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Research Article

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Screening of *Marrubium alysson L.* extract for pharmacological activity

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ABSTRACT

The main objective of this study was to investigate the pharmacological activity of M. alysson L. extract collected in Egypt using experimental animal models. M. alysson L. used in this study was collected from Northern Sinai in Egypt and was air-dried in the shade and ground to a fine powder (3 Kg) which extracted by maceration with 90 % methanol, filtered, and evaporated using rotary evaporator till dryness. In this study, Diabetes mellitus was induced in male albino rats by intraperitoneal injection of Alloxan. The antipyretic activity was evaluated using pyrexia induced by yeast injection. Acetic acid writhing test was used to evaluate the analgesic activity, and rat paw edema method was used to evaluate the acute anti-inflammatory activity. The antiulcer activity of the extract was evaluated using indomethacin-induced ulcer method. Finally, metabolic cages were used to study the diuretic activity of the extract. Results showed that M. alysson L. extract in a dose of 100 mg/kg exhibited improvement in the antioxidant activity. In addition, the extract caused a significant reduction in elevated temperature after two hours as well as reduced number of acetic acid writhing movements, indicating a significant analgesic effect. Furthermore, the extract significantly reduced the carrageenan-induced paw edema through an anti-inflammatory effect. In terms of gastro protection, M. alysson L. reduced the number of ulcers induced by indomethacin and possess diuretic properties. In conclusion, M. alysson L. extract collected in Egypt showed antioxidant, antipyretic, analgesic, anti-inflammatory, antiulcer as well as diuretic activity.

Key words: Acetic acid writhing, Antioxidant, Antipyretic, Antiulcer, *Marrubium alysson L.*

INTRODUCTION

Natural products have been used for the prevention and treatment of various diseases for thousands of years. In modern medicine, natural products have made huge contributions towards drug discovery. A large number of anticancer, antibacterial, antiviral, anti-inflammatory, and analgesic drugs were derived directly or indirectly from natural products. Plants continue to provide bioactive compounds that are used as novel drug leads. Family Labiatae, also known as Lamiaceae (the mintfamily) contains approximately 200 genera and more than 3,000 species. Plants belonging to this family have been used for thousands of years in the form of infusions and tinctures to treat various diseases [1].

Marrubium L. is a genus of flowering plants in Family Labiatae [2].The genus *Marrubium L.* is represented by 97 species which are widely spread over the temperate and warm regions [3]. Genus *Marrubium L.* is a rich source of

many classes of compounds such as labdane diterpenes, flavonoids, phenylpropanoids and volatile oils [2].Several species of genus *Marrubium L.* are used in folk medicine for the treatment of jaundice, diabetes, fever, cough, and asthma [4-7].

Marrubium alysson L. (M. alysson L.) is commonly distributed in Egypt and its Arabic name is 'Hashisha Rabiah' [8], while the common English name is 'White horehound'[2]. In addition, it was found on the Mediterranean coastal strip from El-Sallum to Rafah, as well as the Sinai desert. It was prepared in the form of decoction as a treatment for asthma and as a diuretic. Also, its tops were used as a flavoring agent in North Africa.

M. alysson L. was reported to have a hypoglycemic effect [2], antiviral and anticancer activities [4]. In addition, *M. alysson L.* leaves and flowers were used in folk medicine for cough, colds and asthma [2]. Therefore, the present study was aimed to study some pharmacological activities of *M. alysson L.* extract that collected in Egypt in experimental animals.

EXPERIMENTAL SECTION

2.1. Materials and methods

2.1.1. Plant collection

The whole plant of *M. alysson L.* used in this study was collected once during February 2012 from Wadi Awlad Aly from ElQussima road in Northern Sinai in Egypt, air-dried in the shade, ground to a fine powder and stored at low temperature (20ºC) until further processing. The plant was kindly authenticated by Dr. Mohamed El-Gebaly at the Department of Plant Taxonomy, Faculty of Science, Cairo University and by comparison with plant description in the flora of Egypt[8, 9]. The voucher specimen was deposited in the herbarium section of Pharmacognosy Department, Faculty of Pharmacy, Suez Canal University, and Ismailia, Egypt under registration number SAA-160.

2.1.2. Extraction

The plant powder (3 Kg) was extracted by maceration with 90 % methanol, filtered, evaporated using rotary evaporator till dryness, and weighed to obtain150 g of the total extract; 10 g was dedicated for studying the pharmacological activity of the plant.

2.1.3. Experimental Animals

Albino mice of 25-30 g body weight were used in this study. In addition, adult male albino rats of 130-150 g body weight were used. The animals were purchased from the Modern Veterinary Office for Laboratory Animals (Cairo, Egypt) and housed in polyethylene cages under controlled laboratory conditions, the same hygienic conditions and on a standard laboratory diet. All experimental protocols were approved by The Animal Care and Use Committee at the Faculty of Pharmacy, Suez Canal University.

2.1.4. Antioxidant activity

Diabetes mellitus was induced to male albino rats by intraperitoneal injection of Alloxan (150 mg/kg body weight, Sigma, USA) [10]. Hyperglycemia was assessed after 72 hours by measuring blood glucose [11]using 24 rats divided into four groups of six animals each. The first group contained the normal rats and served as a negative control, while the second group contained the diabetic rats and served as a positive control. Both group one and two received 1 ml saline. The third group contained the diabetic rats that received 7.5 mg/kg body wt. of vitamin E (Pharco Pharmaceutical Co., Cairo, Egypt) as reference drug. Finally, the fourth group contains the diabetic rats that received (100 mg/kg) of the *M. alysson L.* extract.

Blood samples were collected after 7 days for estimation of blood glutathione levels using Biodiagnostic kit (Biodiagnostic Co., Giza, Egypt).The restoration of blood glutathione levels (reduced due to induction of diabetes) was taken as a measure of antioxidant activity.

2.1.5. Hypoglycemic activity

Diabetes mellitus was induced to male albino rats by intraperitoneal injection of Alloxan (150 mg/kg body wt.) [10]. Hyperglycemia was assessed after 72 hours by measuring blood glucose levels and after 2 weeks and 4 weeks[11]. Twenty four rats were divided into four groups of six animals each. The first group contained the normal rats and served as a negative control, while the second group contained the diabetic rats and served as a positive control. Both group one and two received 1 ml saline. The third group contained the diabetic rats that received 100 mg/kg

body wt. of metformin (Chemical Industries Development Co., Giza, Egypt)[12] as a reference drug. Finally, the fourth group contained the diabetic rats that received (100 mg/kg) of the *M. alysson L.* extract.

At the end of each study period, blood samples were collected from the retro-orbital venous plexus through the eye canthus of anesthetized rats after an overnight fasting period. Serum was isolated by centrifugation and the blood glucose level was measured[11].

2.1.6. Antipyretic activity

Eighteen male albino rats were divided into three groups of six animals each. The first group contained rats that received 1 ml saline and served as control. The second group contained rats that received 20 mg/kg body weight of paracetamol (Misr Co., Cairo, Egypt) as a reference drug. The third group contained rats that received (100 mg/kg) of the *M. alysson L.* extract.

Pyrexia was induced by intramuscular injection of 1 mg/100g body weight of 44 % yeast suspension[13]. The normal rectal temperature was recorded before the beginning of the experiment. After 18 hrs, the temperature was recorded for all groups to serve as the baseline of elevated body temperature, to which the antipyretic effect will be compared. One and two hours later, other records of temperature were determined.

2.1.7. Analgesic activity

Male albino mice were used for testing the analgesic activity. Animals were acclimatized to the laboratory conditions for at least one hour before testing. The analgesic activity was estimated using acetic acid induced writhing test, the extract was administered orally at a dose of (100 mg/kg). Thirty minutes later 0.6% acetic acid was injected intraperitoneally (0.2% ml/mouse) and each mouse was then placed in an individual clear plastic observation chamber and the total number of writhes/30 minutes was counted for each mouse[14].

2.1.8. Acute anti-inflammatory activity

This effect was determined according to the method described by Winter et al [15]. Eighteen albino rats were divided into three groups of six animals each. The first group contained the rats that received 1 ml of saline and served as control. The second group contained the rats that received 20 mg/kg indomethacin (Egyptian International Pharmaceutical Industries Co., Cairo, Egypt) as a reference drug. The final group contained the rats that received (100 mg/kg) of the *M. alysson L.* extract.

One hour later, all the animals received a sub plantar injection of 0.1 ml of 1% Carrageenan (Sigma, USA)solution in saline in the right hind paw and 0.1 ml saline in the left hind paw[15]. Four hours after drug administration, the rats were sacrificed; both hind paws were excised and weighed separately.

The percentage of edema produced and that of edema inhibition due to drug administration were respectively calculated as follows:

Edema = wt. of right paw - wt. of left paw ×100/wt. of left paw

Edema inhibition = (Mc – Mt) × 100/Mc

Where Mc is the mean edema in control rats and Mt is the mean edema in drug-treated animals; the % of change was then computed.

2.1.9. Antiulcer activity

The antiulcer activity was carried out according to Corell et al.[16]. Twelve male albino rats were divided into two groups of six animals each. The first group contained rats that received 20 mg/kg indomethacin (Egyptian International Pharmaceutical Industries Co., Cairo, Egypt) as a positive control. In this group, rats were fasted for 18 h but given water and *lib*, then indomethacin was administered orally through gastric gavage. The rats were sacrificed4 h after indomethacin administration. The stomachs were removed, fixed in 10% formalin, dissected along the greater curvature and washed in normal saline. Subsequently, the stomachs were observed with the help of a magnifying lens, and their external and internal surfaces were studied and observed for any eroded or ulcerated areas and the number of ulcers were counted and compared to those of the second group[17].

The second group contained rats that received (100 mg/kg) of the *M. alysson L.* extract. In this group the treatment schedule was once a day and it was continued for five days. After five days, rats were kept 18 h fasting and then indomethacin was given orally, and after 4 h rats were sacrificed and the procedure repeated as the first group and the number of ulcers compared with that of group one.

2.1.10. Diuretic activity

Eighteen albino rats were divided into three groups of six animals each. Animals were held separately in metabolic cages, fasted for 18 hrs and given water only. The first group contained the rats that received 1 ml of saline and served as a normal control. The second group contained the rats that received 0.7 mg/kg furosemide (Egyptian International Pharmaceutical Industries Co., Cairo, Egypt) as a reference drug. The final group contained the rats that received (100 mg/kg) of the *M. alysson L.* extract.

Immediately after drug administration, a funnel was placed at the bottom of each cage to allow the urine to pass through it into a collecting tube. After 2 hrs, 4 hrs and after a further 24hr the urine was transferred into a graduated cylinder and its volume was recorded[18].

2.1.11. Toxicity study

The toxicity of the *M. alysson L.* extract was tested using four doses (100, 250, and 500 mg/kg) (three mice for each dose). Three control mice were kept under the same conditions without any treatments. The animals were observed for 24 hrs for any symptoms of toxicity and mortality rates in each group [2].

2.1.12. Data analysis and statistics

Data are expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was applied to determine the significance of the difference between the groups using SPSS software, version 2 (Graphpad Software, Inc., San Diego, USA).

RESULTS DISCUSSION

3.1. Results

3.1.1. Antioxidant activity

Alloxan administration resulted in a significant reduction of the glutathione concentration **(Figure 1)**. *M. alysson L.* extract in a dose of 100 mg/kg caused improvement in the antioxidant activity and nearly normalized the level of glutathione **(Figure 1)**.

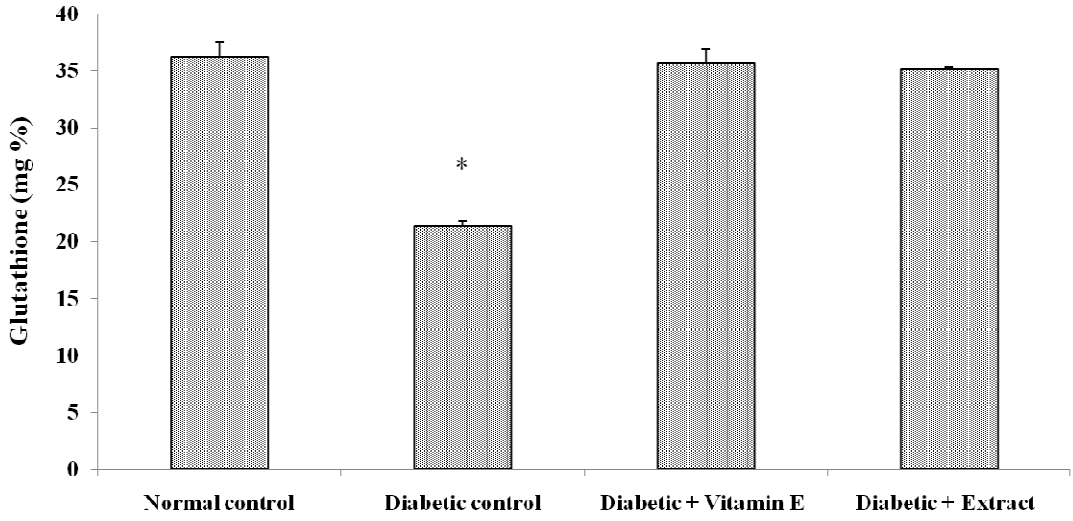

Figure 1. Effects of *M. alysson* extract (100 mg/kg/day for 7days) and vitamin E (7.5 mg/kg/day for 7days) on reduced glutathione in diabetic rats

*Results are expressed as mean ±S.E.M. and analyzed using one-way ANOVA (n = 6). * Statistically significant difference compared to the normal control group at p < 0.10.*

3.1.2. Hypoglycemic activity

Diabetic rats showed a significant increase in the levels of plasma glucose when compared to normal rats as shown in **Table 1**. Oral administration of *M. alysson L.* extract showed a hypoglycemic activity but the effect was not significant when compared to the values at zero time.

Table 1. Hypoglycemic activity of *M. alysson* extract (100 mg/kg) and Metformin (100 mg/kg) on blood glucose level of Diabetic albino rats

Time	Normal control	Diabetic non treated	Diabetic treated with M. alysson extract (100 mg/kg)	Diabetic treated with metformin (100 mg/kg)
Zero	81.6 ± 1.9	246.9 ± 8.2	258.3 ± 8.7	261.9 ± 10.1
2weeks	83.4 ± 2.1	251.3 ± 9.14	189.1 ± 7.2	143.7 ± 6.4*
4weeks	82.5 ± 1.8	263.2 ± 9.7	159.2 ± 6.9	83.6 ± 2.4*

*Results are expressed as mean ±S.E.M. and analyzed using one-way ANOVA (n = 6). * Statistically significant different from the values in day 0 in each corresponding group at p < 0.01.*

3.1.3. Antipyretic activity

M. alysson L. extract in a dose of 100 mg/kg showed a significant reduction in the elevated temperature after two hours when compared to the raised and the control groups as shown in **Table 2**. On the other hand, paracetamol (20 mg/kg) administration significantly reduced the elevated temperature after one hour and after two hours **(Table 2)**.

Table 2. The antipyretic effect of *M. alysson* extract (100 mg/kg) and paracetamol (20 mg/kg) on Pyrexia induced by yeast suspension in albino rats

Group	Induced rise in temperature	Body temperature change	
		One hour	Two hours
Control(1 ml saline)	38.7 ± 0.03	38.9 ± 0.02	39.2 ± 0.04
Paracetamol(20 mg/kg)	39.1 ± 0.05	38.2 ± 0.03*	37.1 ± 0.01*
M. alysson extract(100 mg/kg)	39.1 ± 0.04	38.7 ± 0.02	38.1 ± 0.02*

*Results are expressed as mean ±S.E.M. and analyzed using one-way ANOVA (n = 6). * Statistically significant different from the values of induced rise in temperature in each corresponding group and control group at p < 0.01.*

3.1.4. Analgesic activity

M. alysson L. extract (100 mg/kg) significantly reduced the writhing number as compared to normal control mice treated with saline only at $p < 0.01$**(Figure 2)**.

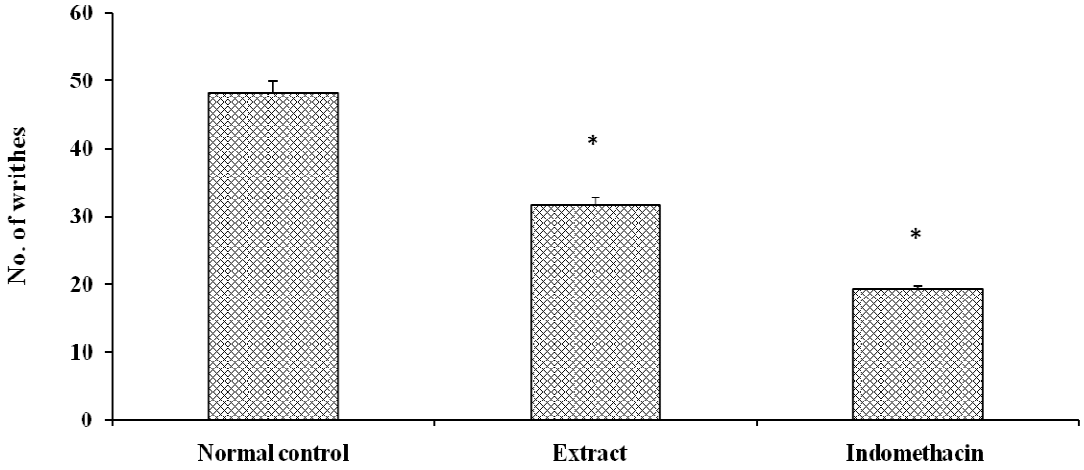

Figure 2. Effects of *M. alysson* extract (100 mg/kg) and Indomethacin (20 mg/kg) on acetic acid writhing in mice. Results are expressed as mean ± S.E.M. and analyzed using one-way ANOVA (n = 6). * Statistically significant difference compared to the normal control group at p < 0.01

3.1.5. Acute anti-inflammatory activity

M. alysson L. extract (100 mg/kg) significantly reduced the carrageenan induced paw edema in rats when compared to the control group **(Table 3)**. The extract showed 42 % of inhibition of the edema. On the other hand, indomethacin showed 63 % of inhibition as shown in **Table 3**.

Table 3. Acute anti-inflammatory activity of *M. alysson* extract (100 mg/kg) and indomethacin (20 mg/kg) in albino rats

Group	Dose (mg/kg)	% Edema	
		Mean ± S.E.	% of Change
Control	I ml Saline	59.8 ± 1.07	—
M. alysson extract	100	34.9 ± 1.2 *	42
Indomethacin	20	22.3 ± 0.6 *	63

*Results are expressed as mean ±S.E.M. and analyzed using one-way ANOVA (n = 6). * Statistically significant different from the values in day 0 in each corresponding group at p < 0.01. % of change calculated as regard to the control group.*

3.1.6. ***Antiulcer activity***

Gastric lesions induced by indomethacin (20 mg/kg) **(Figure 3)** were significantly reduced by pretreatment with 100 mg/kg of *M. alysson L.* extract.

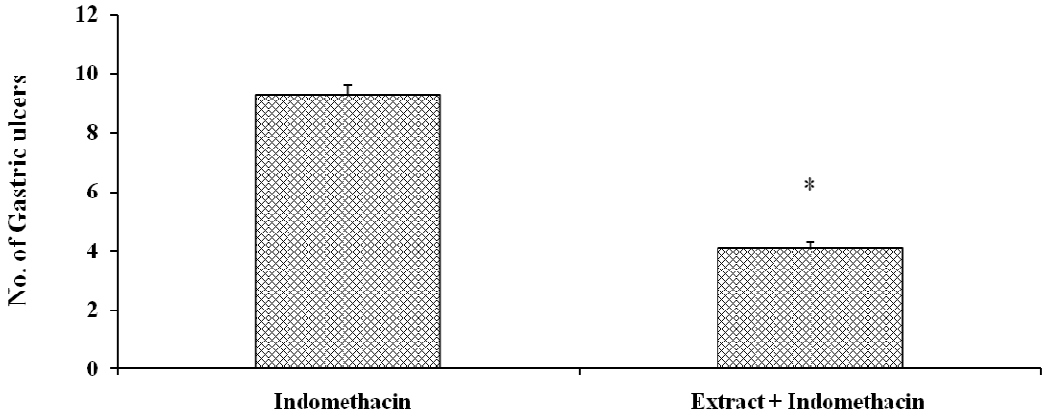

Figure 3. Effects of *M. alysson* extract (100 mg/kg) and Indomethacin (20 mg/kg) on gastric ulcers induced by indomethacin in albino rats. Results are expressed as mean ± S.E.M. and analyzed using one-way ANOVA (n = 6). * Statistically significant difference compared to the indomethacin group at p < 0.01

3.1.7. Diuretic activity

M. alysson L. extract (100 mg/kg) significantly increased the volume of urine after 24 hrs in rats when compared to the control group **(Table 4)**. Furosemide (0.7 mg/kg) significantly increased the volume of urine after 2, 4 and 24 hrs in rats when compared to the control group (**Table 4).**

Table 4. Diuretic activity of *M. alysson* extract (100 mg/kg) and furosemide (0.7 mg/kg) in albino rats.

Group	Dose (mg/kg)	Volume of urine (ml)		
		2 hrs	4 hrs	24 hrs
Control	1 ml Saline	0.8 ± 0.01	1.2 ± 0.03	6.4 ± 0.2
M. alysson extract	100	1.1 ± 0.04	3.1 ± 0.06	9.3 ± 0.5*
Furosemide	0.7	3.6 ± 0.2*	6.7 ± 0.5*	15.2 ± 0.7*

Results are expressed as mean ± S.E.M. and analyzed using one-way ANOVA (n = 6). * Statistically significant different from the control group at p < 0.01.

3.1.8. Toxicity study

The toxicity study revealed the non-toxic nature of *M. alysson L.* extract at doses up to 500 mg/kg. Mice did not show any drug-induced physical signs of toxicity and no deaths occurred.

3.2. Discussion

M. alysson L. (Lamiaceae) is commonly found in Egypt along the Mediterranean coast [2].It has been used in traditional medicine for various purposes. It is reported to have hypoglycemic, anti-inflammatory, antifungal, antiviral, and cytotoxic effects. Therefore, the purpose of this study was to evaluate some pharmacological activities of *M. alysson L.* extract collected in Egypt on experimental animals.

The current study showed that treatment with *M. alysson L.* extract in a dose of 100 mg/kg caused a significant increase in the blood level of glutathione in diabetic animals compared to diabetic non treated animals. On the other hand, *M. alysson L.* extract did not cause a significant decrease in the blood glucose level but showed minor reduction after 2 and 4 weeks. These findings are similar to those reported by Essawy and coworkers where *M. alysson L.* extract was given to high cholesterol-fed rabbits and showed a significant decrease in the free radical concentrations [2]. In another study by Yousefi and coworkers, *M. alysson L.* extract suppressed the elevated malondialdehyde levels in serum and in-vitro experiments, thus providing further proof of its antioxidant activity.[19].

In this study, we also investigated the analgesic, antipyretic, and anti-inflammatory activities of the plant. *M. alysson L.* extract exerted a significant antipyretic activity against pyrexia induced by yeast injection. In addition, it also showed significant analgesic and anti-inflammatory activities.

The anti-inflammatory action of *M. alysson L.* was proposed to be due to suppression of free radicals that are produced during inflammation and suppression of macrophages that modulate the inflammatory process. The antioxidant and anti-inflammatory properties of the plant were reported to be due to its content of phenylpropanoid glycosides [2], diterpenes, and flavonoids [20].

The present study also demonstrated the gastro protective effect of the extract on indomethacin-induced ulcers in rats. Concerning diuretic properties, to the best of our knowledge, this is the first report on the diuretic properties of *M. alysson L.*, and it could therefore be of benefit for hypertensive patients.

CONCLUSION

M. alysson L. extract collected in Egypt showed antioxidant, antipyretic, analgesic, anti-inflammatory, antiulcer as well as diuretic activities at a dose of 100 mg/kg. This study indicates that the plant is a promising natural source of drug molecules that needs further investigation to isolate its bioactive compounds and to help get a better understanding of its mechanism of action.

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