

Ameliorating effect of Csyera and Nutgrass Oil on renal histopathology of male rabbits exposed to heat stress

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Abstract

This study evaluated the effects of Csyera oil and nutgrass oil alone or in combination on body weight, renal function and histopathological changes in the kidney in male local rabbits exposed to heat stress. Twenty-four male rabbits were randomly divided into four groups (n=6). Control group received normal saline (0.2 ml/kg), Csyera oil group (0.2 ml/kg), nutgrass oil group (0.2 ml/kg) and combined treatment group received both oils (0.1 ml/kg each) for 12 days. Co-administration of Csyera and nutgrass oils at 0.1 ml/kg has resulted in significant increase in body weight ($P < 0.05$) and a marked reduction in blood urea nitrogen levels ($P < 0.01$) with decreases in Creatinine and Malondialdehyde (MDA) concentrations. These improvements were more pronounced than with the higher single doses of oil. Histopathological examination revealed thickening of renal capsule in control group, glomerular cell vacuolation and partial loss of Bowman's space in Csyera oil group and interstitial fibrosis with infiltration of inflammatory cells in nutgrass oil group. In the combined treatment group, there was obvious renal protection, with little shedding of tubular epithelium and better preservation of renal architecture. In general, combined administration of Csyera and nutgrass oils at lower doses showed better protective and restorative effects against heat induced renal damage than individual treatments.

Keywords: Kidney, Csyera oil, Nutgrass oil, Heat stress, Blood urea nitrogen, Rabbits.

خلاصة:

قيمت هذه الدراسة تأثير زيت الكتيرا وزيت السعد، عند إعطائهما منفردين أو مجتمعين، على وزن الجسم ووظائف الكلى والنسيج الكلوي لدى ذكور الأرانب المحلية المعرضة للإجهاد الحراري. تم توزيع 24 أرنبًا عشوائيًا على أربع مجموعات (عدد = 6): مجموعة سيطرة تلقت محلول ملحي عادي (0.2 مل/كغ)، ومجموعة زيت الكتيرا (0.2 مل/كغ)، ومجموعة زيت السعد (0.2 مل/كغ)، ومجموعة علاجية مشتركة تلقت كلا الزيتين (0.1 مل/كغ لكل منهما) لمدة 12 يومًا. أدى إعطاء زيت الكتيرا وزيت السعد معًا بجرعة 0.1 مل/كغ إلى زيادة ملحوظة في وزن الجسم ($P < 0.05$) وانخفاض كبير في مستويات نيتروجين اليوريا في الدم ($P < 0.01$)، بالإضافة إلى انخفاض في تركيز الكرياتينين والمالونديالدهيد. وكانت هذه التحسينات أكثر وضوحًا من تلك التي لوحظت مع الجرعات الأعلى من كل زيت على حدة. كشف الفحص النسيجي المرضي عن زيادة في سماكة محفظة الكلى في المجموعة الضابطة، وتكون فجوات في خلايا الكبيبات وفقدان جزئي لحيز بومان في مجموعة زيت الكتيرا، وتليف خلالي مع ارتشاح للخلايا الالتهابية في مجموعة زيت السعد. في المقابل، أظهرت مجموعة العلاج المركب دليلًا واضحًا على حماية الكلى، حيث لوحظ تساقط طفيف للخلايا الظهارية الأنبوبية وتحسن في الحفاظ على بنية الكلى. وبشكل عام، أظهر الاستخدام المشترك لزيتي الكتيرا والسعد بجرعات منخفضة تأثيرات وقائية وعلاجية فائقة ضد تلف الكلى الناتج عن الحرارة مقارنةً بالعلاجات الفردية. الكلمات المفتاحية: التهاب الأذن الوسطى، مقاومة المضادات الحيوية، محافظة ذي قار، تحديد البكتيريا.

الكلمات المفتاحية : الكلى، زيت الكتيرا، زيت نبات النوتغراس، الإجهاد الحراري، نيتروجين اليوريا في الدم، الأرانب.

Introduction:

Gum tragacanth oil (also called Csyera oil) is obtained from the tragacanth plant, which is a member of the largest genus of flowering plants in the family Leguminosae, mainly found in Western Asia [1]. A number of Astragalus species like A. tragacantha, A. gummifer and A. adscendens are identified as gum sources and more than 20 gum producing species are recorded [10]. Gum tragacanth is widely used in pharmaceutical formulations as

suspending agent, cosmetic products and food applications [9]. Recent studies have reported antiviral and antibacterial activities of tragacanth [22]. Csyera oil contains several bioactive constituents such as polyphenols, flavonoids, tannins and polysaccharides and poly-D-galacturonic acid has been identified as a major component. Cyperus rotundus is a member of the Cyperaceae family and is commonly known as a nutgrass or purple nutsedge [6], [21]. It is endemic to India, but it

is considered one of the most invasive and destructive weeds in the world [11]. It has a wide distribution because of its excellent adaptability to various environmental conditions such as temperature, altitude, soil pH and moisture. The plant normally grows in dense clusters and can reach heights of 100 cm [24]. *C. rotundus* has been used in traditional medicine for a long time and modern pharmacology [5]. In Ayurvedic medicine, its rhizomes have been reported to have astringent, diuretic, diaphoretic, analgesic, aromatic, emmenagogue, anti-inflammatory and anti-oxidant activities [7], [14]. Previous phytochemical studies on *C. rotundus* have revealed several bioactive compounds such as sesquiterpenoids, alkaloids, flavonoids, tannins, starch, glycosides and furanochromones [12].

Materials and methods:

The study was conducted for a period of 12 days starting from October at the Department of Public Health, Faculty of Veterinary Medicine, Al Qassim Green University. Twenty four healthy male rabbits of local breeds with an average body weight of 1376-1379 g were used. Animals were purchased from the local markets and randomly divided into four experimental groups, with six rabbits in each group. All animals were acclimatized for a period of one week before the start of experiment. Two natural oils were tested, Csya oil 0.2 ml/kg B.W and Nutgrass oil 0.2 ml/kg B.W. and combined treatment with Csya oil 0.1 ml/kg B.W and Nutgrass oil 0.1 ml/kg B.W and Control group received normal saline (0.2 ml/kg). Both oils were used in their natural form at 100% purity and were obtained from the Imad Corporation Vegetable Oils Production Facility near Mosul, Iraq. All treatments were administered through a gastric tube.

Experimental Design

- Following acclimatization, the rabbits were randomly assigned to four equal groups (n = 6 per group).
- **Group 1 (Control):** Received daily oral doses of normal saline.
- **Group 2 (G2):** Received 0.2 ml/kg B.W. of Csya oil.
- **Group 3 (G3):** Received 0.2 ml/kg B.W. of Nutgrass oil.
- **Group 4 (G4):** Received a combination of 0.1 ml/kg B.W. of Csya oil and 0.1 ml/kg B.W. of Nutgrass oil.

All treatments were given once daily for 12 consecutive days. Water and concentrated pellet feed were provided to rabbits throughout the study period. The animals were kept in a controlled environment (32–35°C; 55–60% relative humidity). The facility was cleaned and disinfected on a weekly basis.

Body weight was recorded at the beginning and end of the experiment using an electronic balance. All animals were fasted for 10 h at the end of the study. Anesthesia was induced with diethyl ether and blood samples were taken by cardiac puncture. Samples were centrifuged at 4000 rpm for 10 min as described by H. J. Hoff [15]. Serum was separated and blood urea nitrogen (BUN), creatinine and malondialdehyde (MDA) levels were determined [18]. Ethics Statement Animal welfare and techniques were reviewed by the Ethics Committee according to the “Guide for the Care and Use of Laboratory Animals” of Al-Qasim Green University, College of Veterinary Medicine.

Histopathological techniques

Kidney tissues were collected from each experimental group immediately after euthanasia of rabbits. The organs were washed extensively with normal saline to remove blood remnants and fixed in 10% neutral buffered formalin for 24 h. Following fixation tissues were dehydrated through graded ethanol series and embedded in paraffin wax. Sections were cut from paraffin blocks at 5 μ m thickness with a rotary microtome. The tissue sections were placed on glass slides and processed according to a routine staining protocol that included rehydration with decreasing concentrations of alcohol and staining with hematoxylin and eosin (H&E). Following staining, the sections were cleared in xylene and examined microscopically for histopathological evaluation, following the procedures described by Bancroft and Gamble [3].

Statistical analysis:

The experimental data were analysed statistically by using a Completely

Randomized Design (CRD) according to the method of D. C. Montgomery [2]. The effects on the measured parameters were assessed in male rabbits administered different oral doses of Csyera oil, Nutgrass oil, and their combination. We assumed the random experimental error was normally distributed with mean zero and constant variance (σ^2).

All statistical analyses were performed using SAS software. Mean comparisons among treatment groups were conducted using SAS, SAS/STAT® 9.4 User's Guide [8] at significance levels of ($P \leq 0.01$ and $P \leq 0.05$) to determine statistically significant differences among group means.

Results:

A statistically significant difference in final body weight was observed between the treatment groups. Group 4 (G4) exhibited a significantly higher mean body weight (1263 ± 2.46 g) compared with the control group (G1), which recorded a mean of 1210.4 ± 2.97 g at the end of the treatment period (Table-1).

Table 1. Mean initial body weight, final body weight, and weight change in male rabbits across experimental groups.

Groups and Parameter	G1	G2	G3	G4
Initial body weight at 1 day / gm	1374.43 $\pm$ 1.52 A	1374.95 $\pm$ 1.70 A	1377.44 $\pm$ 1.83 A	1377.36 $\pm$ 1.85 A
Final body weight at 12 days / gm	1210.49 $\pm$ 2.97 A	1240.30 $\pm$ 2.44 AB	1243.52 $\pm$ 1.68 AB	1263. $\pm$ 2.46 B

Description: G1 = Control group (normal saline); G2 = 0.2 ml/kg B.W. of CSY oil; G3 = 0.2 ml/kg B.W. of NUT oil; G4 = 0.1 ml/kg B.W. of CSY oil + 0.1 ml/kg B.W. of NUT oil. Values within the same row bearing identical letters indicate no statistically significant difference among treatments.

Biochemical parameters:

As presented in Table 2, the biochemical analysis revealed significant reductions in blood urea nitrogen (BUN), creatinine, and malondialdehyde (MDA) concentrations in the G4 treatment group compared with the other experimental groups. The BUN level in G4 was 30.40 ± 0.23 mg/dl, markedly lower than the values recorded for G1, G2, and G3

(46.10 ± 0.29 , 44.35 ± 0.28 , and 33.86 ± 0.26 mg/dl, respectively). Serum creatinine concentration in G4 was 0.67 ± 0.04 mg/dl, compared with 0.96 ± 0.08 , 0.92 ± 0.07 , and 0.77 ± 0.06 mg/dl in G1, G2, and G3, respectively. Similarly, MDA levels were significantly reduced in G4 (2.78 ± 0.25 mg/dl) relative to the control and other treatment groups, which exhibited values of 4.30 ± 0.20 mg/dl.

Table-2. Serum concentrations of blood urea nitrogen (BUN), creatinine, and malondialdehyde (MDA) in male rabbits across experimental groups following treatment

Groups and Parameter	G1	G2	G3	G4
Blood urea nitrogen	46.10 ± 0.29 A	44.35 ± 0.28 AB	33.86 ± 0.26 C	30.40 ± 0.23 D
Creatinine	0.77 ± 0.06 AB	0.96 ± 0.08 A	0.92 ± 0.07 B	0.67 ± 0.04 C
Malondialdehyde	4.30 ± 0.20 A	3.66 ± 0.14 C	3.82 ± 0.18 B	2.78 ± 0.25 D

Description: G1 = Control group (normal saline); G2 = 0.2 ml/kg B.W. of CSY oil; G3 = 0.2 ml/kg B.W. of NUT oil; G4 = 0.1 ml/kg B.W. of CSY oil + 0.1 ml/kg B.W. of NUT oil. Values are presented as mean $\pm$ standard error (SE). Means within the same row sharing identical superscript letters do not differ significantly at ($P \leq 0.01$ and $P \leq 0.05$) according to Duncan's Multiple Range Test.

Histopathological:

Figure A. Histological section of the kidney of a male rabbit from the control group Stained with H&E stain, sectioned 400X.

Figure B. Histological section of kidney of rabbits treated with CSY oil (0.2 ml/kg body weight for 12 days) stained with H&E stain. There is marked vacuolation of glomerular cells

Histopathological analysis of kidney tissue from control rabbits, stained with Hematoxylin and eosin (H&E) and observed at 40 $\times$ magnification, demonstrated different structural changes. The renal capsule was markedly thickened due to increased proliferation of fibrous connective tissue. This marked fibrotic expansion led to compression and damage of the adjacent renal structures suggesting early pathological remodelling in the cortical region.

with partial loss of bowman's space in the section. There is also mild thickening of the parietal layer of Bowman's capsule and sloughing of tubular epithelial cells.

Figure C. Histopathological section of kidney of rabbits treated with NUT oil (0.2 ml/kg body

weight for 12 days) stained with H&E. The tissue shows interstitial fibrosis with inflammatory cell infiltration, consistent with an active degenerative and inflammatory response.

Figure D. Histological section of kidney of rabbits treated with combination of CSY and

NUT oils (0.1 ml/kg body weight each for 12 days) stained with H & E. Focal sloughing of tubular epithelial cells is shown in this section with less structural disruption than single oil treatments.

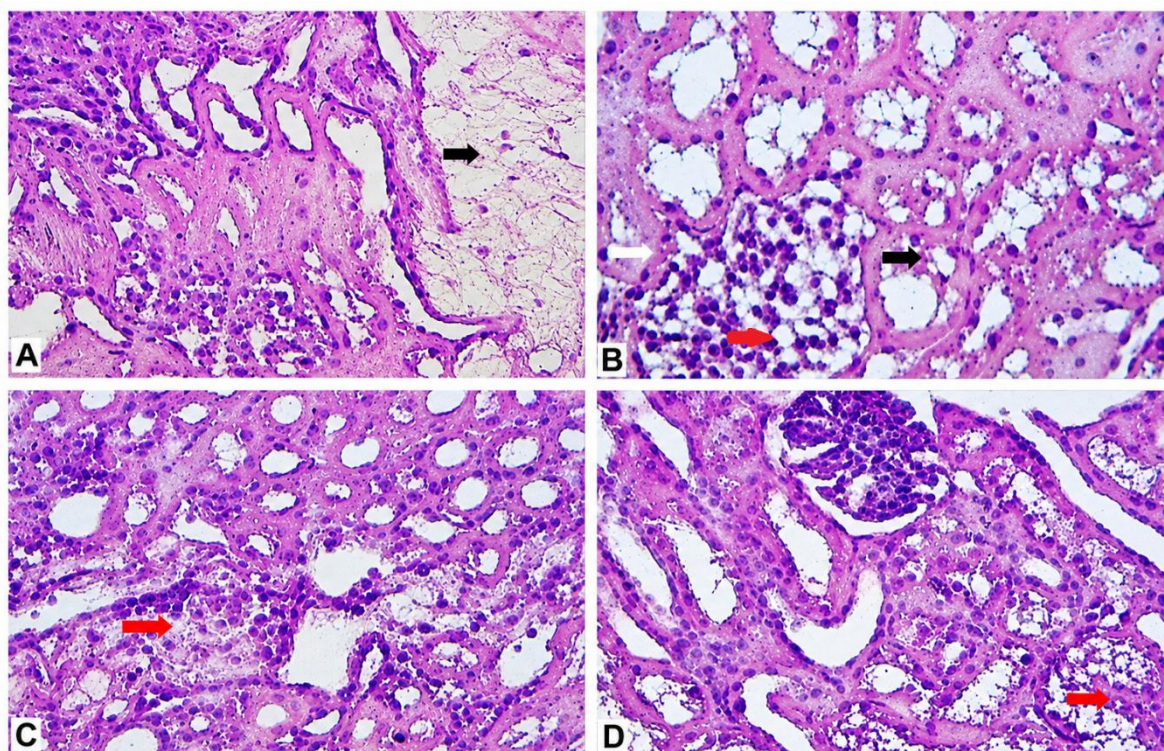


Figure A. Histological section of the kidney of a male rabbit from the control group Stained with H&E stain, sectioned 400X. This section shows significant thickening of the renal capsule due to severe proliferation of fibrous connective tissue which results in compression and collapse of adjacent renal structures (black arrow).

Figure B. Histological section of the kidney from a male rabbit treated with Csyera (CSY) oil at a concentration of 0.2 ml/kg body weight, stained with H&E (400×). The section shows pronounced vacuolation of glomerular cells leading to partial disappearance of Bowman's space (red arrow). Mild thickening of the parietal layer of Bowman's capsule is also evident (white arrow), along with sloughing of tubular epithelial cells (black arrow).

Figure C. Histological section of the kidney from a male rabbit treated with NUT oil at a concentration of 0.2 ml/kg body weight, stained with H&E (400×). The section shows prominent interstitial fibrosis accompanied by infiltration of inflammatory cells (red arrow), indicating an active degenerative and inflammatory response within the renal parenchyma

Figure D. Histological section of the kidney from a male rabbit treated with a combination of Csyera (CSY) and NUT oils at a concentration of 0.1 ml/kg body weight, stained with H&E (40×). The section shows focal sloughing of tubular epithelial cells (red arrow) but overall preservation of renal architecture compared to the single oil treated groups.

Discussion

The results in table 1 showed that rabbits in G4 had significant increase in body weight compared with G1, G2 and G3 after administration of 0.1 ml/kg B.W. of combined Csyera and Nutgrass oils. This increase in body weight could be due to the bioactive compounds of both oils, mainly polyphenols and lipophilic flavonoids, which have been shown to improve metabolic efficiency and modulate lipid metabolism [4], [23], [30]. These compounds might help to improve the nutrient utilization and reduce the oxidative stress which could ultimately help to improve the growth performance under heat stress conditions [20], [28], [29].

Table 2 shows that the concentrations of blood urea nitrogen and creatinine were significantly lower in G4 than the other groups. This reduction shows improvement in renal function as well as decrease in nephron workload in the rabbits fed the combined oils. The nephroprotective effects of Csyera and Nutgrass oils may be due to their high

polyphenolic and flavonoid contents, which have potent antioxidant effects [16], [19]. These antioxidants protect renal cortical epithelial cells from oxidative damage by neutralizing free radicals [25], [26], [27]. Previous studies [13], [17] reported that plant-derived antioxidants have protective effects on renal tissues by reducing oxidative stress and sparing nephron integrity.

Conclusion:

The combined administration of Csyera and Nutgrass oils was more favorable than the higher single dose of oil (0.2 ml/kg) in male rabbits exposed to heat stress at a dose of 0.1 ml/kg body weight. The combined treatment improved body weight, renal function, decreased oxidative stress markers and preserved kidney histological structure. These results indicate that the combined effects of Csyera and Nutgrass oils provide better protection against heat-induced renal damage than the individual administration.

Full name	Abbreviation
Csyera Oil	CSY Oil
Nutgrass Oil	NUT Oil
Milligram/Desi Litters	(Mg/dl)
Body Weight	B.W.
Milligram/ Milliliters	Mg/Ml

Reference:

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