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Kaff-E-Maryam (*Anastatica hierochuntica* L.): Evaluation of Gastro-Protective Activity and Toxicity in Different Experimental Models

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Abstract

Context: *Anastatica hierochuntica* L., (Brassicaceae) is distributed throughout Arabian Peninsula, and elsewhere. It is locally called "Kaff-e-Maryam". All parts of the plant are used in folk medicine.

Objective: "Kaff-e-Maryam" is used to treat stomach cancer and stomach problems, infections, and to ease childbirth. There are no reports on its role in protection of gastric mucosa against toxic damage and nothing is known about its toxic potential. Ethanol treated rats were investigated in detail. The gastroprotective activity of "Kaff-e-Maryam" extract was evaluated in rats while toxicity studies were done in Brine shrimp and mice.

Materials and methods: Ethanol extract of the whole plant was prepared and animals were treated with the standard necrotizing agents. Different doses of the extract were used for pharmacological and toxicity evaluation.

Results: The group of rats treated (gavage) with necrotizing agents including 80% ethanol, caused damage to the stomach wall. The depletion of stomach-wall mucus, concentration of proteins, nucleic acids, and NP-SH groups occurred. The extract treatment caused protection against the changes induced by ethanol. Histopathological studies supported the findings. In brine shrimp toxicity test as well during acute and chronic toxicity studies in mice, *A. hierochuntica* treatment showed low toxicity.

Discussion and conclusion: Pretreatment with *A. hierochuntica* extract offered protection against toxic damage to stomach wall; thus supporting the folklore claim. The extract was found to exert its defensive role through its free radical scavenging and prostaglandin inducing activities. Based on the results of current study, the use of *A. hierochuntica* was found to be safe in the given doses. The toxicity studies revealed *A. hierochuntica* extract in the given dose range, was not toxic.

Keywords: *Anastatica hierochuntica* L; Brassicaceae; Gastro-protective activity in rats; Biochemical effects; Histopathological changes; Toxicity; Brine shrimp; Mice

Introduction

There is a global increase in the use of medicinal plants for health reasons. In developing countries herbal drugs and traditional remedies are relatively more popular because of cultural acceptability and belief that being natural, they are safe and non-toxic. However, toxicity of such drugs is yet not well understood [1-3]. *A. hierochuntica* L. (Family: Brassicaceae) locally called 'Kaff-e-Maryam', is a well known desert zone medicinal plant. Novel melanogenisis inhibitor flavonoids with antioxidant potential were isolated from it [4,5]. Kaff-e-Mayam is a monocarpic annual plant species characterized by topochory/ombrohydrochory type of seed dispersal [6-8] (Figure 1).

Figure 1: Kaff-e-Maryam in Saudi Arabia. (Courtesy of: http://barakabirth.com/blog/).

All parts of *A. hierochuntica* are famous and used in folk medicine to provide cure against various disease states [9,10]. Based on its frequent medicinal use and popularity among the people of central Asia, Africa, Arabian Peninsula, and else-where, Kaff-e-Maryam was selected for present study. The plant is available on sale throughout Saudi Arabia in mini vegetable markets, road side, and on the shops of medicinal plant sellers [10] (Figure 2).

A decoction of Kaff-e-Maryam (*A. hierchuntica*) has been in use against stomach upset, as antispasmodic, against fatigue, uterine haemorrage, menstrual cramps, depression. It is believed to be a cure for arthritis inflammations, infections, diabetes, asthma, viral and autoimmune diseases. It is also used for pain relief after surgery [8,11-14]. Its freshly prepared decoction is commonly used as local disinfectant, to ease childbirth, as liver tonic, to stop vomiting, to treat mouth ulcers, and provide cure against stomach cancer [15,16]. All parts of the plant were reported to possess antimicrobial activity [17]. Several new type and interesting compounds were isolated from *A.*

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Figure 2: Kaff-e-Maryam with courtesy of Free-Photos <www.all-free-download.com>.

hierochuntica which were found to be biologically active [4,14]. Some reports suggested the plant to be cardio tonic; however, nothing is sure about its curing effects on stomach injury, and its toxic potential [15,18].

In the present study, gastro-protective activity of *A. hierochuntica* was evaluated in rats while preliminary toxicity evaluation was done by conducting Brine shrimp toxicity test [19]. In addition, acute and chronic toxicity studies were performed in mice [20,21], and the results are presented in current communication.

Material and Methods

Anastetica hierochuntica L. under present study was collected from Madina Al-Munawara, Uuhd area (Saudi Arabia) where it is locally called "Kaff-e-Maryam". Other popular names of the plant are: Rose of Jerico, Sanggul Fatimah, St. Mary's flower, and Palestinian tumble-weed [22]. The plant under investigation was identified and voucher specimens were kept on record at the Herbarium of the Research Centre for Medicinal, Aromatic, and Poisonous Plants, King Saud University, Saudi Arabia.

Plant extract and dose

The powdered plant material was subjected to preliminary chemical analysis, and extracted with ethanol. Later the solvent was removed under reduced pressure by using rotary evaporator. The ethanol free brownish crude extract thus obtained was kept in a refrigerator and used in the present study [10]. The experiment was conducted in Wistar albino rats of same age, weighing 150 to 200 g following official protocol for animal experiments approved by the Experimental Animal Research Committee as described in our earlier reports [21]. All animals were maintained under controlled conditions of temperature, light (12 h dark, 12 h light), and humidity. The animals were fed with Purina chow diet and had free access to water.

The animals were randomly assigned to the control and treatment groups which were fasted for 36 h with water ad libitum. The animals were treated with the standard necrotizing agents who earlier induced gastric lesions [23-26]. The dose selected was based on the routine dose level of plant extract under investigation which stretched out between 125 to 500 mg/kg. In the present study, Kaff-e-Maryam extract suspended in water was administered (gavage) to the fasted rats in the treatment groups while the control was given equal amount of vehicle [18,21].

Cytoprotection studies

In cytoprotection experiments, the drug treatment was given 30 minutes before treatment with 80% ethanol, 0.6 M HCl, 25% NaCl, 0.2 M NaOH or indomethcin. The animals were sacrificed 1 h after treatment with the necrotizing agents under anesthesia using ether. The stomach of each animal was excised and opened along the greater curvature. In each case stomach was washed with normal saline and by using a binocular magnifier the gastric lesions were quantified. The injuries were scored according to the method defined earlier [27].

Gastric wall mucus determination

The glandular segments from the stomachs were removed and weighed. Each segment was right away transferred to 1% Alcian blue solution (in sucrose solution, buffered with sodium acetate, pH 5). The excess dye was removed by rinsing with sucrose solution. The dye complexed with the gastric wall mucus was extracted with magnesium chloride. A 4 ml quantity of the blue extract was then shaken with equal volume of diethyl ether. The resulting emulsion was centrifuged and the absorbance of aqueous layer was measured at 580 nm. The quantity of Alcian blue extracted per gram of net glandular tissue was calculated following standard procedure [28].

Estimation of nonprotein sulfhydryl groups (NP-SH)

Gastric mucosal NP-SH was measured according to the method described earlier [29]. The glandular stomach was removed and homogenized in ice cold 0.02 M ethylenediamine tetraacetic acid (EDTA). The homogenate were mixed with distilled water and 50% trichloroacetic acid (TCA) and centrifuged. The supernatants were mixed with Tris buffer, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added and the sample shaken. The absorbance was read within 5 min. of addition of DTNB at 412 nm against a reagent blank with no homogenate [24].

Estimation of proteins and nucleic acid contents

The proteins and nucleic acids levels in stomach were determined by the following procedure: The stomach from the animals were quickly excised, frozen in liquid nitrogen, and stored at -20°C till analyzed for total proteins and nucleic acids (RNA, DNA). Protein estimation was done by following the standard procedure [30] while method described by Bregman [31] was used to determine the levels of nucleic acids.

Tissues were homogenized and suspended in ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted with ethanol. DNA was determined by treating the nucleic acid extract with diphenylamine reagent and the intensity of blue colour was measured at 600 nm. RNA was quantized by treating the nucleic acid extract with orcinol and the green colour was read at 660 nm. Standard curves were used in order to estimate the nucleic acids [24].

Indomethacin-induced gastric ulcers

Indomethacin was suspended in 1% carboxymethylcellulose in water (6 mg/ml) and administered to the fasting rats in a dose of 30 mg/kg (0.5 ml/100 g). The rats in control group were similarly fasted and treated with an equal amount of vehicle [32]. The stomachs of the animals were removed, rinsed with normal saline, and studied according to the standard method [33].

Toxicity studies

Brine shrimp toxicity test: For the preliminary assessment of toxicity, standard Brine shrimp lethality assay was followed. The

ethanol extract solutions with concentration 100 ppm and 1000 ppm were tested as described earlier [19,34,35].

Acute toxicity and chronic toxicity studies in mice: The toxicity experiments were conducted according to the recommendations of World Health Organization for herbal drugs [20]. Swiss albino mice, home bred, aged six to seven weeks, weighing about 22-27 g, fed on Purina Chow diet, and free access to water were used in both acute and chronic toxicity studies. Throughout the study, all animals were kept under controlled temperature, relative humidity, and automated light cycle [18,21].

In acute toxicity experiment, a total of 20 mice were randomly assigned to a control and three treatment groups (5 mice in each group). The ethanol extract was suspended in water and orally given (gavage) to the three groups as follows: 0.5 g/kg, 1 g/kg and 3 g/kg body weight, while the control group received only water. All the treated animals were observed for 24 h for any sign of toxicity [21].

For chronic toxicity studies 10 male and 10 female mice were separately assigned to the control group in different cages. Similarly, 10 male and 10 female mice were separately allotted to the treatment group. The male and female animals in the treatment group received 100 mg/kg/day dose of the extract suspended in drinking water which is 20% of the pharmacologically active dose [1,21,36]. The treatment continued for three months. Throughout the study, all animals were observed for external symptoms of toxicity, mortality, and body weight changes. At the end of treatment visceral condition and vital organ weight were compared with the respective control group mice. The results were substantiated by histopathological observations.

Coulter counter was used to analyze WBC, RBC, hemoglobin, haematocrit, platelets and MCV. The chemical analysis for: alanine aminotransferase (ALT/GPT), aspartate aminotransferase (AST/GOT), CK-MB, urea, creatinine and glucose was done by enzymatic colorimetric method by using test combination reagents and spectrophotometer [21,37]. Both, the control as well as chronically treated male animals were analyzed for spermatogenic dysfunction by using sperm abnormality test [1,38].

Histopathological studies

The tissues from heart, lungs, liver, kidney and testis were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Sections of about 5 µm thicknesses were cut by using American Optical Rotary Microtome. The samples were stained with haematoxylin and eosin. The preparations were analyzed by independent observer under microscope and compared with the control. The tabular presentation of the histopathology results was adopted following our earlier published procedure using the following approved key [18,24]: Key: Normal (-), Little effect (+), Appreciable effect (++), Severe effect (+++), Intensively severe effect (++++).

Statistical analysis

Chi-square test, Student-Newman-Keuls Multiple Comparisons test, and Student's t-test were used for analysis and comparison of the treatment groups with the respective control group animals. The values $P<0.05$ were taken as statistically significant.

Results

Gastric lesions induced by necrotizing agents

The results of the present study regarding gastro-protective activity are depicted in Tables 1-7.

Pretreatment with *A. hierochuntica* ethanol extract caused a statistically significant protection against the damage caused by 80% ethanol, 0.06 M HCl, 0.2 M NaOH, and 25% NaCl, in a dose dependent mode (Table 1).

Gastric wall mucus determination

Ethanol treatment decreased the gastric-wall mucus in all treated rats (Table 2). Pretreatment with *A. hierochuntica* inhibited the ethanol induced depletion of stomach-wall mucus in a dose dependent manner.

Effect on protein and nucleic acid concentration

The treatment with ethanol significantly reduced protein and nucleic acid concentration of stomachs of treated animals as compared

No.	Treatment & Dose (mg/kg body weight)	Lesion score of rats treated with different necrotic agents 1 ml/rat. (Mean ± S.E.)			
		80% EtOH	0.2M NaOH	0.6M HCl	25% NaCl
1	Control (Distilled water)	14.53 ± 0.66	13.30 ± 1.64	12.65 ± 0.45	14.94 ± 0.97
2	*A. hierochuntica* (125)	12.95 ± 0.51	12.85 ± 0.77	13.20 ± 0.34	12.82 ± 0.83
3	*A. hierochuntica* (250)	5.75 ± 0.47*	7.64 ± 0.57**	7.52 ± 0.38***	6.85 ± 0.43*
4	*A. hierochuntica* (500)	2.24 ± 0.51***	2.15 ± 0.44***	2.60 ± 0.40***	2.10 ± 0.67***

*P<0.05 ; **P<0.01; ***P<0.001. (Student-Newman-Keuls Multiple Comparisons Test).
- Six animals were used in each group.
- Significant relative to respective control.
- Groups 2, 3 and 4 were statistically compared with group 1.

Table 1: Effect of *A. hierochuntica* ethanol extract treatment on the induction of gastric ulcers by various necrotic agents in rats.

No.	Treatment and dose (mg/kg body weight)	N (Rats)	Gastric wall mucus (Alcian blue µg/g wet glandular tissue)
1	Control (Distilled water, 1 ml/rat)	5	419.35 ± 14.41
2	80% Ethanol (1 ml/rat)	5	281.68 ± 12.88***
3	*A. hierochuntica* (125)+80% ethanol (1 ml/rat)	5	298.49 ± 15.36
4	*A. hierochuntica* (250)+80% ethanol (1 ml/rat)	5	326.30 ± 9.69*
5	*A. hierochuntica* (500)+80% ethanol (1 ml/rat)	5	378.56 ± 10.57**

*P<0.05 , **P<0.01 , ***P<0.001. (Student-Newman-Keuls Multiple Comparisons Test).
- Group 2 was statistically compared with group 1.
- Group 3, 4 and 5 were statistically compared with group 2.

Table 2: Effect of *A. hierochuntica* extract treatment on the induction of changes in gastric wall mucus by 80% ethanol in rats. (Mean ± S.E.).

to the control (Table 3) *A. hierochuntica* pretreatment was found to prevent ethanol caused damage in a dose dependent way.

Effect on gastric mucosal NP-SH concentration

The administration of ethanol significantly decreased NP-SH concentration in the gastric mucosa (Table 4). Pretreatment with *A. hierochuntica* significantly prevented the NP-SH depletion caused by ethanol in a dose dependent order.

Effect on malondialdehyde concentration in gastric lesions

The administration of ethanol significantly increased the concentration of malondialdehyde in gastric ulcers (Table 5). Pretreatment with the extract was found to deplete malondialdehyde concentration in a dose dependent manner.

Effect of indomethacin-induced gastric mucosal damage

Pretreatment with *A. hierochuntica* was found to protect the gastric mucosal damage induced by indomethcin (Table 6).

Histopathological examination of gastric lesions

The treatment with ethanol caused considerable damage in the form of necrosis, erosion, inflammation, hemorrhagic mucosal lesion, and congestion in the stomach walls of treated animals. However, no neutrophillic infiltration was evident (Table 7). The area involved was mainly the glandular segments. There was interluminal bleeding in these animals.

Pretreatment with *A. hierochuntica* provided a statistically significant and dose-dependent protection against the action of ethanol.

Effect on brine shrimp lethality

The ethanol extract of *A. hierochuntica* caused mortality in Brine shrimps as 36.66% and 96.66% at the extract concentrations of 100 ppm and 1000 ppm, respectively. The lethal concentration (LC50) was calculated to be 100 ppm.

No.	Treatment and Dose (mg/kg body weight)	Protein (mg/100 mg)	RNA (µg/100 mg)	DNA (µg/100 mg)
1	Control (Distilled water)	15.86 ± 0.22	407.25 ± 9.81	365.88 ± 5.50
2	80% Ethanol (1 ml/rat)	12.63 ± 0.21***	185.67 ± 5.56***	142.82 ± 9.15***
3	*A. hierochuntica* (125)+80% ethanol (1 ml/rat)	14.46 ± 0.50	281.74 ± 7.83*	250.35 ± 16.62
4	*A. hierochuntica* (250)+80% ethanol (1 ml/rat)	14.97 ± 0.19**	393.47 ± 14.69**	283.86 ± 14.51**
5	*A. hierochuntica* (500)+80% ethanol (1 ml/rat)	15.88 ± 0.17***	379.43 ± 12.57***	342.33 ± 7.99***

*P<0.05, **P<0.01, ***P<0.001. (Student Newman Keuls Multiple Comparisons Test).
- Group 2 was statistically compared with group 1.
- Group 3, 4 and 5 were statistically compared with group 2.

Table 3: Effect of *A. hierochuntica* extract treatment on the protein and nucleic acids levels in the stomach walls of rats treated (gavage) with 80% ethanol.

No.	Treatment and dose (mg/kg body weight)	N (rats)	Malondialdehyde concentration (nmol/g wet tissue)	NP-SH concentration (µmol/g tissue)
1	Control (Distilled water)	5	50.54 ± 1.55	1.878 ± 0.043
2	80% Ethanol (1 ml/rat)	5	74.42 ± 1.17**	0.869 ± 0.038
3	*A. hierochuntica* (125)+80% Ethanol (1 ml/rat)	5	69.65 ± 2.44*	0.856 ± 0.055
4	*A. hierochuntica* (250)+80% Ethanol (1 ml/rat)	5	65.48 ± 2.49*	0.985 ± 0.040
5	*A. hierochuntica* (500)+80% Ethanol (1 ml/rat)	5	60.73 ± 1.48**	1.266 ± 0.048

*P<0.05 , **P<0.01 , ***P<0.001. (Student-Newman-Keuls Multiple Comparisons Test).
- Group 2 was statistically compared with group 1.
Group 3, 4 and 5 were statistically compared with group 2.

Table 4: Effect of *A. hierochuntica* extract treatment on malondialdehyde and NP-SH concentrations in gastric ulcers induced by 80% ethanol in rats. (Mean ± S.E.).

No.	Treatment and dose (mg/kg body weight)	N (rats)	Lesions score (mean ± S.E.)	Percent Inhibition %
1	Indomethacin (30)+Distilled water	5	41.27 ± 4.85	-
2	Indomethacin (30)+*A. hierochuntica* (125)	5	33.16 ± 4.61*	20
3	Indomethacin (30)+*A. hierochuntica* (250)	5	17.48 ± 5.50**	58
4	Indomethacin (30)+*A. hierochuntica* (500)	5	4.15 ± 1.58***	90

*P<0.05 , **P<0.01 , ***P<0.001. (Student-Newman-Keuls Multiple Comparisons Test).
- Group 2, 3, and 4 were statistically compared with group 1.

Table 5: Effect of *A. hierochuntica* extract treatment on indomethacin-induced gastric mucosal damage in rats.

No.	Treatment and dose (mg/kg body weight)	Lesion score (mean ± S.E.)	Percent inhibition
1	80% Ethanol (1 ml/rat)	20.14 ± 2.66	-
2	Indomethacin (2.5)+80% Ethanol (1 ml/rat)	23.27 ± 2.15	-
3	*A. hierochuntica* (250)+80% Ethanol (1 ml/rat)	8.30 ± 1.20**	62
4	*A. hierochuntica* (500)+80% Ethanol (1 ml/rat)	1.75 ± 1.07***	91
5	*A. hierochuntica* (250)+80% Ethanol (1 ml/rat)+Indomethacin (2.5)	16.80 ± 1.25*	25
6	*A. hierochuntica* (500)+80% Ethanol (1 ml/rat)+Indomethacin (2.5)	13.15 ± 1.50**	42

*P<0.05 , **P<0.01 , ***P<0.001. (Student Newman Keuls Multiple Comparisons Test).
- Group 3 and 4 were statistically compared with group 1.
- Group 5 and 6 were statistically compared with group 2.
- Five animals were used in each group.

Table 6: Effect of *A. hierochuntica* extract on gastric lesions induced by combined ethanol and indomethacin treatment in rats.

Acute toxicity studies

At 3 g/kg treatment the animals showed decreased locomotor activity. Among other behavioral changes writhing and pilo erection were observed in the treatment group as compared to the control. No animal died during the test.

Chronic toxicity studies

The results of chronic toxicity studies are presented in Tables 8-13. Throughout the chronic treatment, all mice in the control and treatment groups remained healthy and active. None of the animal died in *A. hierochuntioca* treated mice. In the control group one mouse with snout alopecia died during the first week of treatment. There were no signs of toxicity in the treatment groups throughout chronic toxicity test as compared to control. The weight gain was normal and comparable to the control group mice. At the end of treatment, visceral condition and vital organs were found normal and comparable to the respective control groups. The results were substantiated by histopathological, hematological and biochemical studies indicating no significant deviations and all parameters were found to be normal and comparable to the control groups. After chronic treatment, *A. hierochuntica* extract treated male showed no increase in sperm abnormalities and the data obtained was normal and comparable to the control male mice.

Discussion

The results of the present investigation demonstrated that 'Kaff-e-

No.	**Treatment and dose** (mg/kg body weight)	Edema	Congestion	Hemorrhage	Necrosis
1	Control (distilled water, 1 ml/rat)	-	-	-	-
2	80% Ethanol (1 ml/rat)	++	+++	++	++++
3	*A. hierochuntica* (125)+80% Ethanol (1 ml/rat)	++	+++	+	+++
4	*A. hierochuntica* (250)+80% Ethanol (1 ml/rat)	++	+++	+	++
5	*A. hierochuntica* (500)+80% Ethanol (1 ml/rat)	+	+	-	-

Key: Normal (-), Little effect (+), Appreciable effect (++), Severe effect (+++), Intensively severe effect (++++).

Table 7: The effect of *A. hierochuntica* on 80% ethanol treatment on the induction of histopathological lesions in the stomach walls of rats.

Treatment (3 months)	**Mortality**								**Lethality (%)**	
	0-30 days		31-60 days		61-90 days		Total			
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Control	0	1	0	0	0	0	0	0	0	5
A. hierochuntica	0	0	0	0	0	0	0	0	0	0

The mortality induced by the drug treatment in mice was not significant as compared to the control. (Chi-square test).

Table 8: Mortality induced in mice by *A. hierochuntica* extract treatment (100 mg/Kg/day) during chronic treatment. (10 male and 10 female mice were used in each group).

Treatment (3 Months)	**Mice**	**Pre-treatment body weight (g)**	**Post-treatment body weight (g)**
Control	**Male**	22.4 ± 1.27	33.7 ± 2.25*
	Female	20.5 ± 1.39	33.3 ± 1.88*
A. hierochuntica	**Male**	21.8 ± 1.58	34.8 ± 2.73*
	Female	20.4 ± 1.32	34.2 ± 2.15*

*P<0.001 (Student's t-test).
- In each case pre-treatment body weight of mice was compared with the post-treatment body weight.
- In each group, 10 male and 10 female mice were used.

Table 9: Quantitative data on body weight changes of male and female mice after chronic treatment (100 mg/kg/day) with *A. hierochuntica* extract. (Mean ± SE).

Treatment & dose 100 mg/kg (3 months)	**Average organs weight (per 100 g body weight)**						
	Heart	**Lungs**	**Liver**	**Kidney**	**Spleen**	**Testis**	**Seminal vesicles**
Control	0.45 ± 0.02	0.68 ± 0.03	5.43 ± 0.18	1.42 ± 0.04	0.53 ± 0.04	0.66 ± 0.03	0.86 ± 0.12
A. hierochuntica	0.44 ± 0.03	0.70 ± 0.05	5.45 ± 0.16	1.46 ± 0.06	0.55 ± 0.08	0.68 ± 0.06	0.88 ± 0.13

P>0.05 (Student t-test).
- Treatment group was compared to the control group.
- Five animals were used in each group.

Table 10: Effect of chronic treatment with *A. hierochuntica* extract on organ weights (per 100 g body weight) of mice. (Mean ± S.E.).

Treatment & duration: 100 mg/kg/day (3 Months)	**Mice**	**Hematological indices (Mean ± SE)**					
		WBC x 10^9/L	**RBC x 10^{12}/L**	**Hemo-globin g/dL**	**Platelets 10^9/L**	**MCV fL**	**HCT ratio (%)**
Control	Male	5.6 ± 0.6	6.5 ± 0.5	13.8 ± 0.4	490 ± 75.0	51.7 ± 0.5	39.3 ± 0.88
	Female	5.5 ± 0.4	6.6 ± 0.3	14.2 ± 0.6	480 ± 64.0	52.1± 0.7	38.8 ± 1.0
A. hierochuntica	Male	5.7 ± 0.5	6.8 ± 0.7	14.6 ± 0.5	485 ± 44.0	51.9 ± 1.0	38.9± 0.89
	Female	5.5 ± 0.6	6.5 ± 0.7	14.2 ± 0.4	470 ± 55.0	51.6 ± 0.7	38.5 ± 1.5

P>0.05, (Student's t-test)
- Five male and five female mice were used in each group.
Treatment group was statistically compared with the respective control group.

Table 11: The effect of *A. hierochuntica* extract (100 mg/kg/day) on the hematological parameters of male and female mice at the end of chronic treatment.

Treatment & duration: 100 mg/kg/day (3 Months)	**Mice**	**Biochemical indices (Mean ± SE)**					
		AST (µL-1)	**ALT (µL-1)**	**CK-MB (µL-1)**	**Creat. (µmole/L)**	**Urea (mmole/L)**	**Glucose (mmole/L)**
Control	M	17.45 ± 0.55	11.35 ± 0.90	140.8 ± 12.50	133.6 ± 10.50	5.85 ± 0.36	5.40 ± 0.80
	F	15.70 ± 0.46	12.30 ± 1.55	145.5 ± 13.40	135.3 ± 9.75	5.79 ± 0.35	5.25 ± 0.75
A. hierochuntica	M	16.96 ± 0.41	12.15 ± 0.75	143.5 ± 13.50	105.5 ± 7.60	5.83 ± 0.62	5.45 ± 1.53
	F	16.60 ± 0.40	11.87 ± 1.41	140.6 ± 14.00	116.0 ± 8.00	5.81 ± 0.40	5.30 ± 1.22

P>0.05, (Student's *t*-test)
- Five male and five female mice were used in each group.
Treatment group was statistically compared with the respective control group.

Table 12: The effect of *A. hierochuntica* extract (100 mg/kg/day) on the biochemical parameters of male and female mice at the end of chronic treatment.

Treatment (3 Months)	**Total sperm screened**	**Sperm head abnormalities (%)**						**Abnormal sperm (%)**
		Swollen achrosome	**Amorphous**	**Microcephali**	**Megacephali**	**Rotated head**	**Flat head**	
Control (Water)	5680	0.48 ± 0.07	0.48 ± 0.08	0.05 ± 0.03	0.08 ± 0.04	0.29 ± 0.07	0.05 ± 0.02	1.28 ± 0.26
A. hierochuntica	5625	0.54 ± 0.09	0.45 ± 0.06	0.06 ± 0.02	0.06 ± 0.03	0.28 ± 0.06	0.05 ± 0.02	1.26 ± 0.28

P>0.05, (Student's *t*-test)
- Five male mice were used in each group.
- Treatment group was statistically compared with the control group.

Table 13: The effect of chronic treatment with *A. hierochuntica* extracts (100 mg/kg/day) on the epididymal spermatozoa in male mice. (Mean ± SE).

Maryam' (*A. hierochuntica*) offered a significant protection against the ulceration caused by ethanol, as well as treatment with: hydrochloric acid, sodium chloride, sodium hydroxide, and indomethacin. Our results are well in agreement with earlier reports on such chemicals causing stomach injury [33,39]. The results of histopathological studies revealed that pretreatment with *A. hierochuntica* inhibited ethanol-induced necrosis, congestion, hemorrhage and edema in gastric mucosa. The stomach of the animals treated with a higher dose of 'Kaff-e-Maryam' showed only mild congestion; otherwise the stomach appearance was comparable to normal control. These finding confirmed the cytoprotective nature of *A. hierochuntica* [24,26,33]. *A. hierochuntica* pretreatment also inhibited the depletion of stomach-wall mucus caused by ethanol treatment [40]. It is worth specifying, that a considerable controversy exists about the role of mucus in the prevention of gastric mucosal injury [41-45]. However, gastric mucus coat is alleged to be important both in preventing damage to the gastric epithelium as well as in facilitating its repair [40,42,46].

In the present study, ethanol treatment significantly reduced the stomach protein and nucleic acid contents of rats, caused by accumulation of toxic free radicals in the mucosal cells [47]. Pretreatment with *A. hierochuntica* offered a dose dependent protection against the deleterious effects of ethanol on the concentration of protein and nucleic acids. It has been well explained by earlier researchers, that ethanol is metabolized in the body and produces injurious free radicals [48]. The protection observed during present study, well designates *A. hierochuntica* extract to contain chemical constituents with profound antioxidant potential. The observed protection is well supported by the isolation of new type of flavonoids: Anastatin A and anastatin B, glucosinolates and their derived isothiocyanate and other compounds from this interesting desert plant. Both, the extract and some of its isolated phytoconstituents were confirmed to possess antioxidant properties which might be held responsible for the gastro-protective activity observed in the present study [12,49]. The results of our present experiment were further supported by earlier reports where new flavonoids of *A. hierochuntica* were found to display free radical scavenging properties [50-53].

Generally, plant polyphenols including flavonoids are broadly described to be successful inhibitors of lipoxygenase and cyclooxygenase pathways, and they are useful to explicate fatty acid peroxidation process [17,45,54]. Several chemical constituents isolated from *A. hierochuntica* and other medicinal plants were established to be free radical scavengers [55-58]. In the present study, *A. hierochuntica* treatment showed pharmacological activities which inhibited the formation of gastric damage and blocked other biochemical changes to occur. For example, the observed protection of protein and nucleic acid contents in the present investigation. This potential may be attributed to the compounds working as free radical scavengers. The results of the present study are full in agreement with the results of earlier experiments [3,51,59]. All such chemical constituents of *A. hierochuntica* make a protective layer guarding gastric mucosal damage not to occur [54,60-62].

A number of novel and biologically interesting phytochemical constituents have been isolated and characterized from A. hierchuntica. The identified chemical constituents of *A. hierochuntica* were found to belong to different groups: volatile oils, glucosinolates, sterols, triterpenes, tannins, flavonoids, and other polyphenols. In addition, the structures of a number of previously unidentified complex compounds were also elucidated [58,63-65]. It is worth mentioning that, the findings of present study are supported by the isolation of eriodictyol, luteolin, kaempferol, quercetin, anastatin A and B, silybin A, isosilybins A and B, hierochins A and B, (2R,3S)-2,3-dihydro-2-(3,4-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-7-hydroxy benzo furan, (+)-dehydro- diconiferyl alcohol, (+)-balanophonin, 1-(4-hydroxy-3-methoxyphenyl)-2-{4-[(E)-3-hydroxy-1-propenyl]-2-methoxyphenoxy}-1,3-propanediol, and 3,4-dihydroxybenz- aldehyde, form *A. hierochuntica* which were established to be strong inhibitors of free radicals generation [51,59,66]. Furthermore, these compounds were also found to inhibit melanogenesy [4,51].

A tea prepared from A. hierchuntica was earlier found to contain several flavonoids, chlorogenic acids, and phenolic compounds with antioxidant properties [59,67]. Furthermore, luteolin-7-glucoside, luteolin-7-glycoside, isovitexin, kaempferol 3-rhamnoglucoside, lucitin; glucosinolates including glucoiberin and glucocheirolin, and sterols were found in different parts of the plant under present study. However, fruits of Kaff-e-Maryum contained glucose, galactose, fructose, sucrose, raffinose and stachyose. Several of the isolated compounds possessed free radical scavenging potential and added support to the findings of present experiment [4,51,57]. Current findings are also in full agreement with earlier pharmacological experiments on glucosinolates and their

derived isothiocyanate present in *A. hierochuntica*. Such compounds also demonstrated profound anti-proliferative activity [50,68-71].

The observed protective effect of *A. hierochuntica* may be attributed to the antioxidant properties of this interesting desert plant. In earlier studies, compounds such as qurecetin, luteolin, and kaempferol derivatives, and anastatins A, and B were found to be anti-ulcerogenic compounds with significant protective activities [50,51,72]. Hence, the observed cytoprotective and antioxidative property of *A. hierochuntica* are ascribed to the presence flavonoids and other antioxidants present in the plant extract under study [57-59,73]. Besides several biological activities, various flavonoids have been reported to be useful in the treatment of certain gastrointestinal disorders and as inhibitors of free radical generation [53,74,75]. Nevertheless, quercetin was earlier confirmed to exercise its gastroprotective effect by its antiperoxidative, antioxidant, and antihistaminic nature [64,66,76].

The administration of ethanol in the present study caused tissue damage as revealed by decreased gastric mucosal NP-SH concentration. It is well known that gastric mucosa contain high concentration of reduced glutathione, the major component of the endogenous NP-SH pool [47,77]. NP-SH are known to be involved in the cytoprotective effects of various drugs [39,44,78]. Reduced levels of endogenous sulfhydryls have been associated with tissue damage by various chemical agents in the previous investigations [77,79]. Pretreatment with *A. hierochuntica* in the present study was found to prevent NP-SH depletion caused by ethanol. These findings indicated the possible involvement of NP-SH in the cytoprotective effect of *A. hierochuntica*. The results of the present study are full in agreement with earlier reports on protection of gastric damage caused by necrotizing agents including ethanol [24,62,78].

In general, nonsteroidal anti-inflammatory drugs including Indomethacin are identified to reduce prostaglandin levels which are known to be involved in cytoprotective process [23,79,80]. In the present study, *A. hierochuntica* treatment offered a significant protection against indomethacin, and indomethcin combined with ethanol induced gastric mucosal damage in rats [32,40,41,81]. Furthermore, there was a significant production of mucus, increase in nonprotein sulfhydryl concentration and glutathione levels [47]. The results of the present experiment also showed protection against hazardous malondialdehyde levels. Such changes were full in conformity with previous reports on prostaglandin production and free radical scavenging potential of plant extracts [41,42,62,80]. It is also well documented that several compounds with potential to generate prostaglandins could protect gastric mucosa against various ulcerogenic agents [41,82-84]. The observed gastro-protective activity of *A. hierochuntica* against indomethacin-induced damage might be ascribed to stimulation of prostaglandin by its phytochemical constituents [2,53,77,85].

The overall results of present study indicated that the cytoprotective effects of *A. hierochuntica* treatment were the consequence of its effect on gastric mucus production, NP-SH concentrations, free radical scavenging, and possible prostaglandin stimulating properties. The exact mechanism of anti-ulcer activity of *A. hierochuntica* treatment is not investigated earlier, however, a large number of poly phenols including flavonoids, have been isolated from this plant which have known protective activity [75,80]. The screening results also indicated the presence of saponins, glucosinolates and other glycosides in *A. hierochuntica* [10,63]. In an earlier study, sulforaphne was confirmed to inhibit extracellular, intracellular and antibiotic resistant strains of Heliocobacter pylori and prevented against benzo[a]pyrene induced stomach tumors [65,70]. Many individual flavonoids such as quercetin, rutin, kaempferol, flavones glycoside, and hypolaetin 8-glucoside, and other A. hierchuntica chemical constituents, have been reported to possess gastric protective effects [69,86-90]. Similarly, saponins were also reported to have antiulcer effects in rats [85]. Based on the earlier isolated chemical constituents and the results of the present study, it appears reasonable to suggest that different polyphenols, flavonoids, glucosinolates and saponins of *A. hierochuntica* may be totally or partially be responsible for its gastro-protective activity. Further studies are warranted on *A. hierochuntica* to elucidate the exact mechanism of the observed protective activity.

On the other side, the results of the preliminary toxicity studies in Brine shrimp lethality test were found to be useful in initial assessment of chemicals and plant extracts for detailed toxicology and pharmacology investigations [19,34]. In an earlier experiment, *A. hierochuntica* extract treatment induced some mitotic index depression in *Allium cepa* meristems [11]. The cytotoxicity shown by *A. hierochuntica* ethanol extract in the present study successfully indicated the need for detailed investigations on the effects of acute and chronic treatment using different official experimental models [18,20,21].

There is a dearth of toxicity data in the literature to support the safety of *A. hierochuntica* ethanol extract. However, in the present study, during acute treatment studies none of the animals died up to 3 g/kg dose level. There were no obvious behavioral changes in the treatment group as compared to the control, except chnges in writhing and decrease in locomotor activity of animals treated with the highest dose of the extract. The decrease in locomotor activity observed in the acute toxicity studies might be attributed to some of the chemical constituents of *A. hierochuntica* possessing such properties.

Throughout chronic treatment, no abnormal signs indicating toxicity were observed and all mice in the treatment group were comparable to the control. There was a statistically significant weight gain by all the animals in the treatment group which was similar to the weight gain by the control group mice. At the end of chronic treatment, there were no signs of any visceral toxicity. The weight of vital organs was normal and comparable to the control. All the observations were substantiated by histopathological studies confirming lack to toxicity. The hematological and biochemical data of the treatment group was similar to that of control group.

The chronically treated male animals were analyzed for spermatogenic dysfunction by using sperm abnormality test which is a reliable method for assessing germ cell mutagenicty and carcinogenicity [1,38]. The sperm abnormality data of the treatment group was similar and comparable to the male mice in the control, hence, ruling out any mutagenic potential of *A. hierochuntica* ethanol extract after chronic treatment. All the results obtained during present toxicity experiment provided preliminary and basic useful support for future experiments on this effective natural drug.

Conclusion

Kaff-e-Maryam (*A. hierochuntica*) was confirmed to be a widely used and popular traditional drug which possessed antioxidant potential and offered gastro protection activity against toxic damage. The acute and chronic toxicity data in mice showed no signs of abnormal and alarming toxicity. Based on the results obtained, A. hierochimtoca could not be classified as a poisonous plant. The present study justified the folklore claim regarding its gastro protective potential. Furthermore, the results of the toxicity studies added support and provided basic toxicity information for future experiments on this potential natural drug.

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Declaration of Interest

All the authors: N.O.Al-Harbi, R.M. Al-Ashban, M.P. Bhandari, and A.H. Shah, hereby declare that the submitted research paper has been prepared for academic reasons and not submitted to any other Journal. The proper guideline and instructions were followed and the authors have no 'Conflict of Interest' with any other Organization. The research work was conducted by following the official protocols, and there is no grant or financial involvements of any type, with any person or organization in this regards. In addition, we take all the responsibilities that literature survey was conducted and the work presented was not published earlier. There is no copying type activities involved and the references given are according to the international protocol with transparency. There is nothing hidden which should to be disclosed. The interest is only to get the paper published as a part of 'services to science' and not for any other reason. We fully agree with the "Declaration of Interest" policy of the Journal of Biology and Medicine. We all are pleased that Professor Dr. Arif H. Shah will be the corresponding author for any future query regarding this research paper.

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