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EFFECTS OF TRADITIONAL FORMULATIONS ON EXTRA GASTRIC MUCUS OF RATS

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Keywords:

"Monzej-e-balgham", Gastric mucus, Iranian traditional medicine, Humor, Alician blue

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ABSTRACT: According to Iranian traditional Medicine (ITM) "Monzej" es are applied to mature and eliminate abnormal humor, correct the abnormal temperament and promote the formation of normal humor, restoring the functions of all organs and the natural body force. "Monzej-e-balgham" (applying the above functions on abnormal phlegm, called "Balgham" in ITM, is used to evacuate morbid matters produced due to excess or putrefaction of phlegm by maturing and eliminating the abnormal "Balgham", leading to control the development of many diseases in human body like gastrointestinal disorders (poor digestion, sour burp and reflux), Alzheimer's disease, stroke, epilepsy, hemiplegia, facial palsy, asthma, bronchitis, atherosclerosis, vitiligo and impotency. Regarding the definition of phlegm in traditional medicine and the characteristics of mucus, it can be argued that one of "Balgham" type could be the secretions of the gastrointestinal cells or mucus. Due to this similarity, it is possible to alter body's mucus with the help of a "Monzej-e-balgham". So, in this research, the effect of tablet and syrup formulations of "Monzej-e-balgham" on stomach mucus secretion in male rats has been evaluated using Alician blue assay. Tablet and syrup of "Monzej-e-balgham" significantly reduced the amount of extra gastric mucus in male rats ($p < 0.001$); but, there was no significant difference between the tablets and syrups effects on gastric mucosa and both formulations were equally effective in reducing excess mucus secretion in the stomach induced by misoprostol induction.

INTRODUCTION: Iranian traditional medicine (ITM) is one of the oldest types of systems of medicine and is a well-known and effective health care system among various traditional systems of medicine which has been in existence from several centuries due to its basic concept of humor (body fluids).

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Humor or "Khelt" in the Persian language as called in the traditional medicine textbooks, is a wet and fluid substance which foodstuffs in the first stage of permutation convert to, including phlegm or "Balgham" (with cold and wet qualities) blood or "Dam" (with hot and wet qualities), yellow bile or "Safra" (with hot and dry qualities) and black bile or "Sauda" (with cold and dry qualities) [1].

These four senses of humor maintain the normal physiological status of the body. According to the Canon of medicine, health is the result of the balance of these humor, and the imbalance between them, excessive amounts of each one and also

abnormal humor can lead to illness [1, 2]. Once the balance between humor is broken, this would cause many different diseases, and cure would be achieved by evacuation of morbid or excess humor from the body. ITM believes in regulation or elimination of the waste humor to cure diseases with "Monzej" es [3]. "Monzej"es are applied for treatment to mature and eliminate the abnormal humor, correcting the abnormal temperament than to promote the formation of normal humor, restore the function of organs and the body natural force [3, 4].

"Monzej-e-balgham", a traditional herbal medicine prescription, has been widely used in Iranian traditional medicine for a long time. It can eliminate the abnormal "Balgham" humor from the body and restore the balance between all humor [4, 5]. "Monzej-e-balgham" has been playing an effective role in various diseases caused by cold and wet qualities (diseases occurring due to phlegm accumulation) such as Alzheimer's disease [6], stroke [7], coronary heart disease [8], overweight [9], vitiligo [3] and premature ovarian failure (POF) [4] in human body according to modern research.

Different formulations of "Monzej-e-balgham" could be found in ITM textbooks, among diverse prescriptions, a combination of roots of *Glycyrrhiza glabra* L., liquorice, (Paplionaceae), fruits of *Foeniculum vulgare* Mill., fennel, (Apiaceae), aerial parts of *Adiantum capillus-veneris* L., maidenhair fern, (Polypodiaceae), fruits of *Vitis venifera* L., large raisins, (Vitaceae), fruits of *Ficus carica* L., fig, (Moraceae), flowers of *Rosa damascene* Herrm., damask *rose*, (Rosaceae) and seeds of *Onopordum acanthium* L., cotton thistle, (Asteraceae) was selected [10]. Traditional formulation of "Monzej-e-balgham" was reformulated into new dosage forms (syrup and tablet forms) for better acceptance and easier usage by patients. Also, standard pharmaceutical controls of the tablets and syrup have been performed to present a qualified formulation for usage [11].

Regarding the definition of phlegm in traditional medicine and the characteristics of mucus, it can be argued that one of the phlegm forms is the secretions of the gastrointestinal cells or mucus. Due to this similarity, it is possible to alter body's mucus with the help of a "Monzej-e-balgham" and reduce the excess gastric mucus.

In the present study, the effect of tablets and syrup formulations of "Monzej-e-balgham" on amount of gastric mucus in male rats have been investigated.

MATERIALS AND METHODS:

Polyherbal Formulations: The "Monzej-e-balgham" keyword was investigated in the main Iranian traditional references, in order to select the appropriate formulation. Different formulations were prepared in the form of syrup and tablets. After selecting the best formulations, physicochemical properties of syrup and tablets were evaluated, and total phenolic compounds were determined as the marker. Accelerated stability studies were carried out for final formulations for six months[11].

Animal Study:

Ethical Issues: The present study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (approval no IR.SBMU.RETECH.REC.1396.852). The animals were monitored and maintained according to the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and guidelines of the Organization for Economic Cooperation and Development (OECD).

Animals: Fifty-four adult male Wistar rats (250-300 g), is considered healthy and free from specific diseases (SPF – Specific Pathogen Free), were obtained from Animal house, Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Science. They were divided into groups of six animals and kept under standard conditions at 22 ± 2 °C and Humidity 70-75% with a light/ dark cycle ratio of 12:12 h, under continuous airflow. The cages were always handled by the same laboratory expert, who was specially trained for this position. They had access to tap water and food freely. Control group animals received the same experimental handling as those of test groups except that the drug treatment was replaced by the administration of appropriate volumes of dosing vehicles.

Induction of Excess Gastric Mucosa: Misoprostol is known to increase the mucus production in superficial epithelial cells. For this reason, gastric mucosa diameter was first increased by misoprostol

(100 micrograms/kg), and then the effect of formulations on extra mucus was determined by Alcian blue staining method using spectrophotometer [12, 13].

Animal Groups and Experimental Design: The rats were assigned to one of nine groups:

Group 1 (W): Rats were labeled as normal control, and from the 1st to the 14th day, they were given water by gavage.

Group 2 (MW): Rats were pretreated daily (during 7 days) with misoprostol, from the 8th to the 14th day they were given water by gavage.

Group 3 (SH1): Rats were pretreated daily (during 7 days) with misoprostol, from the 8th to the 14th day they were administered excipients of syrup (glycerin, sodium benzoate and potassium sorbate).

Group 4 (SH2): Rats were pretreated daily (during 7 days) with misoprostol, from the 8th to the 14th day they were administered excipients of the tablet (croscarmellose sodium)

Group 5 (M): Rats were pretreated daily (during 7 days) with misoprostol.

Group 6 (S1): Rats were pretreated daily (during 7 days) with misoprostol, on the 8th day they received "Monzej-e-balgham" syrup.

Group 7 (S7): Rats were pretreated daily (during 7 days) with misoprostol, from the 8th to the 14th day they received "Monzej-e-balgham" syrup.

Group 8 (T1): Rats were pretreated daily (during 7 days) with misoprostol, on the 8th day, they received "Monzej-e-balgham" tablet.

Group 9 (T7): Rats were pretreated daily (during 7 days) with misoprostol, from the 8th to the 14th day they received "Monzej-e-balgham" tablet.

Misoprostol (a prostaglandin E1 analog) was dispersed in hydroxyl propyl methylcellulose (1:100) and dissolved in distilled water. Misoprostol was administered at a dose of 100 µg/kg/day [13]. Tablets of "Monzej-e-balgham" were dissolved in distilled water and administered to animals with a dose of 285mg/kg/day and syrup with a dose of 3.2.ml/kg/day.

The animals were fasted for 24 h but were allowed free access to water till 2 h prior to the experiments. All drugs were freshly prepared at room temperature, and treatments were administered by gavage. At the end of the treatment period, the animal food was removed for 24 h; then, all animals were sacrificed with an overdose of ketamine and xylazine, followed by euthanization by cervical decapitation. Immediately their stomachs were removed and transferred to the nitrogen tank for 24 h. The samples were stored in -80 ºC freezer until mucus measurement.

Measurement of Gastric Wall Mucus Content: Gastric wall mucus was determined by the Alcian blue method [12]. The standard curve of Alcian blue was plotted using different concentrations, and the absorbance of each stomach of the rats was measured regarding the corresponding equation obtained from the standard curve. The collected samples were removed from the freezer and opened along the lesser curvature, weighed and immersed in 0.1% w/v Alcian blue solution for 2 h. Extra dye was then removed by two successive rinses in a 0.25M sucrose solution. The dye complex with gastric wall mucus was extracted with 0.5M $MgCl_2$ for 2 h. The blue extract was then shaken extremely with an equal volume of diethyl ether, and the resulting mixture was centrifuged at 3000 rpm for 15 min. The optical density of Alcian blue in the aqueous layer was read versus a blank at 580 nm using a spectrophotometer. The quantity of Alcian blue extract per gram stomach was then calculated regarding the standard curve of Alician blue, which corresponded to the gastric wall mucus.

Statistical Analysis of Data: All analyses were recorded as Mean ± S.E.M (n=6). The statistical significant differences of the *P*-value between groups were assessed according to Tukey's Post Hoc significance difference and one-way analysis of variance (GraphPad Prism 8). A value of $P<0.05$ was considered significant.

RESULTS AND DISCUSSION: The equation correspondent to the standard curve of Alcian blue was used to measure the gastric mucus content of rats (y= 0.0088 - 0.125). The results of Alcian blue assay in treatments and control groups of rats have been shown in **Fig. 1**.

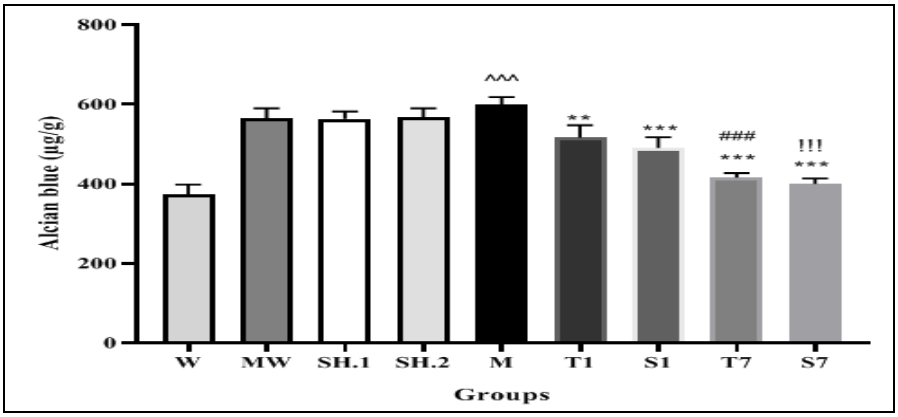

FIG. 1: ABSORPTION RATE OF ALCIAN BLUE IN DIFFERENT GROUPS OF RATS TESTED. Data are reported as mean ± standard error of the mean (n=6). W: Distilled water receiving group, MW: Misoprostol and distilled water receiving group, SH1: Sham1 group, SH2: Sham 2 group, M: Misoprostol receiving group, T1: Single-dose tablets group, S1: Single-dose syrup group, T7: Tablets receiving group (7 days), S7: Syrup receiving group (7 days).^ Significantly different group M to group W ($p<0.001$), **, *** Significantly different treatment groups (T1, S1, T7, S7) to group M ($p<0.01$) & ($p<0.001$), ### Significantly different group T1 to group T7 ($p<0.001$), !!! Significantly different group S1 to group S7 ($p<0.001$).

In order for better presenting the difference between the groups based on the results of **Fig. 1**, the comparison between the tablet and syrup groups with the control groups was presented separately **Fig. 2-4**.

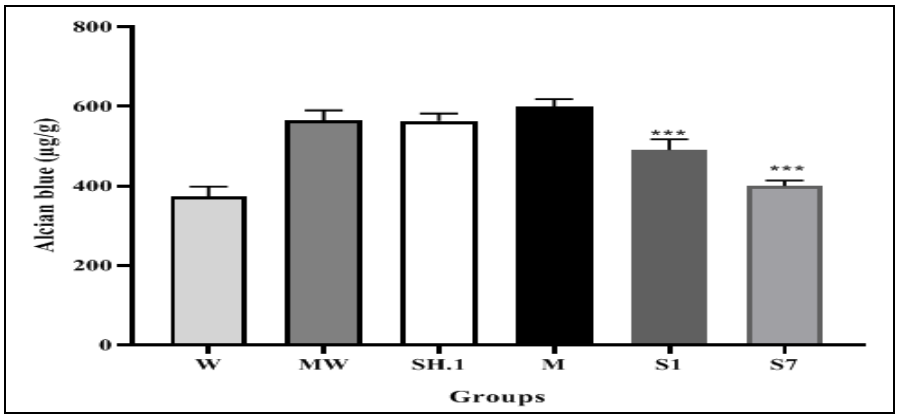

FIG. 2: COMPARISON OF ALCIAN BLUE ABSORPTION RATE IN SYRUPS AND CONTROLS GROUPS. Data are reported as mean ± standard error of the mean (n=6). W: Distilled water receiving group, MW: Misoprostol and distilled water receiving group, SH1: Sham1 group, M: Misoprostol receiving group, S1: Single-dose syrup group, S7: Syrup receiving group (7 days). (p *** <0.001): Significant difference of syrup groups (S1 and S7) with group M, (p ^^^ <0.001): Significant difference of group W with group M and (p !!! < 0.001): Significant difference between group S1 and group S7.

As shown in **Fig. 2**, misoprostol administration in different groups of rats significantly increased mucus compared to the distilled water group alone (p ^^^ <0.001). On the other hand, the misoprostol group alone did not show a significant difference with the misoprostol + distilled water group and sham1 group. Based on the results, the syrup of "Monzej-e-balgham" significantly reduced the gastric mucus at day 8 and day 15 in the treatment groups compared to the misoprostol group (p ***<0.001). Also, the results showed that prolonged administration of syrup of "Monzej-e-balgham" significantly reduced mucus compared to the single dose of the drug (p !!!<0.001).

As presented in **Fig. 3**, misoprostol administration in different groups of rats significantly increased gastric mucus in comparison to the distilled water group alone (p ^^^ <0.001), Misoprostol alone did not show a significant difference with the misoprostol + distilled water and sham 2 groups. According to the results, it was found that the tablets of "Monzej-e-balgham" reduced mucus at day 8 and day 15 in the treatment groups compared to the misoprostol group (p ** <0.01 and p *** <0.001). Significantly, the results of long-term administration of tablets of "Monzej-e-balgham" reduced mucus compared to the single dose (p ### <0.001).

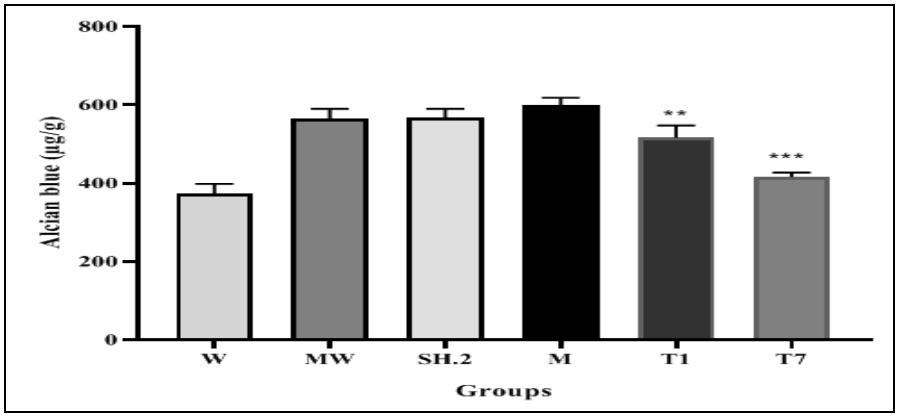

FIG. 3: COMPARISON OF ALCIAN BLUE ABSORPTION RATE IN TABLETS AND CONTROLS GROUPS. Data are reported as mean ± standard error of the mean (n=6). W: Distilled water receiving group, MW: Misoprostol and distilled water receiving group, SH2: Sham2 group, M: Misoprostol receiving group, T1: Single-dose tablets group, T7: Tablets receiving group (7 days). (p ** <0.001 and p *** <0.001): Significant difference between tablet groups (T1 and T7) with group M, (p ^^^ <0.001): Significant difference between group W and group M and (p ### <0.001): Significant difference between group T1 and group T7.

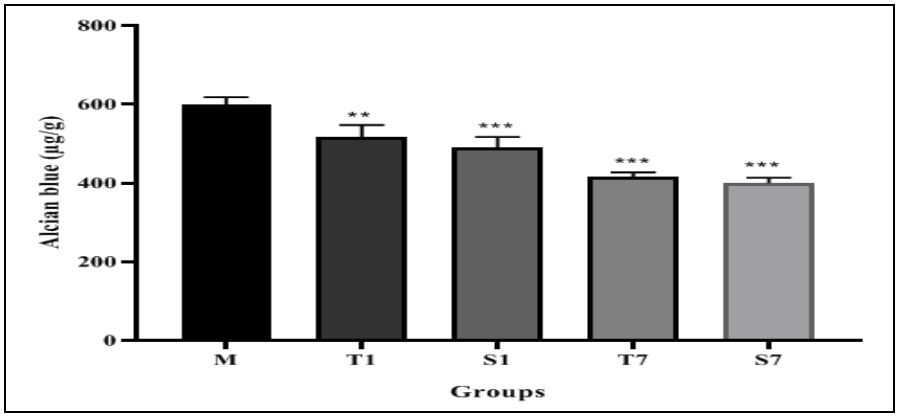

FIG. 4: COMPARISON OF ALCIANE BLUE ABSORPTION IN TREATMENTS GROUPS. Data are reported as mean ± standard error of the mean (n=6). M: Misoprostol receiving group, T1: Single-dose tablets group, S1: Single-dose syrup group, T7: Tablets receiving group (7 days), S7: Syrup receiving group (7 days). (p ** <0.001 and p *** <0.001): Significant difference between treatment groups (T1, S1, T7 and S7) with group M, (p ### <0.001): Significant difference between T1 group and group T7 and (p!!!<0.001: significant difference between group S1 and group S7**.**

As shown in **Fig. 4**, there was a significant difference between the syrup treatment groups on the 8th and 15th day (S1 and S7) as well as the 8th and 15th day (T1 and T7) tablets ((p!!!<0.001) and (p###<0.001) respectively). This indicates the effect of the drug is increased by repetition on different days compared to the single dose. But there was no significant difference between the syrup and tablet groups on eight days (S1 with T1) and on the fourteenth day (S7 with T7).

Based on the Canon of medicine, there are four senses of humor in the human body: Phlegm or **"**Balgham**"**, Blood or **"**Dam**"**, Yellow bile or **"**Safra**"** and Black bile or **"**Sauda". Each of them; is related to a pair of qualities: cold and wet, hot and wet, hot and dry, and cold and dry, respectively [14, 15]. Equilibrium quantity and quality of these humor are important for maintaining the optimum human health [5]. The Iranian traditional medicine system makes an effort to propose the best possible ways by which a person can lead an optimum healthy life. In the Canon of medicine, majority of the diseases are caused by the endogenous factors by excessive accumulation of abnormal humors [2].

"Monzej**"** therapy is used to correct abnormal temperament and to promote the generation of normal humor. Many diseases are caused by abnormal accumulation of "Balgham" humor, which may itself make the body unable to eliminate the produced abnormal fluids. Maturing and removing abnormal body fluids can correct the abnormal temperament, promote the formation of normal body fluid and restore the body force of nature [16]. "Monzej-e-balgham" has been used in Iranian traditional medicine to provide a basis for healing and treatment of various diseases caused

with wet and cold natures (diseases occurring due to phlegm accumulation) such as diseases with symptoms which are today known as Alzheimer's disease [6], stroke [7], coronary heart disease [8], overweight [9], vitiligo [3] and premature ovarian failure (POF) [4].

Regarding the definition of phlegm in traditional medicine and the characteristics of mucus, it can be argued that one of the phlegm forms is the secretions of the gastrointestinal cells or mucus. Due to this similarity, body's mucus could be influenced by the help of a "Monzej-e-balgham".

In this research, the effects of tablets and syrup formulations of "Monzej-e-balgham" on the reduction of stomach mucus secretion induced by misoprostol have been evaluated in male rats.

Cytoprotective PGs like PGE1, PGE2 and PGI2 of gastric and duodenal mucosa is responsible for mucus production and maintaining cellular integrity of the gastric mucosa and so increase mucosal production in superficial epithelial cells [12, 17]. The capacity of several prostaglandins analogous to control and prevent the gastric ulceration, known as cytoprotection, is a very important therapeutically property. There are several mechanisms by which exogenous prostaglandins may promote healing of ulcers. The PGE1 (misoprostol) and PGE2 analogous have been studied clinically. It has been proved that they could significantly decrease the acid and bicarbonate secretion and increase mucosal regeneration and cell proliferation [18] and this may occur *via* an effect on epithelial regeneration and stimulation or modulation of the factors involved in ulcer healing such as angiogenesis, epithelial cell regeneration, wound contraction, and blood flow [13].

It's been proven in this study that pretreatment with misoprostol significantly increased the gastric mucus content in rats (M group compared to W group).

On the other hand, the results of the present study about the effect of tablet and syrup formulations on gastric mucus levels in different groups of rats indicated that consumption of tablets and syrups of "Monzej-e-balgham" reduced gastric mucus secretion induced by misoprostol in rats. It has been shown that there was a significant difference between the control groups, including the misoprostol group alone (M group) or with distilled water (MW group).

It was also found that there was no significant difference in the amount of mucus secretion in the misoprostol receiving groups and the recipient's groups of the tablets and syrups excipients indicating that the excipient of the prepared formulations induced no effects on the gastric mucus secretion in the rats**.**

Notable in the results, there was a significant difference between single-dose and repeated doses, which may indicate a relationship between the concentration and duration of administration and the effect of the drug**.**

CONCLUSION: Finally, it can be concluded that there was no significant difference between the formulation of tablets and syrups of "Monzej-e-balgham" in their effects on the gastric mucosa and both formulations could be equally effective in reducing excess mucus secretion in the stomach induced by misoprostol induction.

On the other hand, the results of the LD_{50} study showed that high doses of "Monzej-e-balgham" were well tolerated by animals. In other words, "Monzej-e-balgham" show slow or no toxicity. However, other aspects of drug toxicity should be evaluated.

Actually, when formulations of "Monzej-e-balgham" were administered, they reduced the excess content of gastric mucus in male rats significantly and kept the gastric mucosa under normal conditions. In fact this treatment can be useful with the reduction of extra mucus and indeed abnormal "Balgham" and prevent various disorders associated with its accumulation. The results of this research proved views of ancient scholars about the relation between "Monzej-e-balgham" property to dissolve phlegm and the conventional view of reducing mucus as a form of "Balgham", with the help of this polyherbal treatment. This traditional product can be used as a conventional formulation for diseases caused by an excess of phlegm.

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