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Evaluation of Systemic Effects to *Pistacia lentiscus* Powder and Tears in Guinea Pigs

Farzana Sadaf[1], Sarah Jameel Khan[1], Sidra Siddiqui[2]*, Sana Siddiqui[2]

[1] Department of Pharmacology, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan.
[2] Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan.

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A B S T R A C T

Gum mastic is the colloquial word for *Pistacia lentiscus L.* (PL). Herbal medicines, dietary supplements, and cosmetics commonly contain its powder (GMP) and tears (GMT). The purpose of this study was to evaluate the sub-acute toxicity of GMT and GMP in guinea pigs given a dose of 0.2857g/kg over a 14-day period. Following the experiment, blood samples were taken in order to evaluate a variety of biochemical markers. The animals were then put to euthanize so that histological examinations of the heart, liver, kidney and spleen could be performed. Gum mastic tears (GMT) or gum mastic powder (GMP) did not cause any harm or mortality in guinea pigs used in a sub-acute toxicity investigation. Histological and macroscopic examinations of the kidney, spleen and heart tissues showed no notable anomalies. The biochemical study found that GMT significantly decreased serum HDL levels ($p < 0.005$) while increasing blood triglycerides ($p < 0.05$), LDL ($p < 0.05$) and the cholesterol/HDL ratio ($p < 0.005$). There was no statistically significant increase in serum cholesterol. On the other hand, after GMP treatment, all lipid profile metrics displayed non-significant changes. GMT and GMP treatments dramatically decreased serum uric acid levels. Three liver enzymes, alkaline phosphatase, γ GT and SGPT, did not change substantially in any group. The results show that both GMT and GMP can be safely taken at the tested dosage since they can heal gout by reducing uric acid. The observed unfavorable changes in the lipid profile suggest that GMT may not be cardio-protective in this animal model. The results confirm the gum mastic powder and tears' safety profile, allowing for their ongoing use in a variety of applications.

Keywords: Gum mastic powder (GMP), Gum mastic tears (GMT), *Pistacia lentiscus*, Sub-acute toxicity, Guinea pig, Histopathology.

Corresponding Author: *Sidra Siddiqui*
Email: sidra.siddiqui@hamdard.edu.pk
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INTRODUCTION

The foundation of traditional healthcare, herbal plants have been used for millennia for their medical, culinary and therapeutic uses. About 80% of people in developing countries still rely on herbal remedies for their basic medical needs (Verma and Singh, 2008). Because they are less likely to cause side effects and toxicity than traditional allopathic treatments, these plants are becoming more and more acknowledged in contemporary drug discovery (Rauf et al., 2017). Mastic or *Pistacia lentiscus*, is a dioecious evergreen shrub that is indigenous to Mediterranean coastal regions, such as Greece, Turkey, Spain and Italy. Because of its strong antimicrobial and antioxidant properties, traditional medicine has used this plant to treat a variety of conditions, including eczema, throat infections and gastrointestinal problems (Rodriguez et al., 2013).

It is mostly grown on the Greek island of Chios and is especially well-known for its fragrant resin. The plant and its extract may provide an alternative source of pharmacoeconomics for the low-cost production of nutraceuticals or nutritional supplements following extensive toxicity testing (Ahmad et al., 2020). Mastic has

been reported to be useful in treating rheumatism and scabies, while the plant's aerial parts have shown diuretic and hypertensive effects (Chekkai et al., 2023). Mastic gum has the potential to be an antiviral agent against influenza A virus (Cho et al. 2021) and has demonstrated bactericidal activity against *Helicobacter pylori* (Marone et al., 2001; Alwadi et al., 2023). The plant's berries are high in anthocyanins, which add to its antioxidant activity and its leaves contain essential oils with antibacterial and antimutagenic qualities (Belhachat et al., 2017). *P. lentiscus* resin has demonstrated anti-inflammatory properties in the treatment of Crohn's disease (Boucheffa et al., 2021). Key components of the essential oil have been found by GC-MS analysis, including spathulenol (17.38%), bicyclogermacrene (12.52%), germacrene D (17.54%) and terpinen-4-ol (9.95%). By preventing leukocyte migration, the essential oil has strong anti-inflammatory effects by reducing paw edema, inflammatory cytokines such as TNF-α and IL-6 and granuloma formation when applied topically (Drioiche et al. 2023).

Terpene-4-ol, α-pinene and β-pinene are all present in the resin itself (Nahida and Siddiqui, 2012). The total medicinal efficacy of the plant is thought to be caused by the combined action of these many compounds (Boucheffa et al., 2021). The plant is a viable natural resource for the culinary and pharmaceutical industries because of its promising chemical makeup and cytotoxic and antiviral qualities (Boke et al., 2024). Although *Pistacia lentiscus* has been used traditionally for a long time, nothing is known about its safety profile. In order to better understand the sub-acute toxicities of gum mastic powder (GMP) and gum mastic tears (GMT), which are generated from the plant's resin, the current study intends to examine the histological, biochemical and toxicological alterations in guinea pigs.

MATERIALS AND METHODS

Experimental details and treatments

Drug

The gum mastic powder and tears were obtained from Pakistan's Hamdard Laboratories (Waqf). Due to its sticky consistency, the powder does not dissolve well in distill water and instead sticks to the spatula when it is dissolved. Tears are off-white crystals found in nature. Both the powder and the tears, when partially dissolved in 10% Tween 80, yield a heterogeneous solution. The powder does not dissolve well in distillation water because of its sticky nature. Therefore, Tween 80 had to be used to make a suspension that could be taken orally. The study used a dosage of 0.2857 g/kg, ten times the usual human dose, to guarantee a reliable assessment. This dosage was chosen in order to detect any possible harmful effects and to give an adequate safety buffer. The reliability and international comparability of the results were guaranteed by the study's rigorous adherence to the chemical testing procedures set by the Organization for Economic Co-operation and Development (OECD) and the World Health Organization (WHO; OECD, 2008).

Animals

The Dr. HMI Institute of Pharmacology and Herbal Sciences offered guinea pigs of the Mongrel breed weighing between 450 and 650 g. This study is not sex-based, and both sexes were included. The animals were kept in individual cages with standard lighting (12 hours of day and night cycles) and temperature settings (22 ± 0.50 C). They had unlimited access to tap water and food throughout the experiment (NRC 2006; NRC 2010).

Experimental Protocol

Studies on the Safety Assessment of Gum Mastic Powder and Tears

(a) Guinea pigs exposed to sub-acute toxicity

In conducting this inquiry, the World Health Organization (Boukeloua *et al*., 2012) and the Organization for Economic Co-Operation and Development (OECD, 2008) adhered to guideline 407 for chemical testing. A set of thirty healthy guinea pigs was split up into A, B and C. Groups B and C received treatment, while Group A served as the control group. 10% tween 80 was given orally to the control group. On the other hand, for 14 days, the treatment groups had gum mastic powder (0.2857g/kg) and gum mastic tears (0.2857g/kg), respectively, once daily. Every day before oral treatment, the weight of each animal was measured, allowing the dose to be determined based on that weight. The animals were observed for two hours following the dose administration to look for any changes in behavior or appearance. (Paraschos *et al*., 2012).

(b) Biochemical analysis on Guinea pigs

At the end of the study, chloroform was administered to all of the animals, as it administered by inhalation after 24 hours of the last dose of treatment and the blood samples were drawn approximately (5ml to 8ml) directly from cardiac puncture with sterile disposable syringes. Serum was centrifuged for 15 minutes at 1500–2000 rpm using a centrifuge machine (Model 80–2, No. 02561, Changzhou Guohua Electric Appliance CO. Ltd, China). The kits were purchased from Diagnostica Merck, a German enterprise. Utilizing a Hitachi U-2000 spectrophotometer, the amounts of triglycerides, Creatinine, Glucose, Urea, Uric acid, SGPT, γ GT, Alkaline Phosphatase, total serum Cholesterol, HDL, LDL and CHOL/HDL Ratio were estimated.

(c) Autopsy

The hearts, livers, spleens and kidneys of recently sacrificed animals were removed for tissue analysis, blotted and then immediately assessed on an advanced electronic balance (Sartorius BP- 211- D).

(d) Histopathological study

Samples of the kidney, spleen, heart and liver were taken immediately after blood withdrawal. After that, the organs were preserved, dried out, washed and imbedded in a mixture of paraffin wax, xylene, graded (80–100%) alcohol and 10% neutral formalin. Hematoxylin and eosin were used to stain the 4-5 µm thick slices that were created using a rotatory microtome (Leica RM 2145). After xylene was used to deparaffinize the sections, they were passed through 80% to 100% alcohol. In the end, a Nikon Advanced Research Microscope (OPTIPHOT Model X2T-21E) equipped with a Nikon Microphotography system (UFX-DX-35) was used to evaluate and take pictures of the tissues.

Statistical analysis

The statistical analysis was performed using the student's t-test. The significance criterion was set at $p < 0.05$, and the results are reported as mean ± SEM.

RESULTS

Toxicological Study of GMP and GMT on Guinea pigs

When guinea pigs were given GMP and GMT (0.285g/kg), they did not exhibit any signs of toxicity or death, and their weight did not significantly increase when compared to the control group (Table 1).

Autopsy

According to Table 1, no discernible changes were found in the heart, spleen, right kidney, left kidney when compared to the control group.

Research of GMP and GMT Biochemistry in Guinea Pigs

The following parameters were examined in Guinea pig serum to determine the impact of GMT and GMP, as indicated in Table 3. The GMT resulted in a substantial drop in the serum HDL level ($p<0.005$) but an increase in blood triglycerides ($p<0.05$), LDL (p, 0.05), and the cholesterol/HDL ratio ($p<0.005$) at a dose of 0.285g/kg. Non-significant increased observed in serum cholesterol level when treated with 0.285g/kg GMT. Yet, when treated with GMP, there was a non-significant increase in triglycerides and the ratio of cholesterol to HDL, as well as a non-significant drop in the levels of LDL, HDL, and cholesterol.

Table 1: GMP and GMT toxicological study after 14 days of treatment in guinea pigs.

Protocols	Tween 80 control: 10%	GMP (g/kg= 0.285)	GMT (g/kg = 0.285)
Administration Route	Orally	Orally	Orally
Weight of body (g)	497.42±27.11	489.01±13.57	498.33±15.26
Weight of Liver (g)	16.118±1.203	16.58±1.432	14.59±0.895
Weight of Heart (g)	1.52±0.08	1.638±0.123	1.588±0.09
Weight of Kidney(R) (g)	2.039±0.123	1.81±0.06	1.95±0.085
Weight of Kidney(L) (g)	2.113±0.169	1.857±0.049	1.946±0.087
Spleen weight (g)	0.601±0.051	0.53±0.023	0.598±0.032
Death rate	Nil	Nil	Nil
Deleterious consequences	Nil	Nil	Nil

The data shows the mean ± standard error for saline control & oral treatment (GMP & GMT 0.285g/kg) rats (n = 6), with *p<0.05.

Table 2: Guinea pigs treated for fourteen days; biochemical analysis of GMT and GMP.

Biochemicals (mg/dl)	Control	Powder of Gum Mastic	Tears of Gum Mastic
Urea	24.99±5.47	22.80±13.02	26.99±7.31
Triglycerides	212.77±6.39	225.65±7.50	228.22±5.60*
HDL	54.75±2.39	50.26±2.45	45.47±1.50**
LDL	23.538±3.497	21.683±7.029	36.566±6.539*
Creatinine	0.79±0.11	1.10±0.22	0.87±0.24
Uric acid	4.85±0.14	4.25±0.11**	4.26±0.11**
SGPT	376.41±17.52	345.44±22.25	370.98±15.05
HDL /CHOL Ratio	2.232±0.09	2.359±0.163	2.828±0.828**
Cholesterol	120.84±3.45	117.07±5.48	127.68±5.70
Gamma GT	214.68±6.41	223.30±16.26	208.32±6.29

$AlkPO_4$	1136.1±31.26	1144.15±46.34	1081.04±37.83
Glucose	94.44±4.6	94.13±7.14	96.26±10.09

*p < 0.05, **p < 0.005 for the mean ± SEM in the group of six oral-treated (GMP and GMT 0.285g/kg) and control (saline) pigs

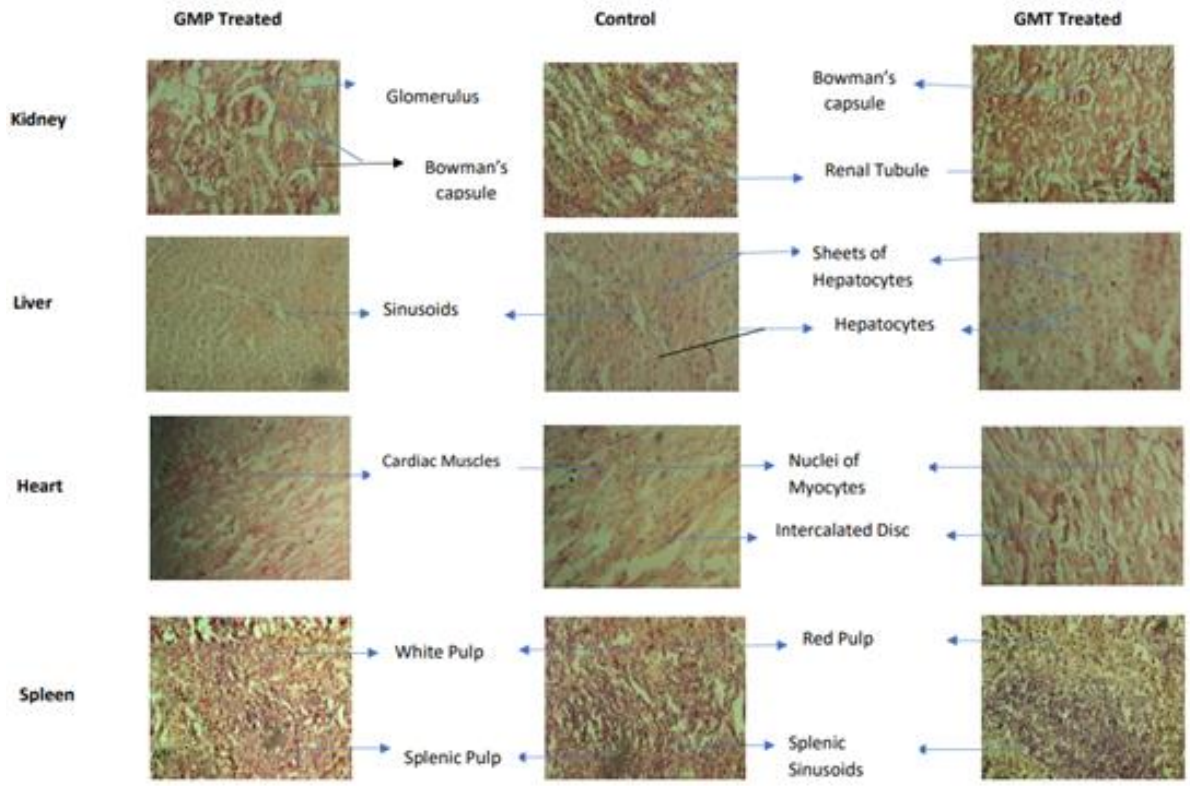

Figure 1: Histological profile of the kidney, liver, heart and spleen in rats treated with GMT, GMP and control

Serum creatinine increased non-significantly and when GMP (0.285g/kg) was used, serum urea reduced non-significantly however, when GMT (0.285g/kg) was used, the urea value elevated. Additionally, serum uric acid was dramatically lowered by the same dosages of GMT and GMP. There was a non-significant increase in γ GT and Alkaline phosphatase, as well as a non-significant drop in serum SGPT and glucose in guinea pigs when treated with 0.285g/kg of GMP. There is non-significant decrease in serum SGPT, alkaline phosphatase and γ GT enzymes in guinea pigs treated with GMT and serum glucose is non-significantly increased.

DISCUSSION

The purpose of the current study was to assess the biochemical effects and sub-acute toxicity of *Pistacia lentiscus* powder (GMP) and tears (GMT) in guinea pigs. According to the findings, there were no symptoms of toxicity or fatality after 14 days of administering both GMT and GMP at a level of 0.285g/kg. The safety profile of these compounds at the tested dose was further supported by macroscopic and histological examination of the kidney, spleen and heart, which revealed no appreciable morphological alterations in these essential organs.

The main finding of this study relates to the effects of GMT and GMP on various biochemical parameters. The most significant result was that both GMT and GMP treatment reduced serum uric acid levels. This finding is in line with the plant's well-known anti-inflammatory properties documented in previous studies and supports *Pistacia lentiscus'* potential as a treatment for conditions including gout and arthritis (Boudieb et al., 2019; Maxia et al., 2011). The non-significant increases in serum creatinine and urea further confirm this, indicating that renal toxicity is not associated with the positive effects on uric acid metabolism.

On the other hand, the impact on lipid profiles revealed a multifaceted, species-specific reaction. Serum HDL significantly decreased whereas triglycerides, LDL and the

cholesterol/HDL ratio significantly increased as a result of GMT treatment. These findings are not consistent with those of other research, such as those conducted by Kannt et al. (2019) and Zitouni et al. (2023), who discovered that mastic supplementation enhanced lipid profiles and hepatic steatosis in various animal models. This disparity suggests a possible species variation in the way *P. lentiscus* influences lipid metabolism, as do the non-significant alterations seen in the GMP group. The overall low toxicity of GMT and GMP is further supported by the non-significant increases in serum glucose levels and liver enzymes (SGPT, γ GT and alkaline phosphatase) for both regimens. This suggests that these compounds do not seriously harm the liver or interfere with glucose homeostasis in guinea pigs when given at the recommended dosage. The results of the study provide important information about *Pistacia lentiscus's* pharmacological activity and safety. The study effectively proved that there was no sub-acute toxicity in guinea pigs at a level that was therapeutically significant. Its effectiveness in treating gout and related inflammatory disorders is demonstrated by the notable decrease in uric acid levels. To comprehend the underlying mechanisms and species-specific variations, more investigation is required to address the unanticipated and undesirable alterations in the lipid profile brought on by GMT. To completely describe the cardio protective function of *P. lentiscus* in other species, future research should concentrate on examining this differential effect. As seen in figures 1, the histopathological investigation reveals no appreciable alteration in the kidney, liver, heart or spleen's cell morphology. The toxicological study in guinea pigs of 0.2587g/kg of GMP and GMT (10 times higher than human dose) showed no indications of harm or death. The guinea pigs that were treated daily with 0.285g/kg of GMP and GMT were also subjected to tissue analysis. When compared to the corresponding control group, the results showed no appreciable changes in the dried weight of the liver, heart, kidney or spleen. When given orally, GMT significantly increased the levels of serum LDL ($p<0.05$), triglycerides ($p<0.05$), and the cholesterol/HDL ratio ($p<0.005$), but it significantly decreased the levels of serum HDL ($p<0.005$) (Table 2, Figure 1). In contrast, the lipid profile values in rats significantly decreased, suggesting a species difference. The significant decrease in uric acid concentration, when treated with GMP ($p< 0.005$) and GMT ($p< 0.005$) revealed that it may be used in the treatment of arthritis and gout (Table- 2). The histopathological examination of GMP and GMT treated guinea pigs at the doses of 0.285g/kg exhibited regular structure and absence of any gross pathological abrasion in organs including kidney, liver, heart and spleen in comparison with their respective saline treated group (Figure 1). Various histopathological features in normal and treated group were similar like in case of kidney, the renal corpuscles appeared normal with condensed smoothed structure of glomeruli, enclosed by fine Bowman's spaces. The slides of liver displayed the Sinusoids which are drifting between hepatocytes. Within each plate, the hepatocytes radiate outwards from a central vein. Dense connective tissue with elastic fibers is present in the cardiac tissue. The cardiomyocytes are straited. They are branched, contain intercalated disks and are usually mononucleated. The parenchyma of the spleen is divided into two functionally and morphologically distinct compartments (red pulp and white pulp) divided by a tissue layer called the marginal zone. The reticular connective tissue supports the red pulp, which makes up the majority of the spleen's stromal tissue, while the white pulp is made up of the marginal zone, lymphoid follicles and the periarterial lymphoid sheath (PALS).

CONCLUSION

One prevalent and painful type of inflammatory arthritis is gout. Only one joint typically the big toe joint is affected at a time. Remission is characterized by periods with no symptoms, while flare-ups are characterized by periods of increased severity. Gouty arthritis is a degenerative kind of arthritis that can result from recurrent bouts of gout. Gout can be efficiently managed and treated using medicine and self-care techniques, despite the fact that there is no cure for it. Although present study on *Pistacia lentiscus* encouraging, the results are based on a sub-acute toxicity study in guinea pigs and more long-term research and clinical trials are required to validate GMT's and GMP's safety and effectiveness for human use.

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AUTHOR CONTRIBUTIONS

FS planned the experiments, FS and SS interpreted the results, SJ, SS and SS made the write up and FS and SS statistically analyzed the data and made illustrations.

CONFLICT OF INTEREST

All authors declare no conflict of interest

DATA AVAILABILITY

Data presented in this study will be available on a fair request to the corresponding author.

ETHICS APPROVAL

The Hamdard University Ethical Review Board HU-ERB granted its approval for the handling of laboratory animals (Ref No. AEC16-02).

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