RESEARCH ARTICLE

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Effects of Csyera Oil and Nutgrass Oil on Liver Function and Histopathology in Heat-Stressed Local Male Rabbits

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Abstract

Objective: To evaluate the protective effects of Csyera oil (gum tragacanth; derived from Astragalus gummifer) and nutgrass oil (Cyperus rotundus), administered individually or in combination, on body weight and liver function in heat-stressed male rabbits. **Methods:** Twenty-four local male rabbits were randomly assigned to four equal groups (n = 6 per group). The control group received 0.2 ml normal saline orally. The second group received Csyera oil (0.2 ml/kg body weight), the third group received nutgrass oil (0.2 ml/kg), and the fourth group received a combination of both oils (0.1 ml/kg each). All treatments were administered orally for 15 consecutive days under heat stress conditions. Body weight was recorded, and liver function was evaluated by measuring serum aspartate transaminase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA). Histopathological examination of liver tissue was also performed. **Results:** The combination treatment significantly increased body weight compared with the control and single-treatment groups ($P < 0.01$). Serum AST, ALT, and MDA levels were significantly reduced in the combined-treatment group compared with all other groups ($P < 0.01$), indicating improved liver function and reduced oxidative stress. Rabbits treated with Csyera oil alone showed mild inflammatory infiltration and vascular dilatation, whereas those treated with nutgrass oil alone exhibited marked collagen fiber proliferation and replacement of hepatic tissue. In contrast, the combined-treatment group demonstrated near-normal hepatic architecture with minimal pathological alterations. **Conclusion:** Combined oral administration of Csyera oil and nutgrass oil at 0.1 ml/kg each was more effective in protecting liver function and reducing heat stress–induced oxidative damage than either oil administered alone at 0.2 ml/kg.

Keywords: Liver- Csyera oil- Nutgrass oil- Aspartate transaminase enzyme (AST)- Heat stress- Rabbits

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Introduction

Csyera oil, also known as gum tragacanth oil, is derived from the tragacanth plant, which belongs to the largest group of flowering plants in the Leguminosae family and is predominantly found in Western Asia (Akhondzadeh, 2001). Several species, including *Astragalus tragacantha, A. gummifer*, and *A. adscendens*, are known to produce this gum, with more than 20 Astragalus taxa reported to yield it. Gum tragacanth is widely used as a suspending agent in pharmaceuticals, cosmetics, and food products [1]. In modern medicine, it has been attributed with antiviral and antibacterial properties [2, 3]. Csyera oil contains a variety of bioactive compounds, including polyphenols, flavonoids, tannins, and polysaccharides, particularly poly-D-galacturonic acid and bassorin [4]. *Cyperus rotundus*, commonly known as purple nutsedge or nutgrass, belongs to the family Cyperaceae [5]. It is considered one of the world's most invasive weeds and is native to India [6]. The plant grows in small clumps reaching up to 100 cm in height and spreads widely due to its adaptability to various soil types, temperatures, altitudes, pH levels, and moisture conditions [7]. It has a broad range of medicinal and pharmacological applications [8, 9]. In Ayurvedic medicine, the rhizomes of *C. rotundus* are recognized for their astringent, diuretic, diaphoretic, analgesic, aromatic, emmenagogue, anti-inflammatory, and antioxidant properties [10-13]. Phytochemical studies have confirmed the presence of alkaloids, flavonoids, tannins, starch, glycosides, furochromones, and several novel sesquiterpenoids in *C. rotundus* [14-17]. Keeping in view the pharmacological potential of both csyera and nutgrass, the current study was designed to evaluate the the effects of csyera oil and nutgrass oil individually and in combine form on body weight and liver function in heat-stressed male rabbits.

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Materials and Methods

Animals

All experimental procedures involving animals were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Al-Mustaqbal University. A total of 24 local male rabbits (aged 2–3 years), weighing between 1376–1379 grams, were used in this study. The rabbits were obtained from local markets and housed under standard hygienic laboratory conditions. They had free access to food and water and were monitored daily for health and welfare. All efforts were made to minimize animal suffering, and the minimum number of animals required for statistical validity was used.

Experimental Design

The study was conducted in the Department of Veterinary Medicine, College of Public Health, Al-Mustaqbal University, starting in October 2022. Before the experiment, all rabbits were given a 7-day acclimatization period. The animals were randomly divided into four groups, with six rabbits per group (n = 6). The experimental treatments involved the use of two different natural oils: csyera oil (gum tragacanth oil) and nutgrass oil (*Cyperus rotundus*). The oils were administered via oral gavage (stomach tube) at the following doses:

• Group 1 (G1, Control): received 0.2 ml of normal saline

• Group 2 (G2): received csyera oil at 0.2 ml/kg body weight

• Group 3 (G3): received nutgrass oil at 0.2 ml/kg body weight

• Group 4 (G4): received a combination of csyera oil (0.1 ml/kg BW) and nutgrass oil (0.1 ml/kg BW)

All oils were 100% pure and obtained from Imad Company for Vegetable Oils, Mosul. The treatments were administered once daily for 15 consecutive days. Each treatment was replicated across six animals per group, and all dosing was performed carefully to ensure accurate delivery and minimal discomfort. The rabbits were administered oral treatments once daily for a period of 15 days using a gastrointestinal (oral) tube. Throughout the experimental period, the animals were fed a standard pellet diet and provided with free access to clean drinking water. The animals were housed at a room temperature of 32–35 °C, which induced heat stress in the rabbits, with relative humidity maintained at 55–60%. The animal facility was cleaned and sanitized weekly to maintain hygienic conditions.

Laboratory analyses

Body weights of all rabbits were recorded at the beginning and end of the experiment using a digital electronic balance to assess any weight changes due to treatment. At the end of the study, the animals were fasted for 10 hours prior to sample collection. Diethyl ether was used to anesthetize the rabbits. Blood samples were collected via cardiac puncture and placed into non-heparinized tubes. The samples were then centrifuged at 4000 rpm for 10 minutes [18], and the separated serum was collected using a micropipette. The serum was analyzed to determine levels of aspartate transaminase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA) using standard biochemical assay kits.

Histopathological Examination

liver tissue samples were collected from rabbits in each group immediately after sacrifice. The tissues were rinsed with normal saline to remove residual blood and then fixed in 10% neutral buffered formalin for 24 hours. Following fixation, the tissues were dehydrated in graded concentrations of alcohol, cleared in xylene, and then embedded in paraffin wax.

Paraffin blocks were sectioned at a thickness of 5 µm using a rotary microtome. The sections were then processed through alcohol solutions, stained with hematoxylin and eosin (H&E), and cleared in xylene. The stained slides were examined microscopically for histopathological alterations, following the standard procedures described by [19].

Statistical Analysis

The statistical analysis of the experimental data was performed using a completely randomized design (CRD), to evaluate the effects of different treatments on the measured parameters in local male rabbits. The data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatment groups. To compare the means, Duncan's Multiple Range Test was applied at two levels of significance ($p < 0.05$ and $p < 0.01$) to assess statistically significant differences between group means [20]. All statistical analyses were conducted using the Statistical Analysis System [21] software. It was assumed that the experimental errors were normally and independently distributed, with a mean of zero and a constant variance (σ^2_e).

Results

Body Weight

Body weight changes (g/day) are presented in Table 1.

Table 1. Effect of Csyera (CSY) Oil and/or Nutgrass (NUT) Oil on Initial and Final Body Weight (mean ± SE) of Local Male Rabbits

Parameters	G1	G2	G3	G4
Initial body weight (g)	1372.43 ± 1.51^{a}	1372.95 ± 1.72^{a}	1374.44 ± 1.82^{a}	1374.36 ± 1.84^{a}
Final body weight (g)	1211.49 ± 2.96^{a}	1241.30 ± 2.43^{ab}	1244.52 ± 1.67^{ab}	1264.00 ± 2.45^{b}

Different superscript letters within the same row indicate statistically significant differences between groups ($p < 0.05$). Values are expressed as mean ± standard error (SE). G1: Control group (received 0.2 ml normal saline orally); G2: Received Csyera (CSY) oil at 0.2 ml/kg body weight orally; G3: Received Nutgrass (NUT) oil at 0.2 ml/kg body weight orally; G4: Received a combination of Csyera + Nutgrass oils at 0.1 ml/kg each orally

Table 2. Effect of CSY oil and/or NUT Oil on Aspartate Transaminase (AST), Alanine Aminotransferase (ALT), and Malondialdehyde (MDA) Levels (mg/dl) in Local Male Rabbits (mean ± SE)

Parameter	G1	G2	G3	G4
AST (mg/dl)	49.31 ± 0.60[a]	33.28 ± 2.32[ab]	32.60 ± 2.33ab	30.61 ± 2.32b
ALT (mg/dl)	40.51 ± 0.23[a]	28.02 ± 0.25[b]	26.20 ± 0.31c	24.98 ± 0.20d
MDA (mg/dl)	4.56 ± 0.22[a]	3.69 ± 0.14c	3.83 ± 0.20b	2.77 ± 0.27d

Note: Different superscript letters within the same row indicate statistically significant differences between groups ($p < 0.01$). Values are expressed as mean ± standard error (SE). G1: Control group (received 0.2 ml normal saline orally); G2: Received Csyera (CSY) oil at 0.2 ml/kg body weight orally; G3: Received Nutgrass (NUT) oil at 0.2 ml/kg body weight orally; G4: Received a combination of Csyera + Nutgrass oils at 0.1 ml/kg each orally

A significant increase ($p < 0.05$) in body weight was observed in Group 4 (G4), which recorded a final weight of 1264.00 ± 2.45 g, compared to Group 1 (G1), which had a final weight of 1211.49 ± 2.96 g.

Biochemical Parameters

The results of AST levels (mg/dl) are shown in Table 2. There was a highly significant decrease ($p < 0.01$) in AST levels in G4 (30.61 ± 2.32 U/L) compared to G1 (49.31 ± 0.60 U/L), G2 (33.28 ± 2.32 U/L), and G3 (32.60 ± 2.33 U/L).

Similarly, the results for ALT levels (mg/dl), also shown in Table 2, revealed a significant reduction ($p < 0.01$) in G4, with a level of 24.98 ± 0.20 U/L, in comparison to G1 (40.51 ± 0.23 U/L), G2 (28.02 ± 0.25 U/L), and G3 (26.20 ± 0.31 U/L).

For MDA levels (U/L), a marker of oxidative stress, a significant decrease ($p < 0.01$) was observed in G4 (2.77 ± 0.27 U/L) when compared to G1 (4.56 ± 0.22 U/L), G2 (3.69 ± 0.14 U/L), and G3 (3.83 ± 0.20 U/L), as shown in Table 2.

Histopathological analysis

Histological examination of liver tissues using hematoxylin and eosin (H&E) staining at 400X magnification revealed notable differences among the experimental groups. In the control group (G1), the liver sections exhibited severe coagulative necrosis, periportal mononuclear cell infiltration, and an increased mitotic rate, indicating significant hepatic damage and cellular stress (Figure 1). Rabbits treated with csyera oil (0.2 ml/kg B.W.) for 15 days (G2) showed mild inflammatory cell infiltration, along with dilatation of the portal vein and hepatic sinusoids, suggesting a partial protective effect of the oil on liver architecture (Figure 2). In the group treated with nutgrass oil (0.2 ml/kg B.W.) for 15 days (G3), the liver tissues displayed marked proliferation of collagen fibers, which had replaced large portions of the hepatocyte population. This indicates significant fibrotic changes and hepatic degeneration (Figure 3). Rabbits receiving the combined treatment of csyera oil (0.1 ml/kg) and nutgrass oil (0.1 ml/kg) (G4) showed near-normal liver architecture, with minimal histopathological changes. There was a clear reduction in necrosis, inflammation, and fibrosis compared to the other groups, suggesting a synergistic hepatoprotective effect of the combined treatment (Figure 4).

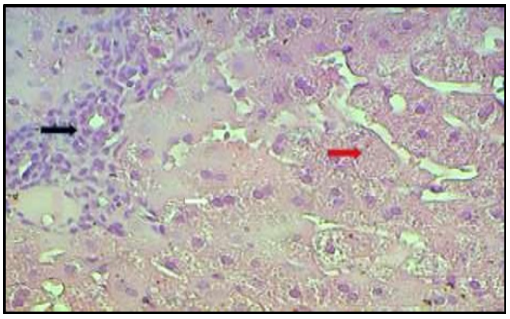

Figure 1. Crosse Section of Liver of Rabbits Control Group Showing Sever Coagulative Necrosis (red arrow), with periportal momonuclear cells infiltration (black arrow), also increase in the mitotic figure (white arrow). Stained with H&E stain, sectioned 40X.

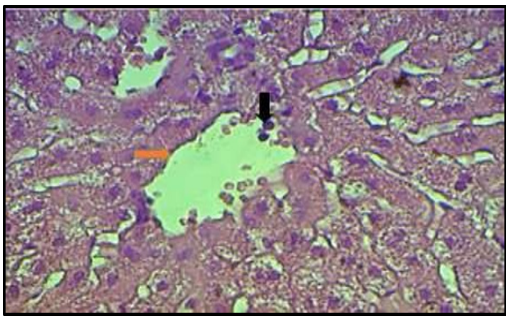

Figure 2. Cross Section of Liver of Rabbit Treated with CYS Oil Concentration of 0.2ml Showing Dilation of the Portal Vein and Sinusoids (red arrow), with slight infiltration of inflammatory cells (black arrow). Stained with H&E stain, sectioned 40X.

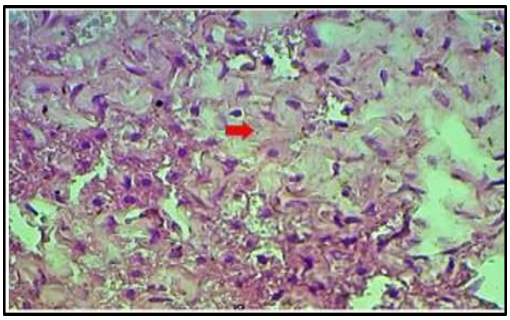

Figure 3. Cross Portion of the Rabbit's Liver Treated with NUT Showing Marked Collagen Fibers Proliferation that Replacement most of the Hepatocyte (red arrow). Stained with, The H&E stain sectioned 40X.

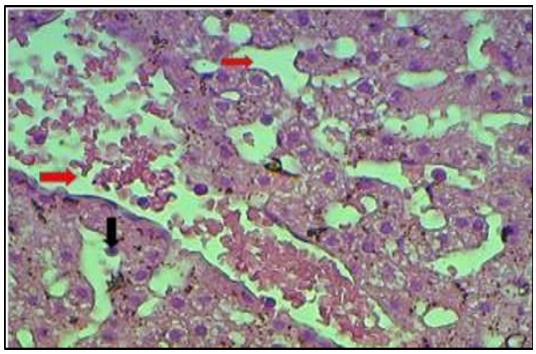

Figure 4. Cross a Portion of Liver of Rabbit CYS + NUT oil concentration of 0.1ml showing marked dilation and congestion of the central vein and sinusoids (red arrow), with mild inflammatory cells infiltration (black arrow). Stained with H&E stain, 400X.

Discussion

The present study demonstrated that supplementation with csyera (CSY) oil, nutgrass (NUT) oil, and their combination exerted a protective effect on liver function in rabbits exposed to heat stress. This was evidenced by increased body weight gain, a significant reduction in liver enzymes AST and ALT and lower levels of MDA, a marker of lipid peroxidation (LPO). Furthermore, histopathological analysis revealed improved liver architecture in treated groups, particularly in the combination treatment group, which showed minimal signs of inflammation, necrosis, and fibrosis compared to the control. In a similar study conducted by [22], *C. rotundus* methanolic extract exhibited hepatoprotective effects in mice by reducing MDA levels and enhancing catalase activity, although it also increased liver enzymes (ALT, AST, and ALP). The extract's interaction with glucophage further modulated oxidative stress markers and altered liver histology. Similar results were also reported by [23, 24] in their study.

The elevated levels of AST, ALT, and MDA observed in the untreated heat-stressed group indicate liver toxicity, oxidative stress, and increased lipid peroxidation, which are consistent with previous reports on the impact of hyperthermia-induced oxidative imbalance [25]. Oxidative stress occurs when there is an imbalance between oxidants and antioxidants in biological systems, leading to cellular damage [26-28]. The antioxidant potential of natural oils like CSY and NUT may counteract this imbalance by enhancing both enzymatic and non-enzymatic antioxidant defenses.

Although not directly used in this study, the hepatoprotective role of sesame oil (SO) has been well documented; it is known to reduce blood pressure, lipid peroxidation, and to enhance antioxidant capacity, making it a relevant reference for the antioxidant potential of similar plant-based oils [29].

Our findings are consistent with those of [13, 30], who reported that natural oils exhibit hepatoprotective activity under oxidative stress conditions. Similarly, [31-33] highlighted the antioxidant and anti-inflammatory effects of nutgrass (*C. rotundus*) oil, which contributed to hepatic protection in rodent models. Furthermore showed that combination therapy involving herbal oils and antioxidant vitamins was more effective than monotherapy in mitigating heat-induced liver damage, supporting our observation that the combined administration of CSY and NUT oils produced superior outcomes in terms of biochemical and histopathological improvements.

In conclusion, the combined administration of csyera oil and nutgrass oil each at a dose of 0.1 ml/kg body weight resulted in more positive outcomes than the individual higher doses of 0.2 ml/kg body weight, particularly in reducing heat stress and protecting liver function in local male rabbits. This suggests that these oils have synergistic potential, and that moderate dosing may optimize their antioxidant and anti-inflammatory effects—offering a promising natural approach for managing heat stress and protecting liver function.

Author Contribution Statement

All authors contributed equally in this study.

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Novelty Statement

This study investigated the combined hepatoprotective effects of csyera (gum tragacanth) oil and nutgrass (*Cyperus rotundus*) oil in local male rabbits under heat stress conditions. It uniquely demonstrates that a moderate combined dose (0.1 ml/kg B.W. each) exerts superior antioxidant and anti-inflammatory effects compared to higher doses, highlighting a synergistic interaction between the two oils. The findings offer a novel, natural strategy for protecting liver function and managing oxidative stress in heat-exposed animals.

Generative AI and AI-assisted technology statement

During the preparation of this article the author(s) used ChatGPT in order to paraphrase the initially written draft to reduce the similarity index, improve the language and readability of the manuscript. The author(s) reviewed and revised the content generated using this tool/service as necessary and assume full responsibility for the final content of the publication.

Conflict of interest

The authors have declared no conflict of interest.

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