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Original Article

# Immunomodulatory Effects of Carob (*Ceratonia siliqua*) Extract in Pregnant Rats: A Histological Evaluation of Embryonic Liver

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## Abstract

**Background**: Carob (*Ceratonia siliqua*) is a polyphenol-containing plant that has anti-oxidant and anti-inflammatory qualities, which can affect maternal immunity in pregnancy. Nevertheless, it has not been studied how it affects maternal immunity and the integrity of embryonic organs. Objective .The current paper aimed to test the immunomodulatory properties of carob extract on pregnant rats affected with induced hepatitis and to examine the histological alterations of embryonic liver tissue. **Methods**: Pregnant female Wistar rats (200-230 g) were chosen randomly and distributed into four groups (n=7/group): control (vehicle only), carob-only (400mg/kg/day carob extract), infected (induced hepatitis by concanavalin A), and carob + infected (carob pretreatment then ConA induced hepatitis). Carob aqueous extract was administered on gestational days 12-18. At gestational day 19 (GD19), hepatic enzyme levels (ALT, AST, ALP, GGT) and pro-inflammatory cytokine levels (IL-6, IL-12, IL-18, IL-29, TNF-α) were assessed in maternal blood using ELISA. Cesarean delivery was used to harvest embryos, and embryonic livers were subjected to histological analysis using hematoxylin and eosin staining. **Results**: Pretreatment with carob significantly attenuated maternal hepatic infection by reducing ALT (345 ± 30 to 120 ± 20 U/L, 65%) and AST (290 ± 25 to 105 ± 18 U/L, 64%). There was a significant decrease in pro-inflammatory cytokines, namely, IL-6 (reduced by 62.5% [320 ± 50 to 120 ± 30 pg/mL, $p < 0.01$]), TNF-α (reduced by 63% [2500 ± 400 to 920 ± 150 pg/mL, $p < 0.001$]), IL-12 (reduced by 50% [110 ± 20 to 55 ± 10, $p < 0.01$]) and IL-18 (reduction by 55% [400 ± 50 to 180 ± 30 pg/mL, $p < 0.01$]). Embryonic livers of carob-treated dams were observed to have preserved hepatic architecture with visible central veins and ordered hepatocyte cords, but those of embryos of damaged dams that did not receive carotenogenic treatment had a significant structural disorganization, cellular necrosis, and architectural distortion. **Conclusion**: Carob extract has strong immunomodulatory activity in pregnant rats, suppressing inflammatory changes and hepatic damage, which is associated with the maintenance of embryonic liver architecture. These effects have not yet been verified in other models, and the mechanisms behind them remain unclear.

**Keywords:** *Ceratonia siliqua*; Pregnancy; Embryonic Liver; Immunomodulation; Cytokines; Histology; Polyphenols

## 1. Introduction

Pregnancy is a distinctive immunological condition characterized by dynamic changes in maternal immune responses that are vital for ensuring embryonic development without compromising host defenses [1]. A significant change in the maternal immune system occurs, including alterations in the cytokine profile and immune cell populations, to develop tolerance to the semi-allogeneic embryo [2]. Disruption of this fine immunological balance by inflammatory states can negatively impact not only maternal health but also embryonic organ development [3, 4].

The cytokines that play a significant role in mediating inflammatory responses are pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IL-12, IL-18, and IL-29 [5]. These cytokines can either bypass the placental barrier or, when elevated in maternal circulation, indirectly damage the embryo's tissue integrity by affecting placental function and nutrient transfer [6]. Such inflammatory insults are especially likely to affect the embryonic liver, which plays a major role in hematopoiesis during gestation [7]. Maternal inflammatory diseases have been linked to changes in hepatic morphology, hepatocyte disorganization, and long-term effects on offspring hepatic function [8].

Carob (*Ceratonia siliqua L.*) is a leguminous evergreen tree native to the Mediterranean region and has been used historically as a source of food and a traditional medicinal remedy [9]. Carob pods, particularly the polyphenols such as gallic acid, (+)-catechin, (-)-epicatechin, and their derivatives, are extraordinarily rich in bioactive compounds that together confer their high antioxidant activity [10, 11]. The biological effects of these polyphenolic compounds are achieved in several ways, including scavenging reactive oxygen species, blocking nuclear factor kappa-B (NF-kB) or mitogen-activated protein kinase (MAPK) signaling pathways, and regulating the production of inflammatory cytokines [12, 13].

Recent studies have shown that carob has therapeutic value in various experimental animals. According to Martić *et al.* [14], carob pulp extract reduced hepatic enzyme levels and inhibited lipid peroxidation in acetaminophen-induced hepatotoxicity. Rašković *et al.* [15] showed that carob supplementation also improved liver morphology and reduced inflammatory markers in diet-induced obese rats. Recent research by

Ahmed *et al.* [16] . Indicated that carob powder, together with thymoquinone, alleviated oxidative stress and reduced pro-inflammatory cytokines in an asthmatic pregnant rat model, suggesting its potential usefulness during pregnancy. Nevertheless, no research has examined the precise effects of carob extract on maternal immune parameters and embryonic liver histology in a setting of liver inflammation.

The immunomodulatory effects of carob have not yet been studied in pregnant models, and whether maternal treatments can influence embryonic organ integrity remains unknown. This is a knowledge gap, as there is growing interest in the use of natural compounds as potential therapeutic agents during pregnancy. Thus, we conjectured that carob extract treatment of pregnant rats would regularize the maternal inflammatory response and, by extension, prevent inflammation-related harm to the embryonic liver. This research set out to assess the immunomodulatory actions of carob extract on maternal parameters of inflammatory reactions in pregnant rats with induced immune-mediated hepatitis and to determine the resultant histological alterations of embryonic liver tissue.

## 2. Materials and Methods

### 2.1 Ethical Approval

The entire experimental procedure was performed in strict compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and was endorsed by the Institutional Animal Ethics Committee (Protocol No. IAEC/2024/05). The animals used were kept in humane conditions during the study, and suffering was reduced as much as possible.

### 2.2 Animals and Mating Protocol

Twenty-eight adult female Wistar rats (200 – 230 g, 10 – 12 weeks old) were obtained from the Animal House Facility at the College of Veterinary Medicine, University of Tikrit, Iraq. The animals were housed in polypropylene cages under controlled environmental conditions (temperature: 22 ± 2 °C; relative humidity: 50–60%; 12-hour light/dark cycle) with ad libitum access to standard rodent laboratory chow and filtered water. After a one-week acclimatization period, female rats were mated with fertile males at a ratio of 2:1. Vaginal smears were examined daily; the presence of spermatozoa was designated as gestational day 0 (GD0), and pregnancy was further confirmed by monitoring body weight gain [17].

### 2.3 Carob Extract Preparation

Mature carob (*Ceratonia siliqua L.*) pods were obtained from local markets. The pods were thoroughly washed, air-dried at room temperature in a well-ventilated area for 7 days, and ground into a fine powder using an electric grinder. To prepare the aqueous extract, 100 g of the powder was macerated in 1000 mL of distilled water at room temperature with periodic agitation every 72 hours. The suspension was filtered through Whatman No. 1 filter paper, followed by a 0.45 µm membrane filter for clarification. The filtrate was concentrated using a rotary evaporator at 40 °C under reduced pressure to obtain the crude extract (yield: 18.5% w/w). The resulting extract was lyophilized and stored at -20 °C until further use. For administration, the lyophilized powder was freshly resuspended in 0.5% carboxymethyl cellulose (CMC) as a vehicle. A dose of 400 mg/kg body weight was selected based on its established efficacy and safety in rodent models [14, 18], representing approximately 1/10 of the No-Observed-Adverse-Effect Level (NOAEL) for carob extracts in rodents [19].

### 2.4 This Experimental Design and Treatment Protocol

After pregnancy confirmation, rats were randomly assigned to four experimental groups (n = 7 per group) using a computer-generated randomization sequence:

**Group I** (Control): Received the vehicle (0.5% CMC, 1 mL/kg, p.o.) daily from GD12 to GD18, and a single injection of normal saline (1 mL/kg, i.v.) on GD18.

**Group II** (Carob Only): Received carob extract (400 mg/kg, p.o.) daily from GD12 to GD18, and normal saline (1 mL/kg, i.v.) on GD18.

**Group III** (ConA- infected): Received the vehicle (0.5% CMC, 1 mL/kg, p.o.) daily from GD12 to GD18, followed by a injection (15 mg/kg, i.v.) on GD18.

**Group IV** (Carob + ConA): Received carob extract (400 mg/kg, p.o.) daily from GD12 to GD18, followed by a ConA injection (15 mg/kg, i.v.) on GD18, administered 1 hour after the last carob dose.

The treatment period (GD12–GD18) was selected to encompass the critical stage of fetal hepatic organogenesis in rats [20].

**Induction of Immune-Mediated Hepatic Infection:** To induce acute hepatic inflammation, Concanavalin A (ConA) was used. ConA is a well-established T-cell mitogen that triggers rapid cytokine release and liver infection within 24 hours [21]. All intravenous injections were administered via the lateral tail vein under brief isoflurane anesthesia.

### 2.5 Sample Collection

On GD19 (24 hours after ConA or saline injection), pregnant rats received anesthesia with ketamine/xylazine solution (80/10 mg/kg, i.p.). The maternal blood was separated from the serum (about 5 mL) by cardiac puncture using plain tubes. Blood samples were clotted at room temperature for 30 minutes, then centrifuged at 3000 rpm for 4 °C. Serum aliquots were stored at -80 °C until biochemical and immunological tests. After the blood sample was collected, pregnant rats were euthanized by cervical dislocation, and embryos were harvested immediately through cesarean section. The viable embryo, resorption sites, and embryo weight were counted. Three random embryos were selected from each litter and subjected to histological examination. Embryonic livers were also dissected under a stereomicroscope and fixed immediately on 10% neutral-buffered formalin.

### 2.6 Maternal Hepatic Enzyme Analysis

Serum markers of hepatic infection were measured using an automated clinical chemistry analyzer (Beckman Coulter AU480, USA). The kinetic UV technique of NADH oxidation was used to measure the activities of Alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Alkaline phosphatase (ALP) was ascertained using the p-nitrophenyl phosphatase technique, and gamma-glutamyl transferase (GGT) was ascertained using the γ-glutamyl-3-carboxy-4-nitroanilide technique. All assays were standardized with commercial standards and performed in duplicate; results are expressed in U/L.

### 2.7 Assessment of Cytokine Concentrations in Maternal Blood

Pro-inflammatory cytokines (IL-6, IL-12p70, IL-18, IL-29, and TNF-α) in the maternal serum were measured using commercially available rat-specific sandwich ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. In brief, 96-well precoated serum samples (diluted 1:2 in assay buffer) were incubated with capture antibody-coated microplates for 2 hours at 37°C, followed by incubation with predetermined recombinant cytokine standards. After three washing steps in phosphate-buffered saline containing 0.05 per cent Tween-20, biotin-conjugated detection antibodies were added and incubated for 1 hour at 37 °C. The solution of horseradish peroxidase (HRP)-conjugated streptavidin was introduced after washing, and the mixture was incubated at 37 °C for 30 minutes. Development was then performed using the colorimetric 3,3,5,5'-tetramethylbenzidine (TMB) substrate, and the reaction was left to develop in the dark for 15 minutes, after which it was stopped with 2N sulfuric acid. Optical density was measured at 450 nm using a microplate spectrophotometer (BioTek ELx800, USA). Cytokine levels (pg/mL) were determined using four-parameter logistic standard curves. Each sample was tested twice. The inter- and intra-assay coefficients of variation were less than 10%. Detection limits: IL-6 (5 pg/mL), IL-12p70 (10 pg/mL), IL-18 (2 pg/mL), IL-29 (15pg/mL), and TNF-α (5 pg/mL).

### 2.8 The Histological Study of Embryonic Liver.

Specimens of embryonic liver were fixed in 10% neutral-buffered formalin for 48 hours at room temperature. Fixed tissues were dehydrated in a graded series of ethanol (70, 80, 90, and 100 per cent), cleared twice in xylene, and embedded in paraffin wax. A rotary microtome (Leica RM2235, Germany) was used to prepare serial sections 5 mm thick, which were then mounted on glass slides. According to conventional histological procedures, sections were deparaffinized with xylene, rehydrated through a descending ethanol series, and stained with Harris hematoxylin and eosin (H and E) [22].

A qualified pathologist who was not aware of the experimental groups performed histological evaluation. The samples of liver were viewed using a light microscope (Olympus BX51, Japan) at magnifications of 1: 100, 1: 200, and 1: 400. The parameters that were assessed were as follows (1) overall hepatic architecture and structure; (2) the arrangement of the hepatocyte cords and morphology of the hepatocytes; (3) the visibility and integrity of the central veins; (4) the distribution of spaces in

a sinusoidal manner; (5) presence and degree of necrosis or degeneration; (6) the presence of the inflammatory cell infiltration; and (7) the presence of the hematopoietic activity (suitable to the gestational age). A digital camera system (Olympus DP72) was used to take representative photomicrographs.

The system of semi-qualitative scoring was used to evaluate the liver histology: 1, normal architecture with corded hepatocytes and visible central veins; 2, mild changes (slight disorganization, minimal affected cells); 3, moderate changes (obvious disorganization, scattered necrotic cells); 3 severe changes (assessed the significant disorganization of the architecture, extensive grade of necrosis or degeneration).

### 2.9 Statistical Analysis

GraphPad Prism (version 9.0; GraphPad Software, Inc., San Diego, CA, USA) was used to perform statistical analyses. The Shapiro-Wilk test was used to assess data normality. Data that follow a normal distribution are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and the Tukey honestly significant difference (HSD) post hoc test were used to compare the groups. This was done using the Kruskal-Wallis test and Dunn's multiple comparison test, which were used to determine nonparametric scores for the histological scores. A p-value that was less than 0.05 was deemed to be statistically significant. To cause statistically significant differences between groups, power analysis (a = 0.05, b = 0.20, effect size = 1.5) was conducted with G*Power software, which also allowed determining the appropriate sample size (n = 7 per group).

The control and carob-only groups of all pregnant rats maintained normal behavior, food intake, and weight gain throughout the gestation period. Due to the administration of ConA on GD18, the damage to the dams in the infected group had clinical manifestations of acute hepatitis that included lethargy, piloerection, hunched posture, and decreased food intake within the period of 6-8 hours. Group IV exhibited a significant reduction in clinical manifestations of infected because of carob - pretreated pregnant rats compared with the non-treated infected group. In all experimental groups, there were no maternal deaths or premature births.

## 3. Results

Table 1 summarizes the results of pregnancy on GD19. There was no significant difference in the number of viable embryos per litter (10.1 – 11.4, p > 0.05). There were no significant differences in mean embryo weight (control, carob-only, carob + infected: 2.82 – 2.95 g), but the embryos of untreated injured dams were slightly but not significantly lower in weight (2.68 ± 0.31 g). Resorption rate was slightly higher in the infected group (8.5) than in controls (4.2), but the difference was not statistically significant (p = 0.08).

**Table 1.** Pregnancy outcomes on gestational GD19 (mean ± SD).

| Group | Viable Embryos/Litter | Mean Embryo Weight (g) | Resorption Rate (%) |
|---|---|---|---|
| **I. Control** | 11.4 ± 1.2 | 2.95 ± 0.28 | 4.2 ± 1.8 |
| **II. Carob Only** | 11.1 ± 1.3 | 2.89 ± 0.25 | 4.5 ± 1.6 |
| **III. ConA-Infected** | 10.1 ± 1.5 | 2.68 ± 0.31 | 8.5 ± 2.2 |
| **IV. Carob + ConA** | 11.0 ± 1.4 | 2.82 ± 0.29 | 5.1 ± 1.9 |

### 3.1 The Levels of Maternal Hepatic Enzymes

Table 2 shows the maternal serum hepatic enzyme levels. Severe hepatocellular infection in pregnant rats induced by ConA administration was evidenced by significant increases in serum transaminases. In the infected group (Group III), ALT levels rose to 345 ± 30 U/L, an increase of 7.7-fold compared with the control (45 ± 8 U/L, p < 0.001). Equally, AST increased to 290 ± 25 U/L, which was 5.8 times higher than the controls (50 ± 10 U/L, p<0.001).

These enzyme levels were considerably reduced by carob pretreatment (Group IV). The ALT decreased to 120 ± 20 U/L, which was 65 % lower than the untreated group (p < 0.01). AST reduced to 105 ± 18 U/L, which was a 64 per cent decrease (p < 0.01). Although the values remained higher than those in controls (p < 0.05), carob pretreatment provided significant protection of the liver.

There were similar patterns in cholestatic markers. The increase in ALP was 3 times (p<0.001) between 80 ± 15 U/L in controls and 240 ± 30 U/L in the infected group. Pretreatment with carob reduced ALP to 130 ± 20 U/L (45.8% inhibition of infected, p < 0.01). GGT was higher in controls 6 ± 2 U/L than in the infected group (18 ± 4 U/L, p < 0.001) and lower in carob pretreatment (5.1 per cent reduction, p < 0.01). It is important to note that the carob-only (Group II) did not show significant changes in any liver enzymes relative to controls (p > 0.05), which supported the claim that carob extract administration had no hepatotoxic effects.

**Table 2.** Serum hepatic enzyme levels of the maternal on the GD19.

| Group | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) |
|---|---|---|---|---|
| **I. Control** | 45 ± 8 | 50 ± 10 | 80 ± 15 | 6 ± 2 |
| **II. Carob Only** | 40 ± 5 | 48 ± 9 | 78 ± 20 | 5 ± 1 |
| **III. ConA-Infected** | 345 ± 30* | 290 ± 25* | 240 ± 30* | 18 ± 4* |
| **IV. Carob + ConA** | 120 ± 20*† | 105 ± 18*† | 130 ± 20*† | 8 ± 3 |

*Data are expressed as mean ± SD (n = 7 per group). ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase. *p < 0.05 vs. Control group; †p < 0.01 vs. Infected group (one-way ANOVA with Tukey's post-hoc test).

### 3.2 Maternal Inflammatory Cytokine Profiles

Cytokine analysis of maternal serum revealed a high level of inflammatory response after ConA administration, which was strongly inhibited by carob pretreatment (Table 3). Pro-inflammatory cytokines were at baseline levels in the control group: IL-6 10 ± 2 pg/mL, TNF-α 22 ± 5 pg/mL, IL-12p70 5 ± 2 pg/mL, IL-18 20 ± 4 pg/mL, and IL-29 < 5 pg/mL.

Strong cytokine responses in ConA administration (Group III) were noted: IL-6 increased to 320 ± 50 pg /mL (p < 0.001 vs. control), TNF-α to 2500 ± 40 pg/mL (113-fold increase, p < 0.001), IL-12p70 to 110 ± 20 pg/mL (22-fold increase, p < 0.001), and IL-18 to 400 ± 50. These results support strong stimulation of the innate and adaptive inflammatory mechanisms.

All of the measured cytokines, IL-6 (62.5% reduction vs. Infected group, p < 0.01), TNF-α (63% reduction, p < 0.001), IL-12p70 (50% reduction, p < 0.01), IL-18 (55% reduction, p < 0.001), and IL-29 (57.9% reduction, p < 0.01) were significantly reduced by carob pretreatment (Group IV). The carob-sole sample showed no significant changes in any cytokines relative to controls (p<.05), indicating that carob extract alone did not cause any inflammatory effects.

**Table 3.** Maternal serum pro-inflammatory cytokine levels on GD19.

| Group | IL-6 (pg/mL) | TNF-α (pg/mL) | IL-12p70 (pg/mL) | IL-18 (pg/mL) | IL-29 (pg/mL) |
|---|---|---|---|---|---|
| **I. Control** | 10 ± 2 | 22 ± 5 | 5 ± 2 | 20 ± 4 | < 5 |
| **II. Carob Only** | 11 ± 3 | 24 ± 6 | 5 ± 2 | 21 ± 5 | < 5 |
| **III. ConA-Infected** | 320 ± 50* | 2500 ± 400* | 110 ± 20* | 400 ± 50* | 38 ± 8* |
| **IV. Carob + ConA** | 120 ± 30*† | 920 ± 150*† | 55 ± 10*† | 180 ± 30*† | 16 ± 4† |

*p < 0.05 vs. Control group; †p < 0.01 vs. Infected group (one-way ANOVA with Tukey's post-hoc test).

### 3.3 Histological Embryonic Liver Findings.

The histological analysis of embryonic liver tissues showed that there were morphological differences (according to the maternal treatment groups) (Figure 1). The summary of the histological scores is in Table 4. Control group (Figure 1A): Damaged embryonic livers. Standard hepatic architecture in control dams was appropriate to GD19. On histological examination, there were well-organized hepatocytes in cord-like structures emanating from visible central veins. Sinusoidal spaces were not destroyed and were regularly distributed. Hepatocytes had normal cell structures with basophilic cytoplasm and centrally placed nuclei. At this stage of gestation, hematopoietic cell clusters, which are typical of embryonic liver, were found throughout the parenchyma. No necrosis, degeneration, or inflammatory infiltration was seen. Mean histological score: 0.14 ± 0.38.

The infected group, as seen in Figure 1B), in striking contrast, embryonic livers of dams subjected to both untreated ConA-induced hepatitis exhibited serious histological defects. The classic hepatic

architecture was drastically impaired, and the organization of the hepatic cord pattern of hepatocytes was lost. Hepatocytes showed cellular disorganization, cytoplasmic vacuoles, and nuclear pyknosis, indicating cellular degeneration and necrosis. The presence of central veins was difficult to detect due to the surrounding tissue disorder. The sinusoidal spaces were irregularly distributed and typically dilated. These results show that inflammatory hepatitis of the maternal adversely affected the embryonic liver integrity. Mean histological score: 2.57 ± 0.51 ($p < 0$, 001 vs. control). In the Carob + infected group (Figure 1C), embryonic hepatic livers of dams pretreated with carob and induced hepatitis showed significantly preserved hepatic archetype. The tissue structure was very similar to embryonic liver morphology, with central veins and hepatocytes observable and organized in recognizable cords. Even though some cells with mild degenerative foci remained apparent, the architectural integrity was significantly better than in the untreated infected group. The distribution was relatively normal in the sinusoidal spaces. These results indicate that maternal carob supplementation showed protective effects on embryonic hepatic tissue despite the incidence of maternal inflammation. The treated Dams with carob extract alone (carob only group), as seen in Figure 1D, the embryonic liver histology of dams treated with carob extract alone showed no difference as compared to the control. The architectural appearance of the hepatocyte was good, with well-organized cords of hepatocytes; central veins were clearly visible, and sinusoidal spaces were not lost. There were no untoward histological alterations, indicating that the carob treatment of the maternal did not compromise the embryonic liver. Mean histological score ($p > 0.05$ vs. control) 0.14 ± 0.38.

**Table 4.** Semi-quantitative histological scoring of embryonic liver tissue in the different experimental groups.

| Group | Histological Score (0–3) | Architectural Integrity | Hepatocyte Organization | Necrosis / Degeneration |
|---|---|---|---|---|
| **I. Control** | 0.14 ± 0.38 | Normal | Normal | Absent |
| **II. Carob Only** | 0.14 ± 0.38 | Normal | Normal | Absent |
| **III. ConA-Infected** | 2.57 ± 0.51* | Severely disrupted | Disorganized | Extensive |
| **IV. Carob + ConA** | 1.00 ± 0.58*† | Moderately preserved | Partially organized | Mild/Focal |

*$p < 0.05$ vs. Control group; †$p < 0.01$ vs. Infected group (one-way ANOVA with Tukey's post-hoc test).

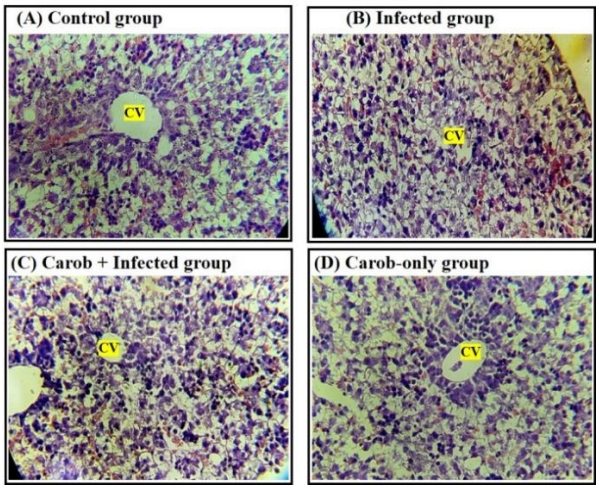


**Figure 1:** Embryonic liver sections stained with hematoxylin and eosin (400x), representative photomicrographs of the stage GD19. (A) Control group: normal embryonic hepatic architecture with well-organized hepatocytes in cord-like structures radiating at the center of the central vein (CV); complete sinusoidal spaces are observed. (B) infected group: A severe architectural disturbance that is characterized by the lack of order in hepatocytes, necrotic and degenerative processes; the central vein is not distinctly defined by the surrounding tissue disorder. (C) Carob + infected group: Significantly normal hepatic architecture with the central vein exposed, with hepatocytes being arranged into familiar cords, but some of the affected cells are also noticeable. (D) control group: Carob-only: Control liver histology. It is confirmed that maternal carob administration has no adverse effects, as embryonic liver histology is normal and similar to that of the control.

## 4. Discussion

The current study shows that the immunomodulatory power on pregnant rats with induced immune-mediated hepatitis is significant and associated with the maintenance of embryonic liver structure. The results provide new evidence of the effectiveness of dietary polyphenols in protecting fetal tissues from the negative effects of maternal inflammation.

Maternal hepatoprotective mechanisms: The significant reduction in maternal hepatic enzyme increases (65% of the increase in ALT and 64% of the increase in AST) in the presence of carob extract indicates that carob extract is a potent hepatoprotection during pregnancy. These results are consistent with previous non-pregnant models. Similar results were noted by Martić *et al.* [14], who found that transaminases significantly decreased after carob pretreatment in acetaminophen-induced hepatotoxicity, whereas Rašković *et al.* [15] found that hepatic parameters were improved in diet-induced obesity. These findings are generalized to the current study, which examines the more complex physiological background of pregnant women, where the liver's vulnerability can be modified by gestational hormonal and metabolic alterations [23].

The mechanisms underlying the hepatoprotective action of carob likely involve multiple pathways, as explained by the presence of its polyphenolic constituents. The most common polyphenols in carob are gallic acid and catechins, both of which have been well documented to have antioxidant properties capable of neutralizing reactive oxygen species generated during liver inflammation [24, 25]. Moreover, the compounds prevent NF-kB activation in hepatocytes and Kupffer cells, thereby inhibiting the transcription of pro-inflammatory genes [12, 26]. This dual action, as an antioxidant and an anti-inflammatory, could be the reason for the strong hepatoprotection observed in our study.

Immunomodulatory and cytokine-reduction: One of the key opportunities of this research is the significant reduction in maternal pro-inflammatory cytokines. The decreases in IL-6 (62.5%), TNF-α (63%), IL-12 (50%), IL-18 (55%), and IL-29 (58%) evidenced a comprehensive immunomodulatory effect. The roles of these cytokines in hepatic inflammation are interconnected: TNF-α triggers hepatocyte apoptosis and recruits inflammatory cells [27], IL-6 enhances the acute-phase response [28, 29], and Th1-mediated cytotoxicity is promoted by IL-12 and IL-18, which synergistically induce IFN-γ [30, 31]. Simultaneous decreases in these mediators indicate that carob blocks numerous branches of the inflammatory cascade.

Our findings are consistent with those of Atta *et al.* [32], who discovered that carob extract increased anti-inflammatory IL-10 and decreased TNF-α and IL-6 in a nephrotoxicity model. On the same note, Aboura *et al.* [13] also established that carob polyphenol-enriched infusions inhibited the expression of inflammatory cytokines in model colitis and obesity. This immunomodulatory profile, observed across a variety of inflammatory states, indicates a fundamental mechanism that inhibits NF-kB and MAPK signaling pathways, which regulate the expression of various pro-inflammatory mediators [12].

Embryonic liver architecture protection: The histological results of embryonic livers are the most important result of this paper. The embryos of dams of untreated hepatitis had hepatic disorganization, necrosis and loss of normal architecture, whereas those of carob-pretreated dams had hepatic integrity that was significantly preserved. This interaction between maternal immunomodulation and embryonic protection in the hepatic context implies that reduced maternal inflammatory load is translated into embryonic tissue protection. This embryonic protection may involve several mechanisms. To begin with, maternal pro-inflammatory cytokines would be reduced, and the attainment of the inflammatory load by the embryo via placental transfer or its indirect effect on placental function would be affected [32, 33]. The fact that maternal IL-6 crosses the placenta and reaches embryonic tissues has been demonstrated by Dahlgren *et al.* [34], supporting the applicability of maternal cytokine regulation. Second, carob polyphenols, in themselves, can travel across the placental barrier to act as direct antioxidants of embryonic tissues, as illustrated with structurally related flavonoids [35]. Third, better hepatic maternal function would sustain the metabolic homeostasis necessary to supply embryonic nutrients and support growth.

Development at GD19 in rat embryonic liver fundamentally involves the maturation of hepatocytes, the formation of hepatic lobular architecture, and the transformation into a metabolic organ rather than a hematopoietic organ [35, 36]. Only maternal inflammatory mediators would disrupt these processes, with effects on hepatic function in the postnatal period. The hepatic architecture of embryos treated with carob indicates that maternal supplementation could protect these processes.

Safety concerns: One of the main issues is that the carob administration in the maternal did not have a negative impact on embryonic liver histology. Carob-only embryonic liver showed no

difference from controls and exhibited normal architecture and no toxicity. This was supplemented by maternal hepatic enzymes and cytokines being the same in the carob-only group, which supports that carob extract is safe at the dose studied during the gestation period. This is in line with the generally accepted safety of carob as a food ingredient and with toxicological findings of no harm at doses up to 4000 mg/kg in rodents [19].

### 4.1 Future Directions

This study has raised several questions that require answers in future studies. Dose-response would also be useful in determining the best therapeutic dosages and safety margins. Postnatal outcome measures, such as tests of hepatic function and long-term health outcomes in offspring, would also be conducted to determine whether embryonic hepatoprotection confers long-term health benefits. The molecular mechanism of protection could be clarified through mechanistic studies of individual signaling pathways (NF-kB, MAPK, Nrf2) and placental delivery of carob components. The evaluation of anti-inflammatory cytokines and regulatory immune cell populations would better portray the immunological image. Lastly, phytochemical fractionation research might identify the bioactive compounds responsible for the noted actions and, eventually, lead to standardized therapeutic preparations.

## 5. Conclusion

Carob extract (*Ceratonia siliqua*) had strong immunomodulatory activity in rats with immune-mediated hepatitis during pregnancy, which was also indicated by a significant decrease in the markers of hepatic damage (ALT reduced by 65, AST reduced by 64%) and pro-inflammatory cytokines (IL-6, TNF-α, IL-12, IL-18, IL-29 reduced by 50 – 63%). Such maternal effects were associated with intact embryonic liver architecture, as indicated by histological analysis of intact hepatocyte organization and visible central veins in embryos of carob-treated dams, but not in embryos of untreated injured dams, which showed clear architectural disruption. Administration of carob to the maternal under isolated conditions did not cause any adverse effects on embryonic liver histology, indicating that the drug is safe at the dose studied during the gestation period in question. These results indicate that carob, with its anti-inflammatory and antioxidant effects mediated by polyphenols, may be a safe dietary supplement that can suppress maternal inflammation and safeguard embryonic liver tissue. Those are, however, only preliminary results obtained in an acute rodent model and need to be confirmed with dose-response studies, assessment of long-term offspring effects, and in other species before any clinical implications are considered. Additionally, studies are needed to clarify the mechanisms underlying these protective effects, including the molecular pathways and the bioactive compounds involved.

## Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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This study was not funded by a particular grant agency, whether in the public, commercial, or not-for-profit sector.

## Conflict of Interest

The authors declare no conflicts of interest.

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## References

[1] Mor, G., Cardenas, I. (2010) The immune system in pregnancy: a unique complexity, *American Journal of Reproductive Immunology* **63**: 425-433.

[2] PrabhuDas, M., Bonney, E., Caron, K., Dey, S., Erlebacher, A., Fazleabas, A., Fisher, S., Golos, T., Matzuk, M., McCune, J.M. (2015) Immune mechanisms at the maternal-fetal interface: perspectives and challenges, *Nature Immunology* **16**: 328-334.

[3] Hsiao, E.Y., Patterson, P.H. (2012) Placental regulation of maternal-fetal interactions and brain development, *Developmental Neurobiology* **72**: 1317-1326.

[4] Mahmoud, D.A., Saleh, S.S. (2021) Determination of the level of homocysteine and antioxidants in the blood serum of women with gestational diabetes, *Solid State Technology* **64**: 1129-1135.

[5] Brenner, C., Galluzzi, L., Kepp, O., Kroemer, G. (2013) Decoding cell death signals in liver inflammation, *Journal of Hepatology* **59**: 583-594.

[6] Pantham, P., Aye, I.L., Powell, T.L. (2015) Inflammation in maternal obesity and gestational diabetes mellitus, *Placenta* **36**: 709-715.

[7] Crawford, L.W., Foley, J.F., Elmore, S.A. (2010) Histology Atlas of the Developing Mouse Hepatobiliary System with Emphasis on Embryonic Days 9.5-18.5, *Toxicologic Pathology* **38**: 872-906.

[8] Ornoy, A., Reece, E.A., Pavlinkova, G., Kappen, C., Miller, R.K. (2015) Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes, *Birth Defects Research Part C, Embryo Today: Reviews* **105**: 53-72.

[9] Biner, B., Gubbuk, H., Karhan, M., Aksu, M., Pekmezci, M. (2007) Sugar profiles of the pods of cultivated and wild types of carob bean (*Ceratonia siliqua* L.) in Turkey, *Food Chemistry* **100**: 1453-1455.

[10] Papagiannopoulos, M., Wollseifen, H.R., Mellenthin, A., Haber, B., Galensa, R. (2004) Identification and quantification of polyphenols in Carob Fruits (*Ceratonia siliqua* L.) and derived products by HPLC-UV-ESI/MS$^n$, *Journal of Agricultural and Food Chemistry* **52**: 3784-3791.

[11] Goulas, V., Georgiou, E. (2019) Utilization of carob fruit as sources of phenolic compounds with antioxidant potential: Extraction optimization and application in food models, *Foods* **9**: 20.

[12] Rahman, I., Biswas, S.K., Kirkham, P.A. (2006) Regulation of inflammation and redox signaling by dietary polyphenols, *Biochemical Pharmacology* **72**: 1439-1452.

[13] Aboura, I., Nani, A., Belarbi, M., Murtaza, B., Fluckiger, A., Dumont, A., Benammar, C., Tounsi, M.S., Ghiringhelli, F., Rialland, M. (2017) Protective effects of polyphenol-rich infusions from carob (*Ceratonia siliqua*) leaves and cladodes of Opuntia ficus-indica against inflammation associated with diet-induced obesity and DSS-induced colitis in Swiss mice, *Biomedicine & Pharmacotherapy* **96**: 1022-1035.

[14] Martić, N., Zahorec, J., Stilinović, N., Andrejić-Višnjić, B., Pavlić, B., Kladar, N., Šoronja-Simović, D., Šereš, Z., Vujčić, M., Horvat, O. (2022) Hepatoprotective effect of carob pulp flour (*Ceratonia siliqua* L.) extract obtained by optimized microwave-assisted extraction, *Pharmaceutics* **14**: 657.

[15] Rašković, A., Martić, N., Tomas, A., Andrejić-Višnjić, B., Bosanac, M., Atanasković, M., Nemet, M., Popović, R., Krstić, M., Vukmirović, S. (2023) Carob extract (*Ceratonia siliqua* L.): Effects on dyslipidemia and obesity in a high-fat diet-fed rat model, *Pharmaceutics* **15**: 2611.

[16] Ahmed, A.F., Cevher, Ş.C., Peker, E.G.G., Balabanlı, B., Ebegil, M. (2024) Synergistic effects of thymoquinone and carob powder versus dexamethasone in the model of asthma in pregnant rats: new insights into their therapeutic effects, *Gazi Medical Journal* **35**: 393-400.

[17] Terry, K., Chatman, L., Foley, G., Kadyszewski, E., Fleeman, T., Hurtt, M., Chapin, R. (2005) Effects of feed restriction on fertility in female rats, *Birth Defects Research Part B: Developmental and Reproductive Toxicology* **74**: 431-441.

[18] Custódio, L., Fernandes, E., Escapa, A.L., Fajardo, A., Aligué, R., Alberício, F., Neng, N.R., Nogueira, J.M.F., Romano, A. (2011) Antioxidant and cytotoxic activities of carob tree fruit pulps are strongly influenced by gender and cultivar, *Journal of Agricultural and Food Chemistry* **59**: 7005-7012.

[19] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2010) *Scientific Opinion on the substantiation of health claims related to pectins and reduction of post-prandial glycaemic responses (ID 786), maintenance of normal blood cholesterol concentrations (ID 818) and increase in satiety leading to a reduction in energy intake (ID 4692) pursuant to Article 13 (1) of Regulation (EC) No 1924/2006*, European Food Safety Authority (EFSA Journal), 10; John Wiley & Sons, Hoboken, New Jersey, USA, https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1747

[20] Si-Tayeb, K., Lemaigre, F.P., Duncan, S.A. (2010) Organogenesis and development of the liver, *Developmental Cell* **18**: 175-189.

[21] Tiegs, G., Hentschel, J., Wendel, A. (1992) A T cell-dependent experimental liver injury in mice inducible by concanavalin A, *The Journal of Clinical Investigation* **90**: 196-203.

[22] Gorovits, B., Azadeh, M., Buchlis, G., Fiscella, M., Harrison, T., Havert, M., Janetzki, S., Jawa, V., Long, B., Mahnke, Y.D. (2023) Evaluation of Cellular Immune Response to Adeno-Associated Virus-Based Gene Therapy, *An Official Journal of the American Association of Pharmaceutical Scientists (The AAPS Journal))* **25**: 47.

[23] Bancroft, J.D., Gamble, M. (2008) Theory and Practice of Histological Techniques, 6th ed., *Churchill Livingstone / Elsevier*, Philadelphia, Pennsylvania, USA, pp. 725

[24] Gao, L., Zhao, Y.C., Liang, Y., Lin, X.H., Tan, Y.J., Wu, D.D., Li, X.Z., Ye, B.Z., Kong, F.Q., Sheng, J.Z. (2016) The impaired myocardial ischemic tolerance in adult offspring of diabetic pregnancy is restored by maternal melatonin treatment, *Journal of Pineal Research* **61**: 340-352.

[25] Hasan, S., Junaid, F., Mahdi, B., Hussein, F. (2025) Therapeutic Applications of Medicinal Plants for the Treatment of Human Intestinal Diarrhea: Review Article, *South Asian Journal of Life Sciences* **13**: 20-24.

[26] Stavrou, I.J., Christou, A., Kapnissi-Christodoulou, C.P. (2018) Polyphenols in carobs: A review on their composition, antioxidant capacity and cytotoxic effects, and health impact, *Food Chemistry* **269**: 355-374.

[27] Koyama, Y., Brenner, D.A. (2017) Liver inflammation and fibrosis, *The Journal of Clinical Investigation* **127**: 55-64.

[28] Schwabe, R.F., Brenner, D.A. (2006) Mechanisms of liver injury. I. TNF-α-induced liver injury: role of IKK, JNK, and ROS pathways, *American Journal of Physiology-Gastrointestinal and Liver Physiology* **290**: G583-G589.

[29] Al-Quraghuli, A.Z.K., Mahmood, D.A. (2025) Determination of high-sensitivity C-reactive protein in chronic kidney disease patients in Al-Hawija General Hospital in Kirkuk City-Iraq, *International Journal of Biological and Pharmaceutical Sciences Archive* **10**: 173-187.

[30] Tilg, H., Moschen, A.R., Szabo, G. (2016) Interleukin-1 and inflammasomes in alcoholic liver disease/acute alcoholic hepatitis and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, *Hepatology* **64**: 955-965.

[31] Bachmann, M., Pfeilschifter, J., Mühl, H. (2018) A prominent role of interleukin-18 in acetaminophen-induced liver injury advocates its blockage for therapy of hepatic necroinflammation, *Frontiers in Immunology* **9**: 161.

[32] Atta, A.H., Atta, S.A., Khattab, M.S., El-Aziz, T.H.A., Mouneir, S.M., Ibrahim, M.A., Nasr, S.M., Emam, S.R. (2023) *Ceratonia siliqua* pods (Carob) methanol extract alleviates doxorubicin-induced nephrotoxicity via antioxidant, anti-inflammatory and anti-apoptotic pathways in rats, *Environmental Science and Pollution Research* **30**: 83421-83438.

[33] Robbins, J.R., Bakardjiev, A.I. (2012) Pathogens and the placental fortress, *Current Opinion in Microbiology* **15**: 36-43.

[34] Dahlgren, J., Samuelsson, A.-M., Jansson, T., Holmäng, A. (2006) Interleukin-6 in the maternal circulation reaches the rat fetus in mid-gestation, *Pediatric Research* **60**: 147-151.

[35] Vanhees, K., Godschalk, R.W., Sanders, A., van Doorn, S.B.v.W., van Schooten, F.J. (2011) Maternal quercetin intake during pregnancy results in an adapted iron homeostasis at adulthood, *Toxicology* **290**: 350-358.

[36] He, J., Cui, H., Shi, X., Jin, Q., Han, X., Han, T., Peng, J., Guo, S., Zhang, L., Zhao, Y. (2022) Functional hepatobiliary organoids recapitulate liver development and reveal essential drivers of hepatobiliary cell fate determination, *Life Medicine* **1**: 345-358.