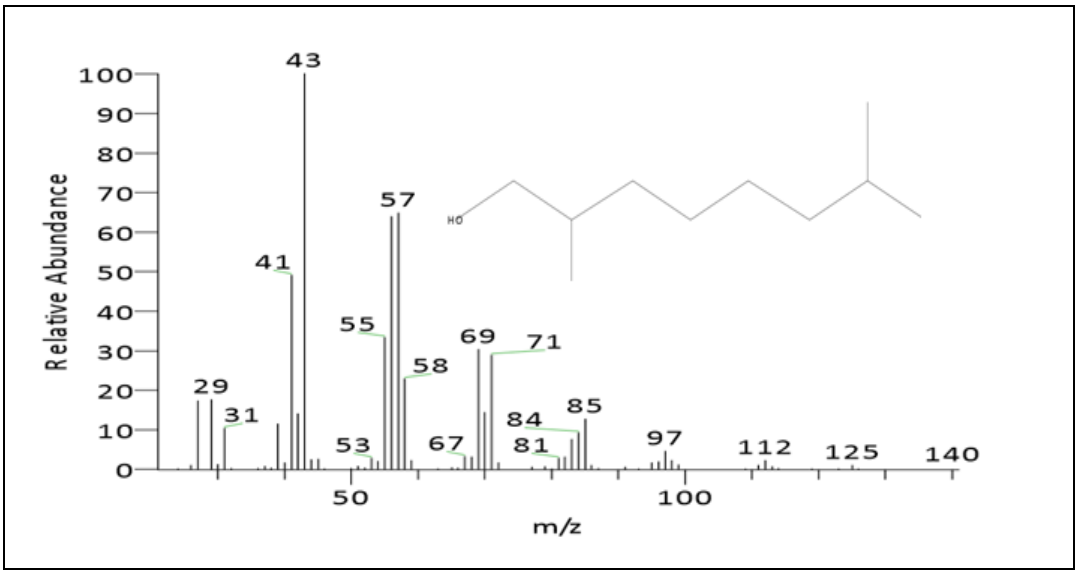


Fig 2: 2,7-Dimethyl-1-octanol

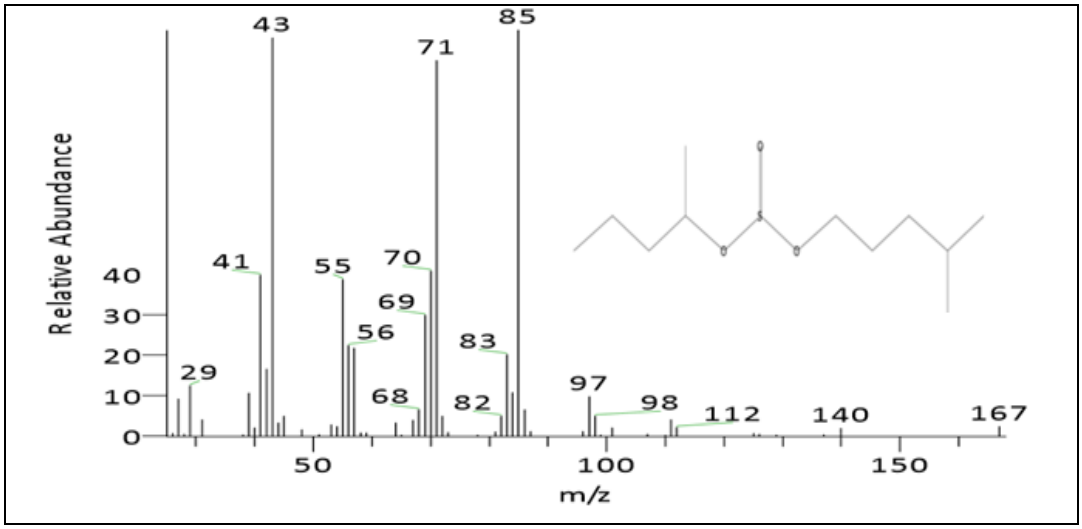


Fig 3: Sulfurous acid, isohexyl 2- pentyl ester

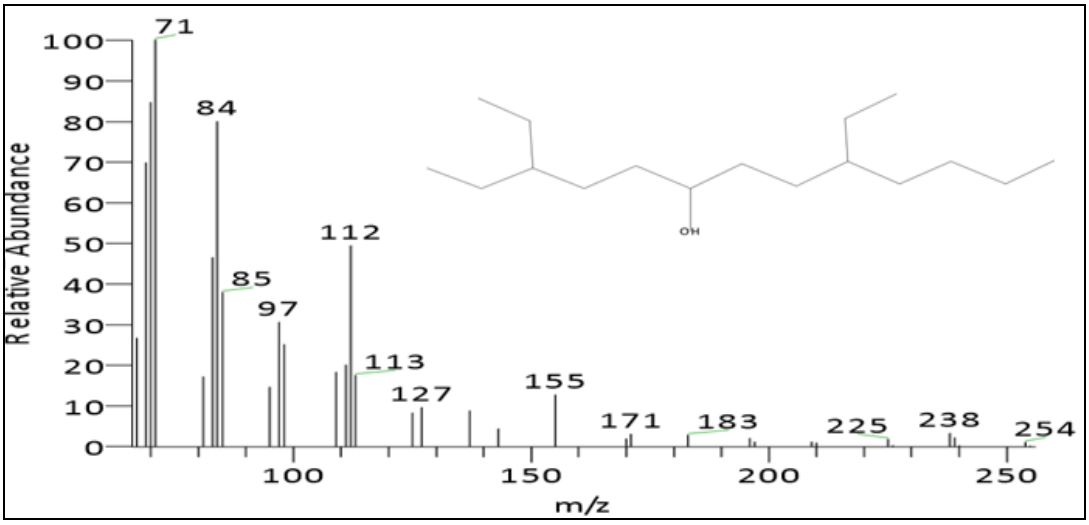


Fig 4: 3,9 Diethyl-6-tridecanol

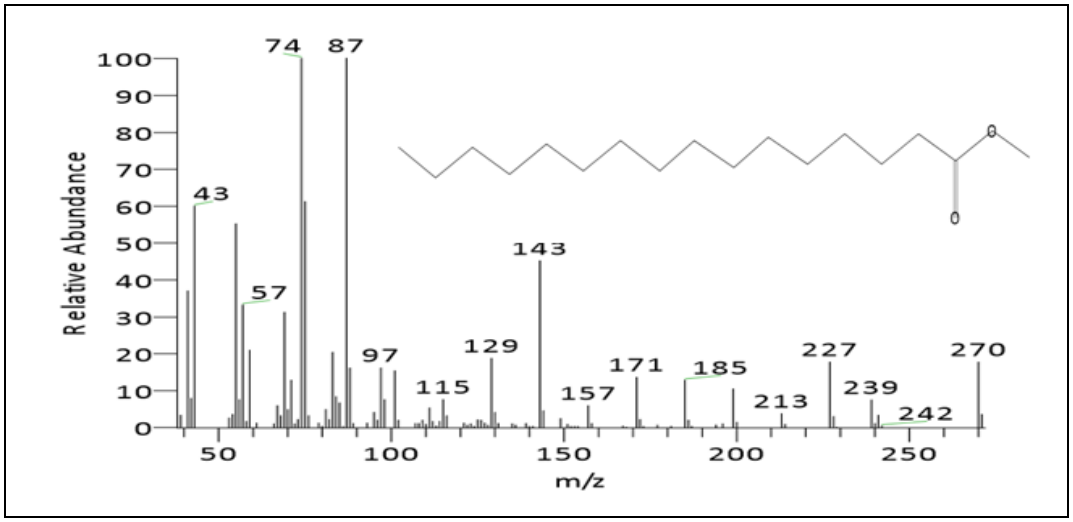


Fig 5: Methyl palmitate

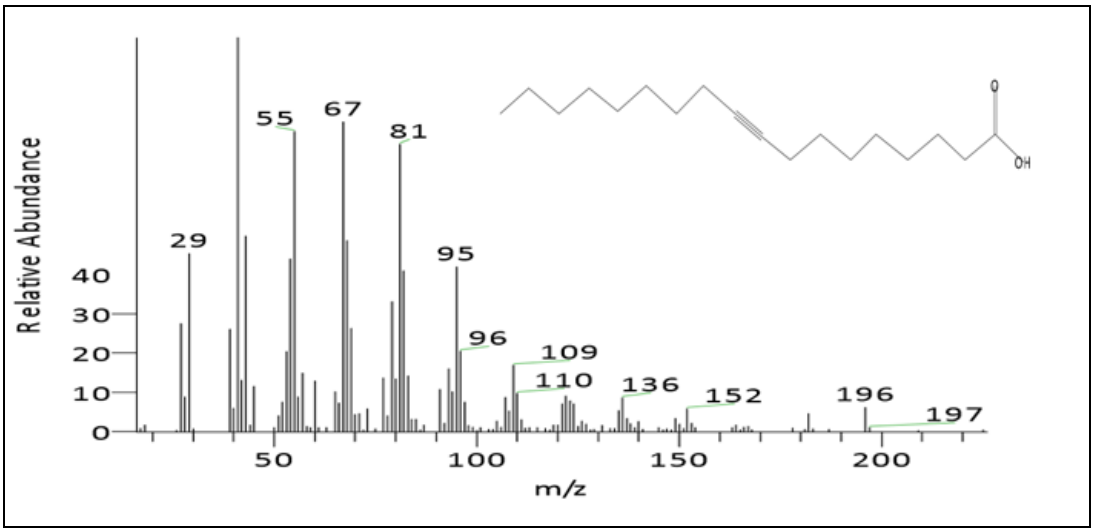


Fig 6: 8-octadecynoic acid Stearolic acid

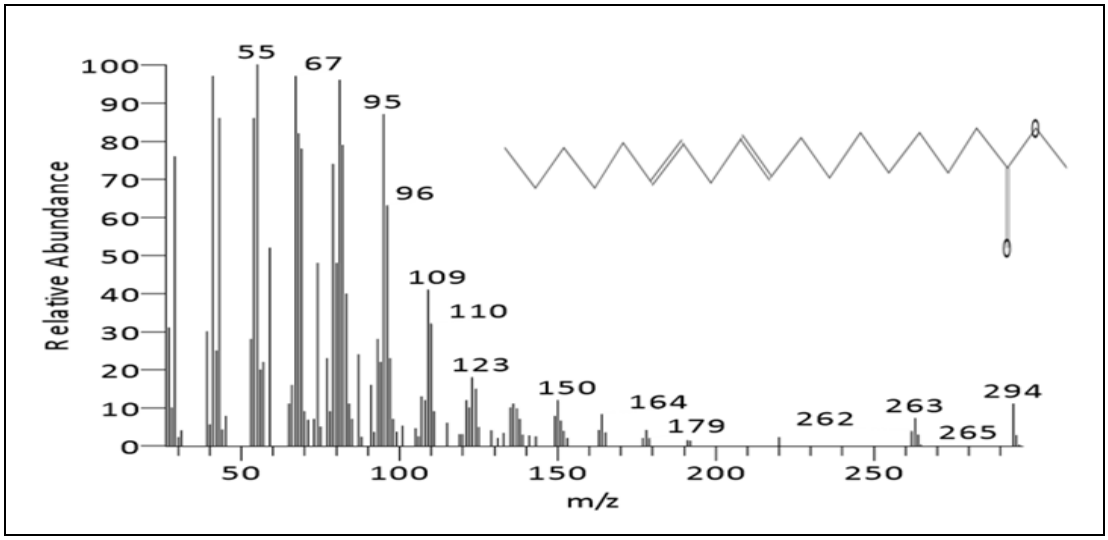


Fig 7: 9,12-octadecadienoic(Z,Z), methyl ester Methyl linoleate

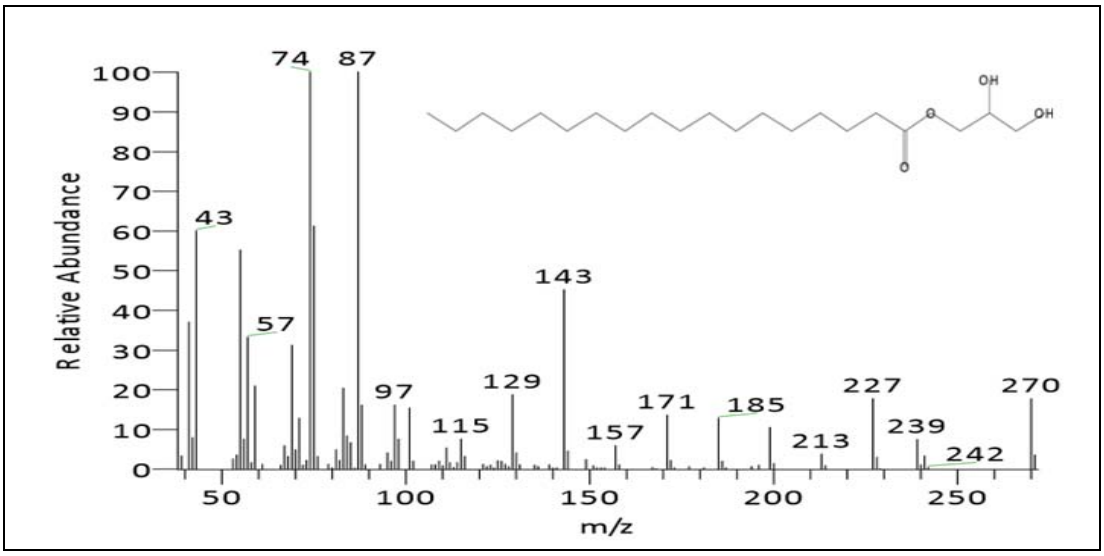


Fig 8: Octadecanoic acid, 2, 3-dihydroxypropyl ester Monostearin Formula

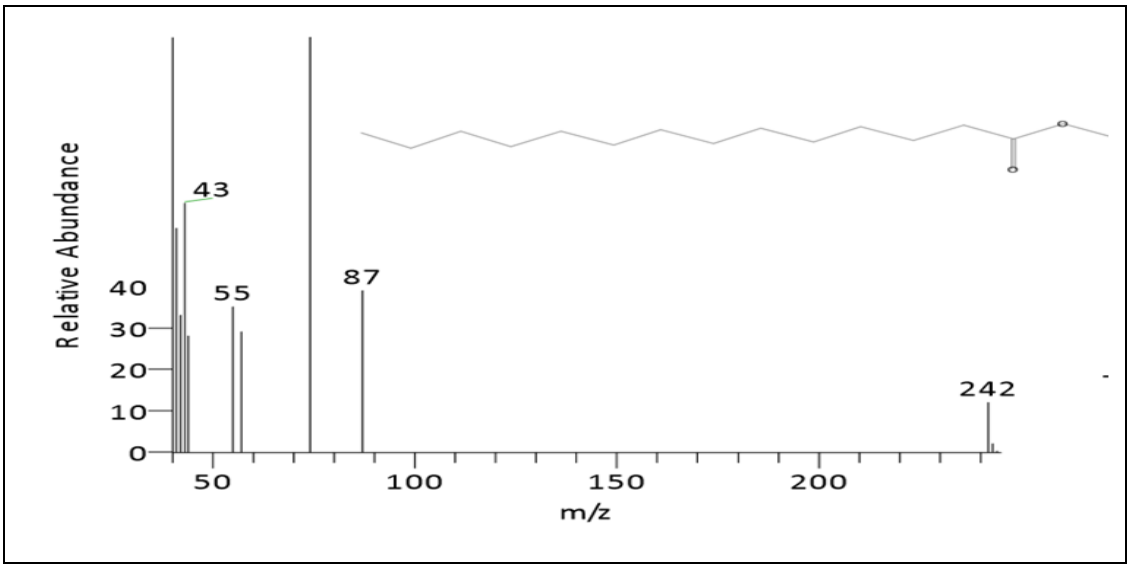


Fig 9: Tetradecanoic acid, methyl ester

Table 1: Phytocomponents identified in unsaponifiable matter of the aerial parts of *Salsola tetrandra* by GC-MS analysis.

No.	RT	Name of the compound	Molecular formula	MW	Peak Area%
1	12.74	Tridecanamine	$C_{13}H_{29}N$	199	1.41
2	16.45	2,7-Dimethyl-1-octanol	$C_{10}H_{22}O$	158	4.19
3	14	Isohexyl-2-pentylester (Sulfurous acid)	$C_{11}H_{24}O_3S$	236	0.85
4	14.45	3,9-Diethyl-6-tridecanol	$C_{17}H_{36}O$	256	6.64
5	33.32	Methyl palmitate	$C_{17}H_{34}O_2$	270	9.91
6	36.53	8-Hexadecynoic acid (Stearolic acid)	$C_{16}H_{29}O_2$	254	6.10
7	36.53	9,12-octadecadienoic(Z,Z), methyl ester (Methyl linoleate)	$C_{19}H_{34}O_2$	294	6.10
8	17.54	Octadecanoic acid, 2,3-dihydroxypropyl ester (Monostearin)	$C_{21}H_{42}O_4$	358	1.31
9	43.78	Tetradecanoic acid, methyl ester (Myristic acid, methyl ester)	$C_{15}H_{30}O_2$	242	0.94

Table 2: GLC of Fatty Acid Methyl Esters from the aerial parts of *Salsola*

Identified compound	No. of carbon atoms	Area %
Lauric acid	C12:0	0.34
Myristic acid	C14:0	0.99
Palmitic acid	C16:0	23.91
Palmitoleic acid	C16:1	1.64
Heptadecanoic (Margaric) acid	C17:0	0.37
Cis-10- Heptadecanoic acid	C17:1	0.52
Stearic acid	C18:0	2.62
Oleic acid	C18:1	5.55
Nonadecanoic acid	C19:0	1.82
Linoleic acid	C18:2	22.31
Eicosanoic (Arachidic) acid	C20:0	1.38
Linolenic acid	C18:3	26.28
11- Eicosenoic acid	C20:1	0.54
Docosanoic (Bhenic) acid	C22:0	3.80
Tricosanoic acid	C23:0	0.79
Tetracosanoic (Lignoceric) acid	C24:0	3.92
Hexacosanoic acid	C26:0	1.72
Octacosanoic acid	C28:0	1.50
*Method Reference=AOAC-996.01 *Limit of detection= 0.01		

Table 3: Effect of various extracts of *Salsola* against Aspirin induced gastric ulcer in rats.

Treatment	Ulcer Index	% of protection
Control	-	-
Aspirin (150 mg/kg)	6.6 ± 0.31	-
Salsola (50 mg/kg) and Aspirin (150 mg/kg)	3.99 ± 0.22	35.33*
Salsola (100 mg/kg) and Aspirin (150 mg/kg)	1.96 ± 0.32	71.26*
Standard drug (Ranitidine) and Aspirin (150 mg/kg)	1.34 ± 0.42	80.63*

Data expressed as mean ± S.E. (n = 6)

* P<0.05 was consider statistically significant when compared to aspirin group

Gross and Histological examinations

The results of gross examinations are presented in table 3. The ulcer areas decreased with plant extracts doses as compared to aspirin-induced ulcer model. It was found that the reduction of the ulcer indexes was dose dependant. Ranitidine was found to produce significant (P <0. 05) reduction in ulcer index (Table 3).

Histological examination of sections of stomach of control rats exhibit normal structure of stomach (Figure 10–A)

Microscopic examination of sections of the stomach of rats treated with an oral dose equivalent to 200 mg/kg of aspirin shows degeneration of the outer third of the mucosa while its lower part shows almost normal structure. In some case, the erosion developed in the superficial third of the mucosa (Figure 10–B).

Consumption of NSAID such as aspirin is associated with the development of gastric erosions or ulcers *via* several mechanisms including a release of salicylic acid which is not ionized by gastric acid (Wallace, 1997; Rainsford, 2001) [35]. Salicylic acid enters and accumulates in the gastric mucosal cells and undergoes ionization. It inhibits cell metabolic functions and permeates H+ ion back diffusion leading to gastrointestinal damage (McCarthy, 2001) [19].

In particular NSAID cause gastric erosionsand delay ulcer healing through various mechanisms including: a) significan tinhibition of biosynthesis of prostaglandins (Wang, et. al. 1989; Brzozowski, 2003) [38, 9] and suppression of bothcyclooxygenase (COX)-1 and COX-2 activity, b) reduction in cell regeneration and inhibition of ulcer contraction (Penney, 1994) [24] and c) decrease in mucosal blood flow in the ulcer margin (Hirose, et. al. 1991) [14]. The inhibition of the COX-1 enzyme impairs the production of protective prostaglandins (PG) and suppresses platelet production of thromboxane, which increases bleeding when an active GI bleeding site is present (Silverstein, et. al. 1995) [18].

Sections of the stomach of rats treated with an oral dose of *Salsola* extract (50 mg/kg) and given a dose equivalent to 200 mg/kg of aspirin and showed few degenerative areas in the outer surface of mucosa while its lower part showed almost normal structure (Figure 11–A). On the other hand, sections of the stomach of rats treated with an oral of *Salsola* extract (100 mg/kg) and given a dose equivalent to 200 mg/kg of aspirin showed little degenerative epithelial cells and the remaining appear more or less like control (Figure 11–B).

Ranitidine, standard drug, which was used at a dose equivalent to 150 mg/kg b.w. also showed a tendency to protect against aspirin-induced gastric damage (Figure 12).

Histochemical study

I-Polysaccharides

Histochemical examination of sections of stomach of control rats showed polysaccharide materials in the gastric mucosa that are mainly localized in the epithelium lining the stomach mucosa. Deep stain is detected in the apical regions of these cells the other cells of the gastric mucosa acquire pale stainability (Figure 13–A).

In rats received an oral dose of aspirin equivalent to 200 mg/kg b.w., the stomach showed the polysaccharides in the surface epithelial and mucous neck cells that acquire a pale stainability (Figure 13–B).

In rats received an oral dose *Salsola* extract (50, 100 mg/kg b.w.) or ranitidine and aspirin equivalent to 200 mg/kg b.w., heterogeneous staining was encountered where the degenerated surface epithelial cells and mucous neck cells were almost devoid of stainable material while the outer half of the mucosa was densely stained (Figure 14–A). Sections of stomach of rats received an oral dose of aspirin (200 mg/kg b.w.) after administration of a dose of *Salsola* extract (100 mg/kg b.w) showed no apparent change in the polysaccharides of the surface epithelium and the mucous neck cells of the gastric mucosa of the treated animals except a few degenerative epithelial cells (Figure 14–B). Examination of sections of stomach of rats given an oral dose of aspirin (200 mg/kg b.w.) after administration of a dose of ranitidine (150 mg/kg b.w) exhibited no apparent change in the polysaccharides of the surface epithelium and the mucous neck cells of the gastric mucosa of the treated animals. The degenerative parts of the mucosa display weak PAS reactivity (Figure 15).

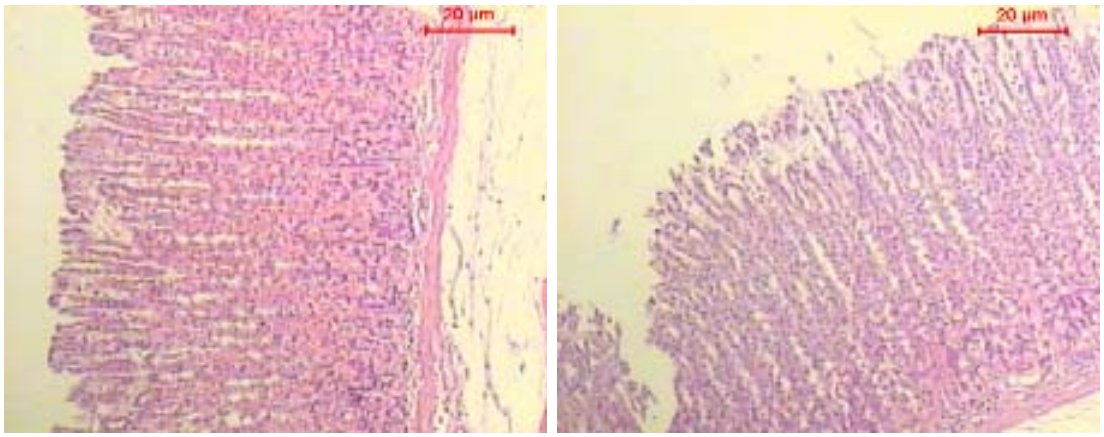
II-Total proteins

Histochemical examination of sections of the control rat stomach showed the protein contents in the various types of the cells of the gastric mucosa generally as intensely dark blue inclusions distributed in both the cytoplasm and nuclei. The surface epithelium acquired strong activity (Figure 16–A).

Administration of an oral dose of aspirin equivalent to 2000 mg/kg b.w. showed that the proteinic inclusions in the inner region of mucosa that includes surface epithelial cells and mucous neck cells displayed diffuse weak stainability while some cells in the outer region of the mucosa showed moderate stainability of its proteinic contents (Figure 16–B). The cells of the gastric epithelium of rats after administration with a single oral dose of *Salsola* extract (100 mg/kg b.w) and aspirin (200 mg/kg b.w.) no apparent changes in the protein material in the surface epithelium. The degenerative areas displayed weak stainability of the proteinic inclusions (Figure 17–A). Sections of stomach of rats that received an oral dose of aspirin (200 mg/kg b.w.) after administration of a dose of *Salsola* extract (100 mg/kg b.w) showed no apparent changes in the protein material of the surface epithelium and the mucous neck cells of the gastric mucosa of the treated animals. A few degenerative epithelial cells exhibited a pale stainability (Figure 17–B).

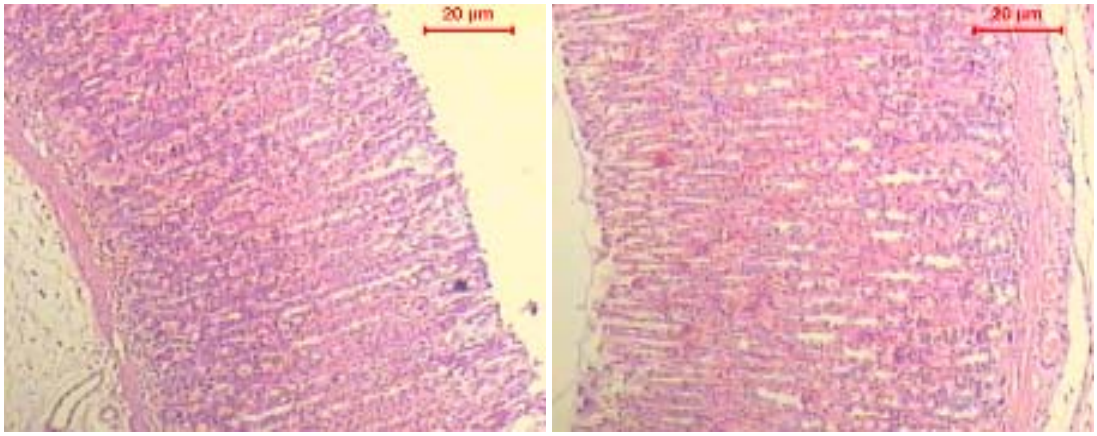
Examination of stomach of rats received an oral dose of aspirin (200 mg/kg b.w.) after administration of a dose of ranitidine (150 mg/kg b.w) showed that the proteinic inclusions in the gastric mucosa cells displayed no change in the surface epithelium and the mucous neck cells of the gastric mucosa of the treated animals. The degenerative parts of the mucosa displayed weak stainability (Figure 18).

Mucus secretion is a crucial factor in theprotection of gastric mucosa from the gastric lesionsand has been regarded as an important defensivefactor in the gastric mucus barrier. A decrease in **t**he synthesis of sulphated mucus glycoprotein hasbeen implicated in the aetiology of gastric ulcer (Younan, *et al.* 1982) [42]. The increase in total carbohydrate ⁄ protein ratio is the direct reflection of mucin activity, whichis indicated by the enhanced level of individualmucopolysaccharides like hexose, hexosamine, fucose and sialic acid (Goel, et. al. 1994) [12]. Decrease in protein contentin the gastric juice also signifies decrease in leakagefrom the mucosal cells indicating mucosalresistance. The wide distribution of adherent mucuscontent in the gastrointestinal tract plays a pivotalrole in cytoprotection and repair of the gastricmucosa (Tanaka, et. al. 1989) [32]. The results showed increased levels ofadherent mucus content of gastric tissue pretreatedwith *Salsola* indicating its cytoprotective action onexperimentally induced gastric ulcer.



A **B**

Fig 10: Sections of stomach of A) control rat shows the normal structure of mucosa, B) rat treated with an oral dose equivalent to 150 mg/kg of aspirin shows degeneration of the outer third of the mucosa (arrow) while its lower part shows almost normal structure (arrow head) at the right. Notice the erosion developed in the superficial third of the mucosa at the left (H & E, Scale Bar = 20 µm).



A **B**

Fig 11: Sections of the stomach of A) rat treated with an oral dose equivalent to 150 mg/kg of aspirin and 50 mg/kg of *Salsola* extract shows few degenerative area in the outer surface of mucosa (arrow) while its lower part shows almost normal structure (arrow head), B**)**: rat treated with an oral dose equivalent to 200 mg/kg of aspirin and 100 mg/kg of *Salsola* extract shows lettel degenerative epithelial cells. The remaining appear more or less like control (H & E, Scale Bar = 20µm).

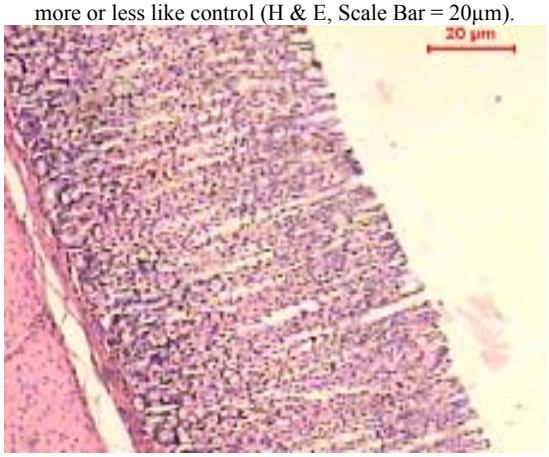
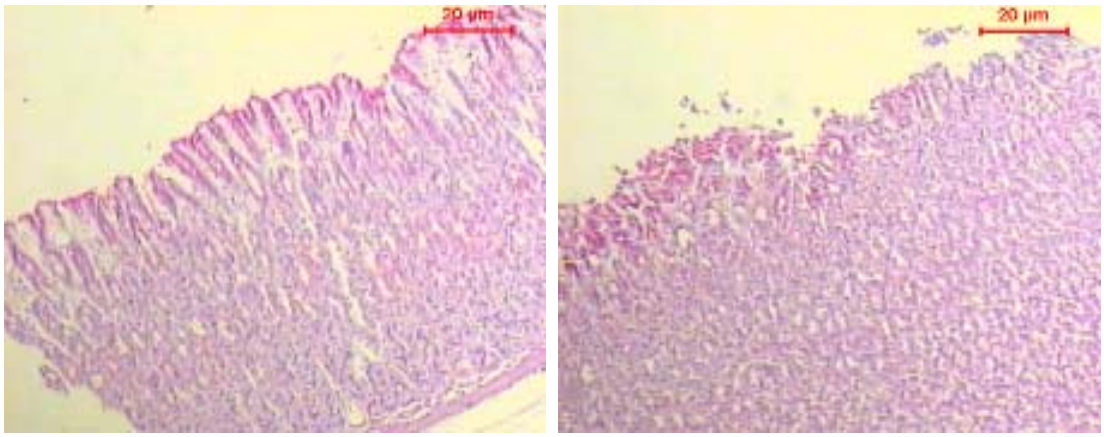


Fig 12: A section of the stomach of rat treated with an oral dose equivalent to 200 mg/kg of aspirin after administration of ranitidine (150 mg/kg) of shows little degenerative epithelial cells. The remaining appear more or less like control (H & E, Scale Bar = 20 µm).



A **B**

Fig 13: Sections of stomach of A): control rat shows the PAS positive materials in the gastric mucosa that displayed a dark pink stainability in the surface epithelial cells and in the mucous neck cells. Notice that the surface epithelial cells give more intense stainability than the mucous neck cells. The other cells of the mucosa acquire pale stainability, B**)**: rat received an oral dose of aspirin equivalent to 150 mg/kg b.w. shows the polysaccharides in the surface epithelial and mucous neck cells acquire a pale stainability (PAS, scale bar: 20 µm)

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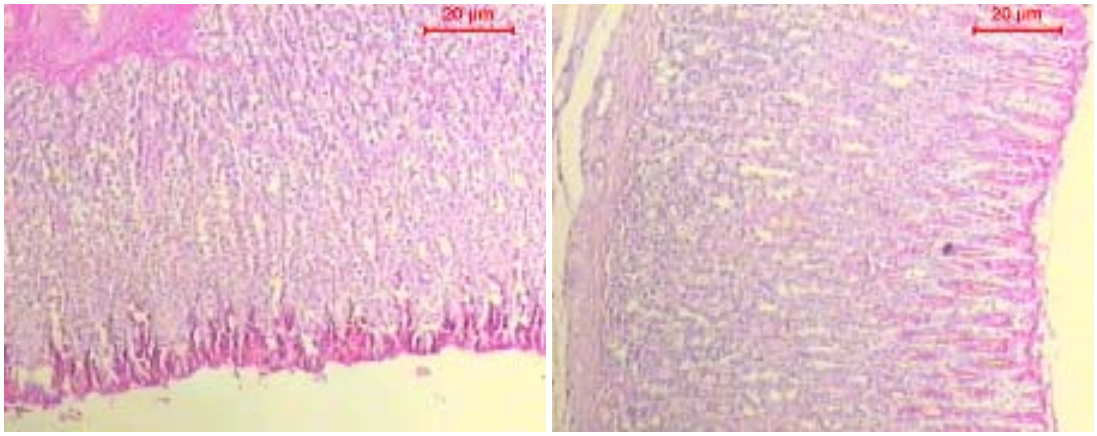


A **B**

Fig 14: Sections of stomach of A): rat received an oral dose of aspirin (150 mg/kg b.w.) after administration of a dose of *Salsola* extract (50 mg/kg b.w) shows heterogeneous staining that encountered where the degenerated surface epithelial cells and mucous neck cells were almost devoid of stainable material while the outer half of the mucosa was densely stained, B**):** rat received an oral dose of aspirin (150 mg/kg b.w.) after administration of a dose of *Salsola* extract (100 mg/kg b.w) shows no apparent change in the polysaccharides of the surface epithelium and the mucous neck cells of the gastric mucosa of the treated animals except a few degenerative epithelial cells (PAS, scale bar: 20 µm).

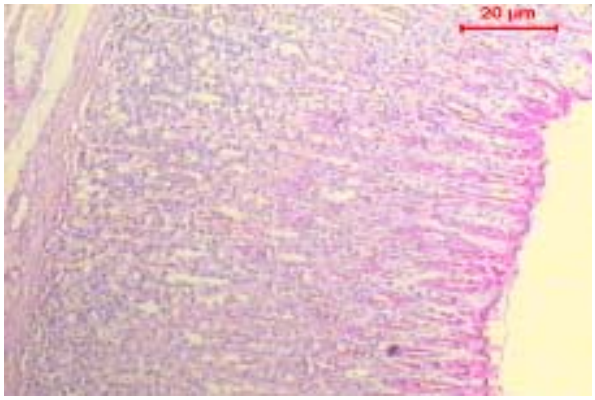
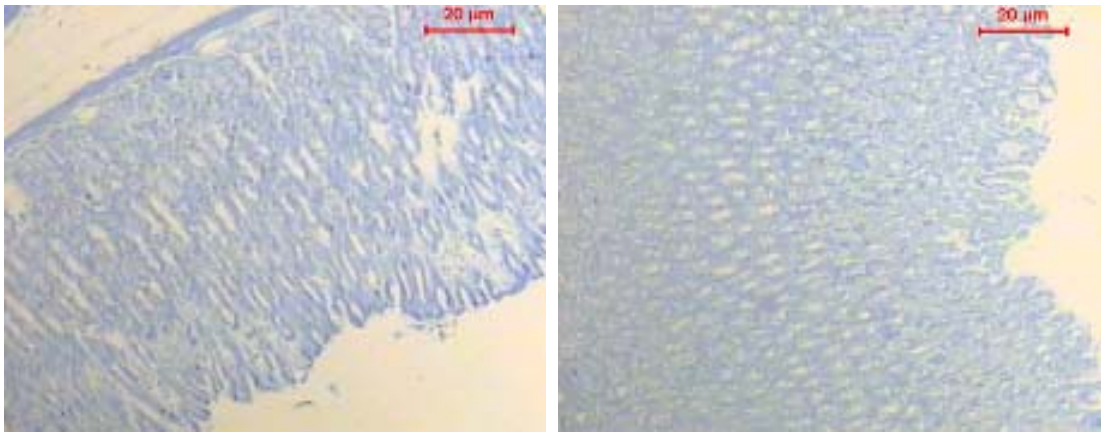
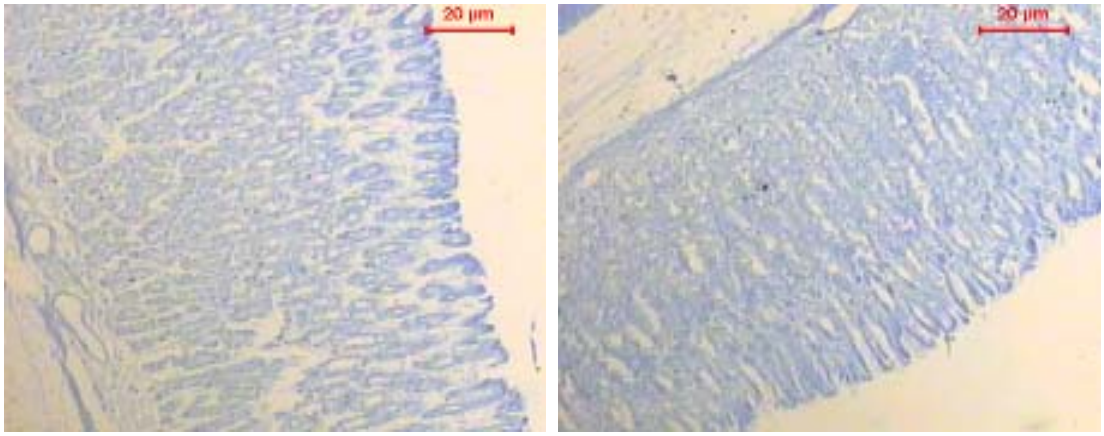


Fig 15: A section of stomach of rat daily received oral dose of aspirin (150 mg/kg b.w.) after administration of a dose of ranitidine (150 mg/kg b.w) no apparent change in the polysaccharides of the surface epithelium and the mucous neck cells of the gastric mucosa of the treated animals. The degenerative parts of the mucosa display weak PAS reactivity (PAS, scale bar: 20 µm).



A **B**

Fig 16: sections of stomach of A) control rat shows the total proteins in the gastric mucosa as intensely dark blue colored inclusions in both the cytoplasm and the nuclei. Notice that the surface epithelium displays a strong reaction of proteins, B**)**: rat received an oral dose of aspirin showsthe proteinic inclusions in the inner region of mucosa displays diffuse weak stainability while some cells in the outer region of the mucosa shows moderate stainability of its proteinic contents **(**Bromophenol blue, scale bar: 20 µm).



A **B**

Fig 17: Sections of stomach of **A):** rat received an oral dose of aspirin (150 mg/kg b.w.) after administration of a dose of *Salsola* extract (50 mg/kg b.w) shows no apparent change in the protein material in the surface epithelium. The degenerative areas displayed weak stainability of the proteinic inclusions, B**):** rat received an oral dose of aspirin (200 mg/kg b.w.) after administration of a dose of *Salsola* extract (100 mg/kg b.w) shows no apparent change in the protein material of the surface epithelium and the mucous neck cells of the gastric mucosa of the treated animals.A few degenerative epithelial cells that exhibit a pale stainability (Bromophenol blue, scale bar: 20 µm)

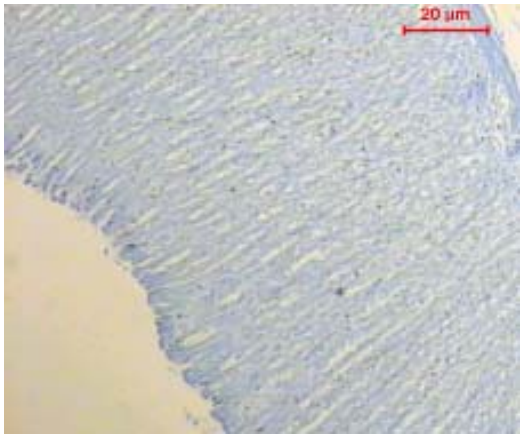


Fig 18: A section of stomach of rat received an oral dose of aspirin (150 mg/kg b.w.) after administration of a dose of ranitidine (150 mg/kg b.w) shows the proteinic inclusions in the gastric mucosa cells. Such inclusions display no change in the surface epithelium and the mucous neck cells of the gastric mucosa of the treated animals. The degenerative parts of the mucosa display weak stainability **(**Bromophenol blue, scale bar: 20 µm**)**.

4. Conclusion

Salsola tetrandra rich in chemical constituents which could be used as a treatment agent. The plant extract also showed a promising biological activity which could be attributed to the presence of the compounds, triterpenes and/or sterols, saponins, alkaloids and coumarins (phenolic compounds) and glycosides as soon as unsaturated long chain fatty acids. 70% alcoholic extract from the aerial parts of the plant exhibited antiulcerogenic effect that induced by aspirin as manifested by the morphometic, histopathological and histochemical examinations.

5. Acknowledgements

This research project was supported by a grant from the deanship of scientific research at Salman bin Abdulaziz University under the research project # 124/T/33.

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