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HISTOPROTECTIVE ROLE OF HONEY AGAINST CYCLOSPORIN INDUCED LUNG TOXICITY IN RATS

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Abstract

CsA is currently extensively utilized in the prevention of organ rejection in transplantation, though, with long-term use it leads to severe pulmonary toxicity via oxidative stress, inflammation, and tissue remodelling. Honey has the reported antioxidant and anti-inflammatory properties that can combat such damage. This paper bases its findings on the protective properties of natural honey on CsA-induced lung injury in rats using histopathological, biochemical, and semi-quantitative scoring methodologies. Twenty-four male albinos were placed in three groups (n = 8) which were CsA (25 mg/kg/day), Control, and CsA + Honey (5 g/kg/day). There was oral medication of treatment over 21 days. Hematoxylin and eosin staining of lung tissue, measurement of serum C- reactive protein (CRP) and lung superoxide dismutase (SOD) activity were done. CsA had a pronounced effect on increasing inflammatory infiltration, congestion, hemorrhage and type II injury of pneumocyte and aggravating CRP and inhibiting SOD activity. Synergetic application of honey had significant numbers of alleviation of these pathological changes and amelioration of biochemical indicators. Honey showed a high histoprotective activity, indicating that it should be considered as a complementary therapeutic agent to reduce CsA-induced pulmonary toxicity.

1. INTRODUCTION

Cyclosporine A (CsA) is an established immunosuppressive agent that is usually applied during organ transplantation and autoimmune diseases [1]. Although CsA has therapeutic benefits, it has been linked to toxicity of vital organs because of oxidative stress and inflammatory processes/mechanisms [2] Although not emphasized in clinical setting, pulmonary toxicity is clinically significant and involves interstitial inflammation, alveolar injury, and loss of antioxidant defenses [3]. There is a growing body of evidence that CsA-induced oxidative stress may overwhelm endogenous enzyme systems and favour cellular injury and inflammatory reactions [4,5]. Honey has been known to be of some medicinal value especially with respect to antioxidant, anti-inflammatory effects as well as immunomodulatory effects. Its bioactive substances such as flavonoids, phenolic acids, vitamins, and enzymes have high free-radical scavenging effects [6-8]. The evidence of protective

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effect of honey is based on experimental studies showing that honey can be used to prevent tissue damage caused by toxic agents and oxidative processes in the body [7]. Nonetheless, there are scanty information on the protective effect of honey against CsA-induced pulmonary toxicity. Thus, the current research will examine the histological, biochemical, and quantitative impacts of honey in treating CsA-induced lung damage.

2. MATERIALS AND METHODS

2.1 Sampling

The healthy male adult albino albino rats were kept in controlled environmental conditions (180220g) and allowed free access to food and water (N=24). Ethical standards and institutional animal care were used in experimental procedures.

2.2 Experimental Design

The rats were seeded out randomly and separated into three groups (n = 8):

- Group A (Control): Was administered with a normal saline orally.
- Group B (CsA): was administered cyclosporine A 25 mg/kg/day orally.

Listing of Groups: Group C (CsA + Honey): orally administered CsA 25mg/kg/day plus honey 5g/kg/day.

Treatments were done via gastric gavage of all the 21 days.

2.3 Histopathological Studies

Tissues of the lungs were collected 24 hours of the last dose. The specimens were abandoned in 10% buffered formalin, processed, embedded in paraffin, sectioned to 4 5mm and stained with hematoxylin and eosin. Microscopic analysis determined the structure of alveoli, cellular infiltration, vascular congestion, hemorrhage, and morphology of type II pneumocytes.

Semistatic histological scoring of the samples was conducted using the semistatic method.

The quantity measured was using a 03 scoring scale:

- Macrophage count
- Congestion
- Hemorrhage
- Type II pneumocyte injury

A blinded pathologist tested 10 high-power fields (HPF) on each slide.

2.5 Biochemical Analysis.

Serum C-Reactive Protein (CRP). The amount of CRP in the serum was measured by rat-specific ELISA kit.

Lung Superoxide Dismutase (SOD) Activity. SOD activity in lung homogenates was determined and was expressed in U/mg protein.

Ethical Issues.The procedures followed were in accordance to the institutional ethical limits that guarantee welfare and humane treatment to animals.

2.6 Statistical Analysis

The data were presented in the form of mean SD. ANOVA was used (one way) and post-hoc test (Tukey) was used to establish significance ($p < 0.05$). SPSS v22 was used for analysis.

3. RESULTS

3.1 Effects of CsA and Honey on Body Weight

CsA significantly reduced weight gain compared to controls, whereas honey co-treatment improved final body weight.

Table 1. Mean Body Weight of Rats Before and After Experiment (Mean ± SD, n = 8)

Group	Initial Weight (g)	Final Weight (g)	% Change
Control (A)	138.2 ± 7.5	166.4 ± 8.1	+20.4%
CsA (B)	136.9 ± 8.0	148.7 ± 7.6	+8.6%
CsA + Honey (C)	137.6 ± 7.9	160.2 ± 7.8	+16.4%

Interpretation: CsA suppressed weight gain; honey partially restored normal growth trajectory.

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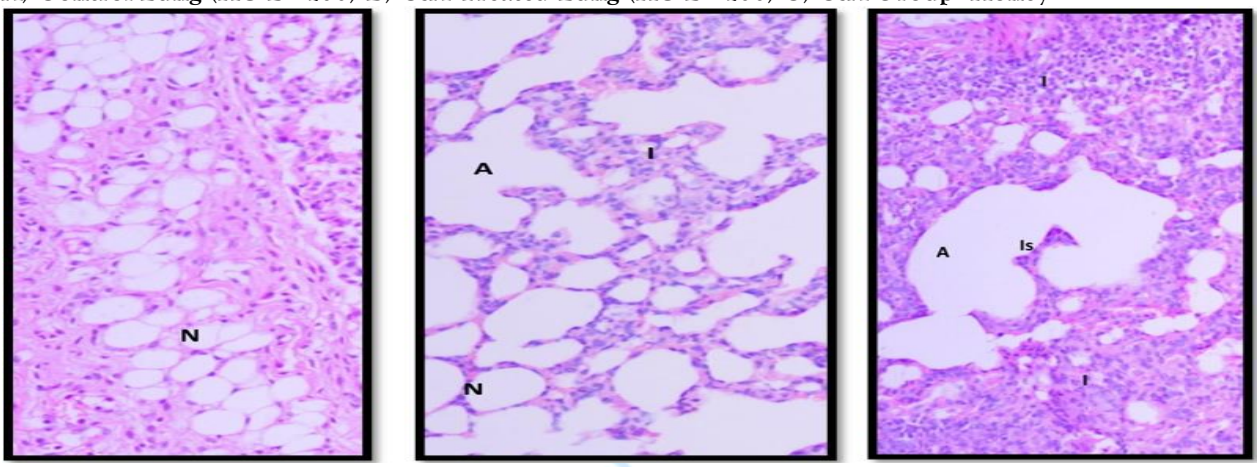
3.2 Histopathological study Findings

By the conclusion of the experiment period, rats were sacrificed 24 hours following the last cyclosporine or honey administration. Thoracic cavity was opened and both lungs were removed and washed thoroughly using normal saline to clean them of blood and debris. The tissues were placed in 10% neutral buffered formalin to be processed routinely and then dehydrated using successive grades of alcohol, cleared using xylene and embedded in paraffin blocks. A rotary microtome was used to achieve serial sections with 4mm of thickness; these sections were stained with haematoxylin and eosin (H&E) under light microscopic analysis. A blinded pathologist assessed all the slides to prevent assessment bias.Histological changes were measured under a semi-quantitative scoring system which measured 4 parameters; macrophage infiltration, vascular congestion, alveolar hemorrhage, and type II pneumocyte injury. Individually, each parameter was rated on a 0-3 scale in terms of severity and the mean score across several high-power fields (HPF) was analyzed statistically. In this experiment no special stains or ultrastructural studies were carried out.

3.3 Comparative Panel: Control, CsA, and CsA+Honey (Side-by-Side)

A) Control Lung (H&E ×100) B) CsA Treated Lung (H&E ×100) C) CsA Group+Honey



3.2.1 Light Microscopy (H&E Staining)

Figure 1(a) —Normal alveolar architecture control sample with thin interalveolar septa and obstructed alveolar spaces.Figure 1(b) —CsA Treated Lung (H&E ×100)

Figure 1 (c) - CsA + Honey Treated Lung (H&E ×100)

(c) CsA + Honey group with better alveolar morphology, less inflammatory foci and partial septal integrity restoration (H&E ×100).

3.2.2 Quantitative Histological Assessment

Table 2. Semi-Quantitative Scoring of Histopathological Changes

Parameter	Control (A)	CsA (B)	CsA + Honey (C)	ANOVA p-value
Macrophages (cells/HPF)	2.1 ± 0.6	11.4 ± 1.3*	4.3 ± 1.0#	<0.001
Congestion Score	0.4 ± 0.5	2.3 ± 0.7*	0.8 ± 0.6#	<0.001
Hemorrhage Score	0.1 ± 0.2	1.6 ± 0.5*	0.5 ± 0.3#	<0.001
Type II Pneumocyte Injury	0.3 ± 0.5	2.0 ± 0.6*	0.7 ± 0.5#	<0.001

*Significantly different from Control ($p < 0.05$).

#Significantly different from CsA ($p < 0.05$).

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Interpretation: CsA caused severe inflammatory and structural injury; honey significantly mitigated each parameter.

3.3 Serum C-Reactive Protein (CRP)

Table 3. Serum CRP Levels (mg/L, Mean ± SD, n = 8)

Group	CRP (mg/L)
Control (A)	1.8 ± 0.4
CsA (B)	6.5 ± 0.7*
CsA + Honey (C)	3.0 ± 0.5#

*Higher than control ($p < 0.05$).

#Lower than CsA ($p < 0.05$).

Interpretation: CsA substantially elevated systemic inflammation; honey halved this response.

3.4 Lung Superoxide Dismutase (SOD) Activity

Table 4. Lung SOD Activity (U/mg protein, Mean ± SD)

Group	SOD (U/mg)
Control (A)	36.7 ± 2.8
CsA (B)	20.4 ± 2.1*
CsA + Honey (C)	31.1 ± 2.5#

*Lower than control.

#Higher than CsA.

Interpretation: CsA depleted antioxidant defenses; honey restored SOD activity toward normal levels.

4. DISCUSSION

The current research established that cyclosporine A (CsA) causes severe pulmonary damage, which is accompanied by inflammatory influx of cells, vascular congestion, hemorrhage, and distortion of the alveolar structure. These results correlate with previous accounts of CsA-induced pulmonary toxicity due to oxidative stress, epithelial malfunction, and inflammatory stimulation [1,3]. CsA was reported to cause reactive oxygen species and disturb the redox homeostasis, causing tissue damage, and reducing antioxidant ability [4]. Considerable decrease in superoxide dismutase (SOD) activity and significant increase in C reactive protein (CRP) in the CsA treated group in present research supports the earlier findings that CsA exposure is associated with oxidative and inflammatory dysfunctions [2, 5].Honey revealed significant protective actions of CsA against the effects of CsA-induced lung injury. The positive effects of CsA + Honey on the alveolar structure, the decrease in macrophage invasion, and the decrease of the congestion and hemorrhage are in accordance with the experimental results that notice the strong antioxidant and anti-inflammatory effects of honey as a product of the CsA group [6]. The phenolic acids and flavonoids present in honey have a broad spectrum of neutralizing free radicals and preventing oxidative damage, which has been proven to be effective in preventing the damage caused by oxidation [11,12] .Similarly to this, these bioactive molecules regulate inflammatory signals and stabilize cell membranes, supporting the preservation of tissue integrity, which has likewise been reported in models assessing honey protective properties against chemically induced toxic effects of lxix, xxx. This marked decrease in CRP levels among the honey-treated rats also indicates suppression of systemic inflammation, which is in line with the immunomodulatory effects of honey that had been previously reported on rats in the literature realm of immunology studies as an area of research . The significant increase in histological score

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further demonstrates the idea that honey can be used to improve tissue damage both by ROS scavenging as well as inhibiting inflammatory infiltration.In addition, the fact that honey can alleviate tissue damage supports previous research that suggests natural honey can be used to improve resistance against oxidative stress as well as the ability to repair damaged epithelium. Taken together, these findings highlight the multi-mechanistic protective nature of honey, which is likely to have clinical applicability in the prevention of pulmonary toxicity in CsA treatment.

5. CONCLUSION

Oxidative stress and inflammation mediate cyclosporine A to cause profound histopathological and biochemical lung injury to rats. Natural honey significantly decreases these harmful effects as potent antioxidant and histoprotective effects. Honey can also be used as a complement to alleviate pulmonary toxicity that comes with CsA.

Conflict of Interest

The authors declared no conflict of interest.

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