Anti-oxidant and Anti-inflammatory activity of Safoof Mohazzil: A traditional, Poly-herbal Unani formulation for Obesity

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Received 17 September 2014, revised 19 December 2014

Inflammation and oxidative stress have been reported in obesity. Safoof Mohazzil, is a traditional formulation prescribed by Unani physicians for weight loss. In the present study, antioxidant and anti-inflammatory properties of Safoof Mohazzil were evaluated using pyrogallol induced hepatotoxicity and carrageenan induced paw edema, respectively, in male Wistar rats. For the antioxidant study, rats were treated with Safoof Mohazzil for 14 days at the doses of 250, 500 and 1000 mg/kg, p.o. On 14th day, 2 hrs after the last dose of Unani formulation, pyrogallol (100 mg/kg) was injected intraperitoneally and on next day the animals were sacrificed for estimation of hepatic oxidative stress markers (malondialdehyde and reduced glutathione). Safoof Mohazzil demonstrated dose-dependent anti-oxidant activity. To assess its anti-inflammatory property, Safoof Mohazzil was administered at the doses of 500 & 1000 mg/kg, p.o. for 7 days and on 7th day carrageenan was administered 1 hr after the last dose of Unani formulation. The change in paw volume was calculated at 1, 3 and 6 hrs. Significant anti-inflammatory activity was found at 1000 mg/kg dose which is the human equivalent of its anti-obesity dose. The antioxidant and anti-inflammatory activity of Safoof Mohazzil support its use in obesity and possibly metabolic syndrome.

Keywords: Safoof Mohazzil, Obesity, Inflammation, Oxidative stress, Metabolic syndrome

IPC Int. Cl.¹: A61K 36/00, A61F 5/01, A01D 20/25, A01D 4/29, G05D 15/00, G01L 1/00, A01D 7/26

Several reactive oxygen species (ROS) such as superoxide (O²•−, OOH•), hydroxyl (OH•) and peroxyl (ROO•) radicals are produced during the oxidative cascades which are involved in pathogenesis of various diseases, such as cancer, cardiovascular diseases, neurodegenerative disorders, atherosclerosis and cataract¹. Exaggerated inflammatory and oxidative response has been reported in adult as well as pediatric obesity which may lead to unregulated food intake²³. Over-production of ROS caused tissue injury by damaging macromolecules and lipid per-oxidation of cell membrane⁴⁵. Additionally, ROS proliferate inflammation by enhancing release of cytokines such as interleukin-1, tumor necrosis factor-α, and interferon-γ. Free radicals are, therefore, considered as important mediators that aggravate inflammatory processes⁶⁷. Thus, free radical scavengers may be used to ameliorate inflammation.

The use of traditional medicine is increasing day by day due to its low cost and less adverse effects in comparison to modern medicines. Natural sources of antioxidants may play a leading role in the development of novel drugs. One such traditional polyherbal formulation, Safoof Mohazzil (SM), is used by Unani physicians for treatment of obesity. In our previous study, anti-obesity effect of this formulation was reported in rats⁸. The formulation contains nankhawah (Trachyspermum ammi (L.) Sprague syn Ptychotis ajowan DC. seed), marzan josh (Majorana hortensis Moench syn. Origanum majorana L. stem), tukhme badiyan (Foeniculum vulgare Mill. seed), zeera siyah (Carum carvi L. seed), lakh maghsool (Kerria lacca syn Coccus lacca), bura armani (Armenian bole) and Berg sudab (Ruta graveolens L. leaf). A brief account of actions of these components has been provided earlier⁹.

Several medicinal plants in Safoof Mohazzil have antioxidant and anti-inflammatory activity

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which could contribute to its antiobesity activity. *Foeniculum vulgare* Mill. (fennel) is an antioxidant, *Kerria lacca* syn *Coccus lacca* is anti-inflammatory and *Ruta graveolens* L. is both an antioxidant and anti-inflammatory. However, literature lacks the scientific data for anti-oxidant and anti-inflammatory activity of this formulation. The present study was supported by Unani Council, Ministry of Health and Family Welfare, Govt. of India to develop a scientific body of evidence for this formulation.

Considering the traditional uses of this formulation, the present was designed to evaluate the anti-oxidant and anti-inflammatory activity of *Safoof Mohazzil* in rats. The antioxidant effect of *Safoof Mohazzil* was evaluated in pyrogallol induced hepatotoxicity in rats whereas anti-inflammatory activity was evaluated in carrageenan induced paw edema model.

**Materials and methods**

*Animals*—Male rats weighing 220–250 gm were obtained from the Central Animal Facility of All India Institute of Medical Sciences, New Delhi, India. The rats were housed in cages not more than four animals per cage under normal laboratory conditions with natural light-dark cycle. The animals were acclimatized for a week before the experiment with free access to water and standard diet. The study was reviewed and approved by Institutional Animal Ethics Committee (Letter No. 463/08 dated 22.02.09).

*Preparation of formulation*—Each 10 gm of the formulation contained *tukhme badiyan* 2 gm, *nan khawah* 2 gm, *zeera siyah* 2 gm, *berg sudab* 2 gm, *lakh maghsool* 1 gm, *marzan Josh* 0.5 gm and *bura armani* 0.5 gm. The preparation process for the formulation has been described earlier.

*Antioxidant activity of Safoof Mohazzil in pyrogallol induced hepatotoxicity*—Antioxidant activity of *Safoof* was evaluated using pyrogallol-induced hepatotoxicity model in rats. The animals were randomly divided into six groups of six animals each. Group I was normal control where no treatment was given. Group II served as pyrogallol control where vehicle and pyrogallol were administered. Group III-V were administered *Safoof Mohazzil* at the dose of 250, 500 and 1000 mg/kg. *Safoof Mohazzil* was suspended in 0.5% carboxy methyl cellulose solution and administered orally by gavage at volume not greater than 1.0 ml/100 gm body weight daily for 7 days. On 7th day, pyrogallol was injected intraperitoneally at the dose of 100 mg/kg 1 hr after vehicle and *Safoof* administration in group II and groups III–V, respectively. In group VI, only 1000 mg/kg was administered orally to study its *per se* effect. Animals were sacrificed after 1 hr of pyrogallol injection and liver was stored at -80°C for estimation of lipid peroxidation and reduced glutathione.

*Biochemical estimation*—Liver samples were homogenized in 10% ice-cold 0.1 M phosphate buffer (pH-7.4) for the assessment of lipid peroxidation and reduced glutathione.

*Lipid peroxidation levels*—Lipid peroxidation was assessed using malondialdehyde (MDA), as an indicator. To 100 µl of liver homogenate; 1.5 ml of 20% (v/v) acetic acid (pH-3.5), 1.5 ml of 0.8% (w/v) of thiobarbituric acid and 0.2 ml of 8.1% (w/v) of sodium dodecyl sulphate were added and then heated at 95°C for 60 min. After cooling the mixture, 5 ml of n-butanol/pyridine (15:1) was added and vortexed. The organic layer was separated by centrifugation at 4000 rpm for 10 min and its absorbance was measured at 532 nm. Tetra-ethoxy propane was used to prepare the standard curve. The concentration of MDA is expressed in nmol/gm wet tissue.

*Reduced glutathione (GSH) levels*—Glutathione was measured according to the method of Ellman. The homogenate was mixed with equal quantity of 10% trichlororacetic acid and centrifuged to precipitate out the proteins. The reaction mixture contained 100 µl of protein free supernatant, 2 ml of 0.3 M phosphate buffer (pH 8.4), and 0.5 ml of 0.04% DTNB in 1% tri-sodium citrate and 0.4 ml of distilled water. Standard GSH was run with every experiment. The absorbance was read at 412 nm in a spectrophotometer within 15 min. The concentration of GSH is expressed as µg/gm wet tissue.

*Anti-inflammatory activity of Safoof Mohazzil in carrageenan induced paw edema*—Carrageenan induced rat paw edema model was used for the assessment of anti-inflammatory activity of *Safoof*. Animals were divided into 4 groups. Group I served as control group received vehicle orally. Group II was administered indomethacin at a dose of 10 mg/kg, p.o as a standard drug. Group III and IV animals received *Safoof* at a dose of 500 and 1000 mg/kg, p.o. The suspension of *Safoof Mohazzil* was prepared in 0.5% carboxy methyl cellulose solution and administered orally for 7 days. On 7th day, 0.1 ml of 1% w/v carrageenan was injected subcutaneously to the planter surface of the right hind paw 1 hr after *Safoof*
Mohazzil. The paw volume was measured using a plethysmometer at baseline (before carrageenan injection) and 1, 3 and 6 hrs after carrageenan injection. The change in paw volume and percentage inhibition was calculated.

Statistical analysis—Results are expressed as means ± SD. One way analysis of variance (ANOVA) with Bonferroni post hoc test was carried out using SPSS statistical software package version 13.0. A P<0.05 was considered as significant.

Results and discussion

Effect of Safoof Mohazzil on the pyrogallol induced oxidative stress in rats—An imbalance between oxidant and antioxidant system leads to the development of oxidative stress which play an important role in drug toxicity, ischemic damage, cancer, cardiovascular, neurodegenerative and age associated diseases. Therefore, prevention of oxidative stress is important for all biological membranes because of their essential role in cellular physiology and maintenance of homeostasis. Therapeutic potential of medicinal plant used in traditional system of medicine may be attributed to their antioxidant property. Hence, an intense search is being carried out for novel antioxidants. In the present study, the antioxidant potential of Safoof Mohazzil, antiobesity Unani formulation was evaluated in pyrogallol induced hepatotoxicity in rats.

Pyrogallol treatment caused significant increase in MDA and decrease in GSH levels in group II in comparison to group I (p<0.001) indicating oxidative stress. These results are in accordance of previous study in which oxidative stress was reported in pyrogallol treated group. Pretreatment with Unani formulation at the doses of 250, 500 and 1000 mg/kg, p.o significantly prevented the increase in MDA levels in comparison to group II (p<0.001). At the dose of 1000 mg/kg, p.o, the formulation did not cause any significant change in the MDA level in group VI in comparison to group I (Fig. 1). The Unani formulation when administered orally for 14 days at the doses of 250, 500 and 1000 mg/kg, significantly prevented the depletion of GSH as compared to group II which indicate the antioxidant potential of Unani formulation. The formulation maintained the GSH level dose dependently. In group VI, Unani formulation did not cause any significant change in the GSH level in comparison to group I (Fig. 2). Safoof Mohazzil is known to contain several medicinal plants. Therefore, at present, it is difficult to identify the constituent responsible for its antioxidant effect.

Effect of Safoof Mohazzil on the carrageenan induced inflammation in rats—Medicinal plants are considered as store-house of chemical substances used to alleviate all ailments of mankind without or minimal adverse effects. Existing anti-inflammatory drugs have numerous adverse effects which create an urgent need for novel anti-inflammatory agents. Numerous studies have reported the anti-inflammatory activity of plant extracts. In the present study, the anti-inflammatory activity of Safoof Mohazzil, a Unani formulation for treatment of obesity was evaluated in carrageenan induced paw edema in rats.

![Fig. 1](image1.png)

**Fig. 1**—Effect of Safoof Mohazzil on MDA level in rat liver. Data represent mean ± SEM. p<0.001; a – as compared to group 1; b – as compared to group II. (Group I: normal control; Group II: pyrogallol control; Group III: Safoof Mohazzil 250 mg/kg; Group IV: Safoof Mohazzil 500 mg/kg; Group V: Safoof Mohazzil 1000 mg/kg)

![Fig. 2](image2.png)

**Fig. 2**—Effect of Safoof Mohazzil on GSH level in rat liver. Data represent mean ± SEM. p<0.001; a – as compared to group 1; b – as compared to group II. (Group I: normal control; Group II: pyrogallol control; Group III: Safoof Mohazzil 250 mg/kg; Group IV: Safoof Mohazzil 500 mg/kg; Group V: Safoof Mohazzil 1000 mg/kg)
More change in paw volume was observed in carrageenan control group at 1, 3 and 6 hrs as compared to control group indicating inflammatory response (Table 1). These results comply with findings of previous studies.\(^\text{21-22}\) Safoof Mohazzil at dose of 1000 mg/kg, p.o (equivalent to human dose) significantly prevented the change in paw edema in comparison to carrageenan control group. No significant change in paw edema was found when Safoof Mohazzil was administered at dose of 500 mg/kg, p.o for 14 days as compared to carrageenan control group (Table 1). The results showed that Safoof Mohazzil at human equivalent dose exhibited anti-inflammatory activity. No anti-inflammatory activity was found at lower dose of Unani formulation. Therefore, Safoof Mohazzil may be active against diseases conditions where inflammation is involved in the pathophysiology.

**Safoof Mohazzil and obesity**—Obesity has reached epidemic proportions in western world as well as India.\(^\text{23-24}\) It has been associated with a low grade pro-inflammatory state and increased oxidative stress.\(^\text{25}\) Although the cause effect relationship of obesity with inflammation and oxidative stress has not been established, the possible role of medicines with antioxidant and anti-inflammatory activity in treatment of obesity is being researched. Rosiglitazone has been demonstrated to possess anti-inflammatory activity.\(^\text{26}\) Supplementation with fruit/ berry/ vegetable juice extract has been demonstrated to reduce systemic inflammation and oxidative stress in obese women.\(^\text{27}\)

The complexity of obesity pathogenesis has precluded a highly effective treatment without producing limiting adverse effects. Natural sources are being explored for alternatives. We studied one such alternative in traditional system of Unani medicine. The medicine known as Safoof Mohazzil is a formulation containing parts of several parts. It has been in use for a long time for treatment of obesity. We have previously demonstrated its antiobesity activity in rats with diet induced obesity.\(^\text{8}\) In the present study, an attempt was made to assess the possible mechanisms of action of the formulation. Adipocyte, which is the source of proinflammatory cytokines, systemic oxidative stress, insulin resistance and increased leptin levels,\(^\text{28}\) could be the potential site of action for Safoof Mohazzil.

**Conclusion**

The results of the present study demonstrate the antioxidant and anti-inflammatory property of Safoof Mohazzil which further support the traditional use of this formulation for treatment of obesity. Further studies are required to elucidate the mechanism responsible for anti-oxidant and anti-inflammatory property, role of individual ingredients and clinical activity of the formulation.

**Acknowledgement**

The study was funded by Central Council for Research in Unani Medicine (CCRUM), Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Ministry of Health & Family Welfare, Government of India. The Unani formulation was prepared by National Institute of Nutrition, Hyderabad, India for CCRUM. Mr Ritesh Kumar and Ms Shalu Arora assisted in execution of the study.

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