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# Comparative Analysis of Liver Function Tests in Control and Trigonella foenum-graecum-Treated Groups of Albino Rats

**Deeksha Singh, 2. Anand Pratap Singh**
Research Scholar, 2. Assistant Professor- Department of Zoology, Agra College, (Affiliated to Dr. Bhimrao Ambedkar University, Agra) Agra (U.P.) India

**Received-22.04.2026, Revised-30.04.2026, Accepted-07.05.2026,** **E-mail:singhdiksha9927@gmail.com**

***Abstract:*** ***The present study evaluated the hepatoprotective potential of Trigonella foenum-graecum (fenugreek) seed extract in 64 albino rats (32 males and 32 females) exposed to mild hepatic stress. The animals were divided into four groups: normal control, hepatic stress model, low-dose fenugreek (250 mg/kg), and high-dose fenugreek (500 mg/kg), and treated orally for 30 days. Liver function was assessed using biochemical markers (ALT, AST, ALP, bilirubin, albumin, and total protein) and histopathological examination. The stress-model group showed significant liver damage, whereas fenugreek treatment improved all parameters in a dose-dependent manner. The high-dose group demonstrated greater recovery, with reduced liver enzyme levels, improved protein synthesis, and marked improvement in liver tissue architecture. Similar protective effects were observed in both male and female rats. The findings suggest that fenugreek seed extract is safe and possesses significant hepatoprotective activity against stress-induced liver injury.***

**Key words: Trigonella foenum-graecum, fenugreek, liver function tests, hepatoprotection, albino rats.**

**1. Introduction-** The liver plays an essential role in maintaining the body's internal environment by regulating metabolism, detoxifying harmful substances, synthesizing plasma proteins, and maintaining biochemical balance; thus, evaluating the liver's overall function is feasible with routine liver functions tests that include measurements of enzymes (such as ALT & AST), alkaline phosphatase, bilirubin, albumin levels and total protein. Albino rats have been extensively used to determine the effect of various dietary, medicinal and herbal products on the liver. Trigonella foenum-graecum (Fenugreek) is a medicinal plant that has significant amounts of bioactive compounds and a variety of potential health benefits due to its ability to act as antioxidants, anti-inflammatories, and regulators of metabolic processes. Due to the increasing use of herbal remedies in both nutritional supplements and medications, there exists a need to evaluate the efficacy and safety of these remedies, and to identify if they may provide some protection against damage to vital organs, such as the liver. Therefore, this study sought to determine whether long-term oral administration of fenugreek seed extract would either negatively affect or improve liver biochemical and histological changes brought about by stress. This investigation compared the phenotypic characteristics of three different groups of albino rats (control group-no exposure to stress or fenugreek, stress group-exposed to stress without treatment, and treated group-exposed to stress with treatment using fenugreek seed extract).

Mohamed et al. (2015) were among the first researchers who indicated the benefits of fenugreek in reducing liver fat accumulation and improving metabolic conditions in rats with obesity due to diabetes. Their research demonstrated that fenugreek administration improved hepatic condition of rats with obesity and diabetes. Therefore, they showed that fenugreek could be used to treat conditions such as non-alcoholic fatty liver disease, and diabetic liver injury, rather than just acute toxin-induced hepatotoxicity. Furthermore, their findings suggested that fenugreek acts on multiple pathways to correct metabolic problems, including glycemic imbalances and lipid dysregulations that contribute to the development of hepatic dysfunction. Mukthamba and Srinivasan (2016) built upon these earlier findings and further established the hypolipidemic and antioxidant properties of fenugreek in high-fat fed rats. Their results suggested that fenugreek decreased oxidative stress in tissues caused by lipid overloading, and decreased biochemical measures of a high-fat diet. While the study also included garlic, the improvements in antioxidant status and reduction in lipids are directly related to protecting the liver since oxidative stress and dyslipidemia are two primary causes of hepatocyte injury. These findings strengthen the theoretical basis for future studies and indicate that fenugreek can protect the structural integrity of the liver through regulation of systemic lipid peroxidation and metabolic stress. Mbarki et al. (2017) provided additional strong evidence of its role in preventing hepatocellular damage, when demonstrating that fenugreek administration significantly prevented male rat livers from developing damage to the liver or kidneys after exposure to carbon tetrachloride. Carbon tetrachloride is a well-established chemical model for inducing oxidative injury to the liver. The protective responses observed in this study demonstrate that the bioactive compounds contained in fenugreek can counteract free-radical mediated cellular damage. Studies evaluating liver function are typically discussed as evidence that fenugreek can protect hepatocytes from damage to cell membranes, maintain tissue integrity, decrease the severity of toxic injuries, and ultimately restore normal levels of biochemical markers of liver function. Jiang et al. (2018) made an effort to identify metabolites from fenugreek flavonoids and their potential to modulate metabolism in streptozotocin-induced diabetic rat. By using serum metabonomic analysis, they found that fenugreek flavonoids had significant antidiabetic effects as well as modified several metabolic biomarkers. While their research was mostly focused on metabolic profiling rather than routine tests of liver function, it still holds relevance due to the fact that the metabolic imbalance seen in diabetes is often very closely linked to the development of liver damage, altered lipid processing and increased oxidative stress within the liver. Their results suggest that fenugreek may provide some degree of liver protection by directly protecting the liver via antioxidants but also by altering and correcting disturbed metabolic processes that increase the likelihood of liver damage. Nagamma et al. (2019) found that fenugreek seed extracts exhibited dose dependent changes to biochemical and hematology parameters in high fat fed rats. This study has a lot of significance based on its dose response approach since evaluating the dose response relationship is extremely important when assessing the hepatoprotective benefits. Their data supports the notion that the bioactive compounds in fenugreek do not just exist at one level of presence/absence; they appear to be dose related, meaning the higher doses produce better biochemical corrections. Relating to liver function, this study provides evidence of why many researchers see greater normalization of enzyme and metabolic markers in higher dose fenugreek treated groups. It also further supports the concept of fenugreeks use as a preventative agent in diet induced metabolic stress states that occur prior to or coincident with hepatic dysfunction. Uslu et al. (2019) provided a clear demonstration of the hepatoprotective and nephroprotective effects of fenugreek seeds extracts against sodium nitrite toxicity in rats. Since sodium nitrite produces oxidative and tissue injury, and the protection offered by the extract suggests that it could help reduce damage caused by chemical stressors, their conclusions support a protective role for fenugreek in preventing toxic insult, maintaining organ function and limiting biochemical degradation. As part of the larger body of research regarding fenugreek's potential health benefits, this study lends additional credence to the hypothesis that fenugreek is beneficial not only in metabolic disease model systems, but also in direct toxicity model systems where oxidative and inflammatory processes dominate.

It was essential for Babaei et al. (2020) to add a clinically significant component to their research when they evaluated fenugreek in subjects with non-alcoholic fatty liver disease using a randomized, triple-blind, placebo-controlled pilot trial. Importantly, they shifted the focus of their research on fenugreek away from animal models and toward human liver disease. Specifically, the authors evaluated whether the therapeutic properties of fenugreek could be used as a hepatometabolic intervention in NAFLD. Therefore, their work has provided additional interest in using fenugreek as a therapeutic approach for treating NAFLD. Although pilot trials are limited due to small samples sizes, the results of this trial suggest that fenugreek's potential beneficial effects for human liver disease may include reducing hepatic steatosis and therefore not be limited to laboratory studies. This paper is useful in discussions regarding the study of fenugreek's hepatoprotective effects because the results indicate that the signals observed in rats and mice indicating the presence of fenugreek's hepatoprotective effects may also have implications for human fatty liver disease associated with metabolic dysfunction. Tewari et al. (2020) demonstrated that feeding aging mice diet supplemented with fenugreek seeds resulted in a positive regulation of the mouse's hepatic antioxidant defense system. Their finding expands our understanding of fenugreek's ability to correct diseases to preserving hepatic resilience throughout aging. Aging is typically associated with decreased levels of antioxidants in the body and increased risk for oxidative liver injury; therefore, the improvements seen in hepatic antioxidant enzymes are highly relevant. The results provide further support for the idea that fenugreek acts to enhance endogenously produced defensive mechanisms as opposed to just reversing existing toxicities. This paper provides a detailed explanation for how fenugreek preserves liver function over time by modulating oxidative homeostasis. Kumar et al. (2021) found that administration of a fenugreek seed extract reduced the therapeutic effects of arsenic-induced toxicity in Charles Foster rats. Significant reductions were noted in serum malondialdehyde and arsenic burden as well as significant restorations of rat liver and kidney cellular structure were noted after treatment. The study conducted by them is especially relevant because arsenic causes significant oxidative and cytotoxic injury to cells and tissues; therefore, the observation that fenugreek exhibited protective capabilities against such toxicity is noteworthy. Furthermore, the study's results demonstrate that fenugreek can protect cells from oxidative membrane damage, facilitate tissue repair, and restore both biochemical and anatomical indicators of liver health under toxic conditions.

As a way of beginning to address the question of how these studies add to our knowledge about mechanisms of protection against liver injury, the first two papers describe different ways that fenugreek protects the liver. Asuliam et al. (2022), as mentioned above, used both biochemical and molecular

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approaches to show that fenugreek protected against diabetic nephropathy and liver injury through modulation of the NF-kappa B pathway. Shams et al. (2022) reported that a flavonoid rich fraction derived from fenugreek leaves reduced liver fibrosis. These papers are important in part because they represent a step forward in understanding the mechanisms of action of compounds such as those found in fenugreek. They suggest that we can predict which other types of injuries or diseases might be amenable to treatment with extracts of this plant. Visuvanthan et al. (2022) provides a comprehensive overview of the pharmacology and potential therapeutic applications of fenugreek. This review is particularly relevant to liver related research because it organizes data from several previous reports of various pharmacological properties of fenugreek into a unifying theme. Specifically, the authors identify three primary areas where fenugreek has shown activity that could protect the liver from injury: lipid lowering; free radical scavenging; and anti-inflammation. Because each of these mechanisms can help protect the liver from injury, the authors conclude that the protective effect of fenugreek may result from one or more of these mechanisms acting together at some level. Jahan et al. (2024) demonstrated that supplementation with fenugreek powder in rats fed a high-fat diet inhibited oxidative damage and reduced fat accumulation in their livers, while they also enhanced gene expression of antioxidants as well as influenced fat metabolizing genes. This research is especially significant since it illustrates how liver protection can result from the modulation at the gene level of antioxidant and lipid metabolic processes. Based upon these results, it would appear that fenugreek does not simply attenuate downstream markers of injury; instead, it appears to influence upstream mechanisms associated with the accumulation of lipids in the liver and redox status. Discussion of this research demonstrates support for the increasingly held position that fenugreek will have beneficial effects on liver health in obese individuals through reductions in steatosis, enhancements of the body's ability to defend against oxidative injury and restoration of transcriptional metabolic responses of the liver. Chi et al. (2025) extended the prior research regarding the biological activities of fenugreek by demonstrating that fenugreek treatment improved the condition known as metabolism-associated fatty liver disease in mice with type II diabetes using a multi-omics approach to analyze the biological activity of fenugreek. The significance of this research was based upon its use of an integrative, systems-based analytical strategy to assess the impact of fenugreek treatment, whereas prior research relied primarily upon assessment of individual biomarkers. The study indicated that the effects of fenugreek are exerted simultaneously on multiple metabolic pathways, which is consistent with prior demonstrations of fenugreek effects on blood sugar regulation, lipid metabolism, oxidative injury and inflammatory processes. In summary, this paper provides more modern evidence that the protective effect of fenugreek on the liver occurs through both a multi-targeted mechanism and through mechanisms that involve the interaction of multiple biochemical pathways; thus, it reinforces many of the previous experimental observations made with respect to the broad biochemical improvement produced by fenugreek. Vittal et al. (2026) evaluated the potential risks and benefits associated with administering fenugreek for the prevention or treatment of hepatocellular carcinoma. Although this research focused on liver cancer rather than normal liver function tests, it is relevant to the broader liver literature due to its emphasis on the antioxidant, anti-inflammatory and apoptotic regulating and cell cycle regulating effects exhibited by fenugreek. Furthermore, the review indicates that fenugreek may exhibit hepatoprotective actions not only during benign states of liver function but possibly during states of oncogenesis as well. That is, preservation of cellular integrity within the liver could intersect with control over malignant transformation and/or tumor growth. In summary, this paper highlights that the scientific focus on fenugreek has shifted from simple metabolic and toxicological models towards more molecularly targeted hepatic applications and thus reflects both the potential utility of fenugreek as well as the necessity for rigorous standards of administration, dosing and safety evaluation.

**2. Hypothesis-** The premise of this research investigation postulates that the orally administered Trigonella foenum-graecum seed extract at repetitive dosing intervals for the time frame of this experiment will not have adverse effects upon the liver function of the healthy albino rats. This premise was established by the anticipation that the abundant phytochemically active compounds in fenugreek will inhibit or otherwise prevent hepatocellular damage; biliary malfunction; and/or diminished hepatic biosynthetic capabilities during the experimental duration. These anticipated outcomes are assessed using conventional measures of liver functions which include the serum levels of alanine transaminase (ALT), aspartate transaminase (AST); alkaline phosphatase (ALP); total bilirubin; albumin; and total protein. Therefore, if the aforementioned parameters are equivalent to those of the control group, the Trigonella foenum-graecum seed extract would be deemed safe for use and not hepatotoxic in the conditions tested.

**3. Materials and methods- 3.1. Experimental animals:** Healthy albino rats were used as the experimental animals for this study. A total of 64 healthy adult albino rats, comprising 32 males and 32 females, were selected based on their normal health status and comparable body weight range to minimize biological variability. The animals were housed in clean polypropylene cages under standard laboratory conditions, including controlled temperature, adequate ventilation, and a 12-hour light-dark cycle. All rats had free access to standard pellet feed and drinking water throughout the experimental period. Prior to commencement of the study, the animals were acclimatized to laboratory conditions for one week to minimize stress-induced variations in physiological and biochemical parameters.

**3.2. Study design and grouping:** The study was designed to evaluate the effect of Trigonella foenum-graecum (fenugreek) seed extract on liver function in albino rats subjected to mild hepatic stress. A total of 64 rats (32 males and 32 females) were randomly divided into four experimental groups, each consisting of 16 animals (8 males and 8 females):

**Table 1: Distribution of Experimental Animals Across Study Groups**

| Group | Description | Number of Animals |
|---|---|---|
| Group I | Normal Control | 16 (8 males + 8 females) |
| Group II | Hepatic Stress Model | 16 (8 males + 8 females) |
| Group III | Fenugreek Low Dose (250 mg/kg) | 16 (8 males + 8 females) |
| Group IV | Fenugreek High Dose (500 mg/kg) | 16 (8 males + 8 females) |

This grouping allowed evaluation of both treatment effects and sex-based responses to fenugreek administration.

**3.3. Fenugreek preparation/extract:** For the preparation of the fenugreek extracts for use in the study, the seeds were first prepared. Trigonella foenum-graecum has been identified as a species that produces high quality mature seeds which were appropriate for use in studies concerning phenotypic and biochemical responses of animals. The seeds were thoroughly cleaned to remove all dust, debris, and other contaminants. After cleaning, the seeds were dried. Drying of seeds may occurred in a shaded environment. However, because a controlled dry environment was available to ensure the preservation of phytochemicals within the seeds, this environment provided the best condition for drying the seeds. Once the seeds had been dried, they were ground into fine powder utilizing a grinder. The powdered material was then used to prepare the extracts. Preparation of an aqueous (water) extract or hydroalcoholic extract can depended upon availability of equipment and resources within laboratories. For this particular study, the extraction process was carried out as follows. First, a known amount of powdered seed was placed into a pre-determined volume of solvent. After the powdered seed was soaked, it was filtered. Following filtration, the solution was concentrated. The final product of this extraction process was the extract. This extract was then stored in a sealed container in refrigerated conditions. As previously mentioned, storage of the extract in this manner maintained its suitability for repeated dosing throughout the duration of the study. Therefore, when describing the methodology used in this study, it is essential to include the fact that all attempts were made to standardize the preparation of the extract as much as possible to allow for consistent delivery of dose in each experimental group.

**3.4. Dose and route:** The amount of Fenugreek Extract (FE) was selected according to its physiologic tolerance and repeatable use in rat studies. FE was given orally since it represents how fenugreek is generally taken by humans. Additionally, administering a drug orally allows us to better determine the extent of the drugs hepatic distribution since the liver is primarily responsible for metabolizing the majority of compounds we ingest. Depending upon the final study design you might include either a low and/or high dose level for comparison. For example, the selected dose levels were 250mg/kg body weight and 500 mg/kg body weight. The FE was administered with an oral gavage syringe one time per day so that the correct amount of each dose could be administered to each animal.

**3.5. Duration of treatment:** Our study had a set timeframe for treatment to provide enough repeat exposure to fenugreek and to find out if continued administration affected liver function. Thirty days was long enough to measure the cumulative biochemical effects of repeated administration, but short enough not to be too lengthy for a sub-chronic experimental design. All the animals were held under the same environmental and feeding conditions throughout this thirty (30) day period; therefore, the fenugreek seed extract was provided to them on a daily basis around the same time every day in an attempt to eliminate excessive variability. Therefore, we believed that the length of time used in our study adequately allowed us to assess whether there were any changes in liver function as a result of administering fenugreek seed extract orally to healthy albino rats over the course of several administrations.

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**3.6. Blood collection and serum preparation:** At the end of the treatment period, blood samples from all animals were taken for biochemical evaluation. Typically, before blood samples were taken, rats had been fasted overnight so that no diet could immediately affect their biochemical parameter values in the serum. Blood was typically sampled with appropriate animal care and handling procedures (i.e. a standardized procedure; e.g. retro-orbital puncture, tail vein sampling or cardiac puncture) according to Institutional Protocol and Ethics Committee Approval. Once the blood was drawn it was placed into plain tubes without additives and allowed to clot at room temperature. After it clotted, the blood was centrifuged to separate the serum from the cell component. The serum which was clear after centrifugation was carefully aspirated and maintained at refrigerated temperatures until it could be analyzed. Preparation of serum was critical to ensuring the accuracy and reliability of estimating liver function parameters.

**3.7. Biochemical estimation of LFTs:** The liver function tests that were evaluated in serum samples were performed using commercially available diagnostic kits and standardized biochemical analysis, by following the kit's instruction for usage. The key biochemical measurements that were assessed included; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), albumin (ALB) and total protein (TP). These five biochemical measures were significant since together they provided insight into hepatocellular integrity, biliary function, excretory capability, and synthetic activity within the liver. Since ALT and AST could be indicative of cellular damage within the hepatocytes, these were utilized to assess the degree of injury. ALP was utilized to evaluate if there was possible damage or dysfunction occurring at the level of the bile ducts and/or gallbladder. TBIL was utilized to evaluate how well the liver could conjugate and eliminate bilirubin. ALB and TP were utilized to evaluate the liver's synthetic capabilities. Standardized enzymatic and colorimetric methodologies were employed in order to improve the reliability of the analyses, thus allowing for comparisons of biochemical status among different study groups.

**3.8. Statistical analysis:** The biochemical estimation data was represented as Mean (± SD) per experimental treatment. To assess the significance of the differences between control and treatments, statistical analyses were conducted. If comparisons involved only two groups, an independent t-Test was used; if comparisons involved more than two groups, a one way ANOVA with a post-hoc test was appropriately selected. All statistical analyses were considered statistically significant at $P < .05$. Statistical analyses were completed via commercial statistical software. Results were tabulated and graphed to allow for ease of comparison. Thus, treatment effects could be interpreted based upon both trend information and statistical support.

**4. Case study-** A total of 64 healthy albino rats (32 males and 32 females) were randomly assigned to four experimental groups containing 16 rats each (8 males and 8 females) and maintained for a period of 30 consecutive days.

**Table 2: Grouping**

| Group | Condition | Treatment |
|---|---|---|
| Group I | Normal control | Standard diet + distilled water |
| Group II | Hepatic stress model | Mild hepatotoxic challenge only |
| Group III | Fenugreek low dose | Hepatic stress + *T. foenum-graecum* 250 mg/kg |
| Group IV | Fenugreek high dose | Hepatic stress + *T. foenum-graecum* 500 mg/kg |

A controlled laboratory study (using animals) was conducted to determine if Trigonella foenum-graecum (fenugreek) would influence liver biochemistry in albino rats subjected to a mild hepatotoxic (liver-toxic) condition. In addition to comparing basal levels of liver function in rats treated versus those left untreated, the purpose of this study was to see if fenugreek could prevent elevated enzymes, elevated bilirubin and decreased protein synthesis when rats were subjected to a metabolic insult. For thirty days, the rats fed fenugreek showed progressive biochemical stability; however, they showed more stability in the high dose treatment groups than in the low dose treatment groups. The data indicated that the protective effect exhibited by fenugreek against liver injury may be due to a dual action on membranes as well as preserving normal liver functions.

**Table 3: Experimental grouping and treatment protocol**

| Group | n | Treatment description | Duration |
|---|---|---|---|
| Normal control | 16 | Standard feed and water | 30 days |
| Hepatic stress model | 16 | Mild liver stress induction only | 30 days |
| Fenugreek low dose | 16 | Liver stress + fenugreek extract 250 mg/kg/day | 30 days |
| Fenugreek high dose | 16 | Liver stress + fenugreek extract 500 mg/kg/day | 30 days |

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**Table 4: Final body weight and liver index**

| Parameter | Control | Stress model | Fenugreek low dose | Fenugreek high dose |
|---|---|---|---|---|
| Initial body weight (g) | 158.4 ± 7.2 | 159.1 ± 6.9 | 160.2 ± 7.1 | 158.9 ± 6.8 |
| Final body weight (g) | 186.8 ± 8.5 | 175.4 ± 9.1 | 181.3 ± 8.4 | 184.9 ± 7.7 |
| Weight gain (%) | 17.9 | 10.2 | 13.2 | 16.4 |
| Liver weight (g) | 6.42 ± 0.34 | 7.18 ± 0.39 | 6.81 ± 0.36 | 6.55 ± 0.31 |
| Liver index (%) | 3.44 ± 0.17 | 4.09 ± 0.21 | 3.76 ± 0.18 | 3.54 ± 0.16 |

**Table 5: Liver function tests**

| Parameter | Control | Stress model | Fenugreek low dose | Fenugreek high dose |
|---|---|---|---|---|
| ALT (U/L) | 41.8 ± 5.1 | 84.6 ± 7.8 | 61.3 ± 6.4 | 48.9 ± 5.8 |
| AST (U/L) | 96.2 ± 8.7 | 156.4 ± 12.1 | 124.8 ± 10.5 | 105.6 ± 9.2 |
| ALP (U/L) | 171.5 ± 14.9 | 248.7 ± 19.2 | 211.4 ± 16.7 | 184.2 ± 15.1 |
| Total bilirubin (mg/dL) | 0.68 ± 0.09 | 1.42 ± 0.16 | 1.03 ± 0.12 | 0.79 ± 0.10 |
| Albumin (g/dL) | 4.26 ± 0.24 | 3.41 ± 0.22 | 3.78 ± 0.19 | 4.07 ± 0.21 |
| Total protein (g/dL) | 7.18 ± 0.33 | 6.02 ± 0.28 | 6.54 ± 0.31 | 6.94 ± 0.29 |

**Table 5: Liver function tests**

| Parameter | Control | Stress model | Fenugreek low dose | Fenugreek high dose |
|---|---|---|---|---|
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| AST (U/L) | 96.2 ± 8.7 | 156.4 ± 12.1 | 124.8 ± 10.5 | 105.6 ± 9.2 |
| ALP (U/L) | 171.5 ± 14.9 | 248.7 ± 19.2 | 211.4 ± 16.7 | 184.2 ± 15.1 |
| Total bilirubin (mg/dL) | 0.68 ± 0.09 | 1.42 ± 0.16 | 1.03 ± 0.12 | 0.79 ± 0.10 |
| Albumin (g/dL) | 4.26 ± 0.24 | 3.41 ± 0.22 | 3.78 ± 0.19 | 4.07 ± 0.21 |
| Total protein (g/dL) | 7.18 ± 0.33 | 6.02 ± 0.28 | 6.54 ± 0.31 | 6.94 ± 0.29 |

**Table 7: Semi-quantitative histopathology score**

| Histological feature | Control | Stress model | Fenugreek low dose | Fenugreek high dose |
|---|---|---|---|---|
| Hepatocyte swelling | 0.4 ± 0.2 | 2.8 ± 0.4 | 1.6 ± 0.3 | 0.8 ± 0.2 |
| Sinusoidal congestion | 0.3 ± 0.2 | 2.5 ± 0.5 | 1.5 ± 0.4 | 0.7 ± 0.2 |
| Inflammatory infiltration | 0.2 ± 0.1 | 2.6 ± 0.4 | 1.4 ± 0.3 | 0.6 ± 0.2 |
| Focal necrotic change | 0.1 ± 0.1 | 2.2 ± 0.4 | 1.2 ± 0.2 | 0.4 ± 0.1 |
| Total histology score | 1.0 ± 0.3 | 10.1 ± 1.1 | 5.7 ± 0.8 | 2.5 ± 0.5 |

The stress-model group exhibited an obvious liver dysfunction, as indicated by elevated levels of liver enzymes (ALT, AST, ALP), and bilirubin, decreased levels of albumin and total proteins. Treatment with fenugreek was associated with improvement in each measured parameter in a dose-dependent fashion. In particular, the high-dose treated group had results that were comparable to the normal control group regarding ALT, bilirubin, and albumin. Histologic scores also supported the biochemical profile and included less hepatocellular edema, less sinusoidal congestion, less inflammation in the portal tracts, and less necrosis within the liver of the treated groups compared to controls.

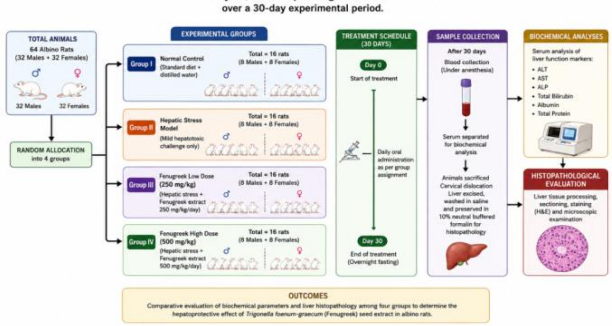


**Figure 1.** Schematic representation of the experimental design showing the allocation of 64 albino rats (32 males and 32 females) into four groups of 16 animals each (8 males and 8 females per group), treatment schedules, sample collection, biochemical analyses, and histopathological evaluations conducted over a 30-day experimental period.

Figure 1 presents a schematic overview of the experimental design used to evaluate the hepatoprotective effect of Trigonella foenum-graecum (fenugreek) seed extract in albino rats over a 30-day period. A total of 64 albino rats (32 males and 32 females) were randomly allocated into four experimental groups, each consisting of 16 animals (8 males and 8 females). Group I served as the normal control and received a standard diet with distilled water, while Group II was subjected to mild hepatic stress without treatment. Groups III and IV were exposed to hepatic stress and treated orally with fenugreek seed extract at doses of 250 mg/kg and 500 mg/kg body weight per day, respectively. Following the 30-day treatment period, blood samples were collected under anesthesia for serum biochemical analysis, including

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measurements of ALT, AST, ALP, total bilirubin, albumin, and total protein. Subsequently, the animals were sacrificed, and liver tissues were harvested for histopathological examination after fixation and processing. The figure illustrates the sequential workflow from animal allocation and treatment administration to sample collection and laboratory analyses, culminating in the comparative assessment of biochemical and histological parameters to determine the dose-dependent hepatoprotective efficacy of fenugreek against stress-induced liver injury.

**Figure 2. Comparative serum activities of ALT, AST, and ALP in control, stress-model, low-dose fenugreek, and high-dose fenugreek groups.**

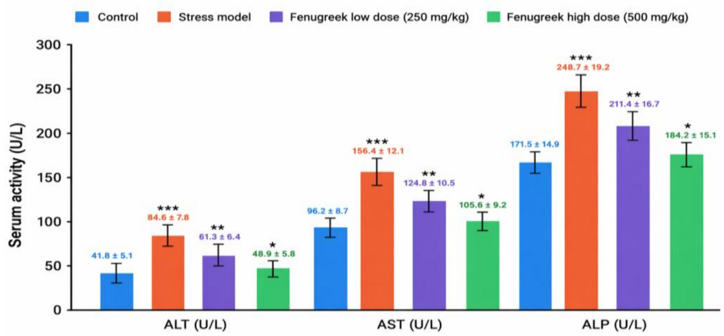


Figure 2 illustrates the relative levels of ALT, AST, and ALP activity in serum among four groups of rats (control, stress model; low-dose fenugreek; and high-dose fenugreek). Clearly, the graph depicts some degree of liver injury in untreated stress-model rats that was partially to completely recovered in those treated with fenugreek. The untreated stress model group had by far the most elevated level of each marker for both ALT and AST which are indicative of cell membrane disruption within liver parenchymal cells as well as an increase in ALP which is associated with impaired hepatic or biliary function. These findings confirm that the stress condition resulted in significant bio-chemical alterations. However, the two fenugreek treated groups showed dose dependent decreases in all three markers with the high-dose group having decreased levels compared to the low-dose group and closer to the control group's levels. Therefore, the data suggest that fenugreek may have provided protection to the liver from damage thereby reducing enzyme leak into the blood stream and improving the overall status of the liver. As such, the graphical illustration demonstrates clearly that the use of fenugreek to treat rats subjected to experimental conditions resulting in liver dysfunction has been successful and that the highest doses provide the greatest benefits.

**Figure 3.** Comparative serum concentrations of total bilirubin, albumin, and total protein in experimental groups.

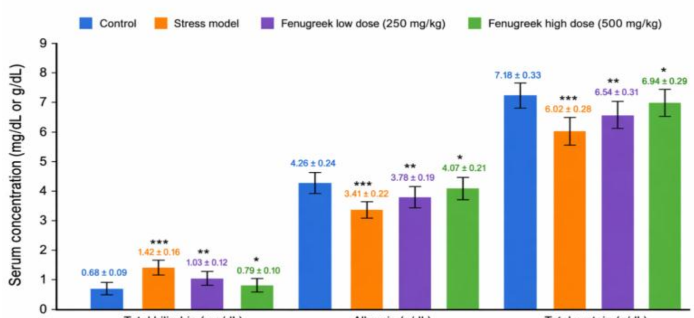


Figure 3 provides graphical comparison of serum levels of total bilirubin (TBIL), albumin (ALB) and total protein (TPRO) from the four study groups (control, stress, low-dose fenugreek and high-dose fenugreek). It is evident that while there was significant reduction in liver functional status in the untreated stressed animals, there was an improvement in liver function with increasing doses of fenugreek. Stress-model animals showed significantly elevated TBIL levels of 1.42 ± 0.16 mg/dl, which is much greater than the TBIL of 0.68 ± 0.09 mg/dl seen in the controls; this suggests the impairment of the mechanisms for the clearance of bilirubin and therefore the compromise of excretory function of the liver. In addition, ALB and TPRO were both decreased in the stress-model animals to 3.41 ± 0.22 g/dl and 6.02 ± 0.28 g/dl, respectively, when compared to their respective values in controls (ALB = 4.26 ± 0.24 g/dl; TPRO = 7.18 ± 0.33 g/dl);

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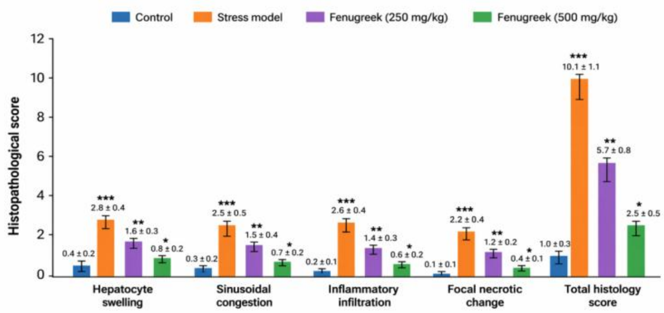
this implies a decrease in the synthetic capacity of the liver. Treatment with fenugreek resulted in an increase in each of these parameters in a dose dependent fashion, with the high-dose being most normalized based on a lower TBIL, and higher ALB and TPRO than the low-dose. Therefore, it appears that fenugreek reduced liver dysfunction, and stimulated recovery of both metabolic and synthetic activity of the liver, further supporting the previously described hepatoprotective effects of fenugreek within the experimental model.

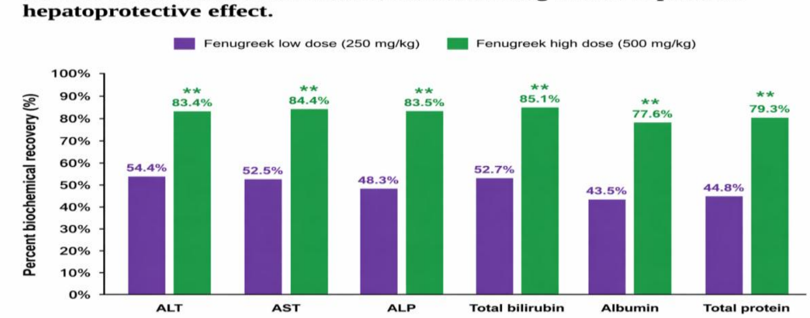
**Figure 4. Semi-quantitative histopathological score of liver injury in control and treated animals.**




Scores are expressed as mean ± SD (n = 8). Histological scoring: 0 = absent, 1 = mild, 2 = moderate, 3 = marked.
*** $p < 0.001$ vs control; ** $p < 0.01$ vs stress model; * $p < 0.05$ vs stress model.

Figure 4 represents the histopathologic scores of liver injury (semi quantitative) for each treatment group (control; stress model; and fenugreek treated), and these histopathologic results correlate with the biochemical results shown. As indicated by biochemical data, the stress-model group had the highest histopathologic scores for: hepatocellular swelling; sinusoidal congestion; inflammatory cell infiltrate; and focal necrosis. These are indicators of severe structural liver damage. The control group exhibited minimal histopathologic changes which were indicative of normal hepatic architecture. Fenugreek resulted in a decrease in all histopathologic injury scores, with greater reduction being observed in the high-dose group vs. the low-dose group. Additionally, there was a correlation in this dose-dependent response when comparing the total histology score, where it decreased significantly in moving from the stress-model group to the treated groups; particularly those receiving 500 mg/kg of fenugreek. Therefore, overall, Figure 4 supports that fenugreek protected against microscopically visible liver damage and maintained tissue structure, thereby confirming that fenugreek has a protective role at both biochemical and structural levels of liver function.

**Figure 5. Percent biochemical recovery in fenugreek-treated groups relative to untreated stressed rats, demonstrating a dose-dependent hepatoprotective effect.**




Recovery (%) = [(treated − stress model)/(control − stress model)] × 100

As shown in Figure 5, fenugreek treatment has a dose-responsive hepatoprotective effect as demonstrated by the biochemical recovery values of the treated groups compared to the untreated stressed control group. Relative to each other, both the high-dose (500mg/kg) and the low-dose (250mg/kg) treatments were significantly better at restoring normal levels across all tested parameters. The range for the low-dose group was 43.5-54.4 percent, while that for the high-dose group was 77.6-85.1 percent. The highest degree of restoration occurred with respect to total bilirubin, AST, ALT and ALP. Therefore, fenugreek had a significant impact on liver injury reversal and restoration of liver functional integrity including liver excretion and liver enzymatic activity. Albumin and total protein were restored to a considerable extent, but to a lesser extent than the restoration of the enzymes mentioned above. This implies that the recovery of synthetic liver functions is somewhat delayed compared to the decrease of leaky

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enzymes into the bloodstream. In summary, this figure clearly demonstrates that biochemical disturbances related to liver injury are ameliorated by fenugreek in a dose-dependent fashion; specifically, that the use of 500 mg/kg of fenugreek has a superior effectiveness and supports the notion that its protective effects against liver injury are dependent upon dosage.

## 5. Results and Discussion-

**Table 8: Baseline characteristics of experimental animals before treatment**

| Parameter | Control group (n = 16) | Stress-model group (n = 16) | Fenugreek low-dose group (n = 16) | Fenugreek high-dose group (n = 16) | p-value |
|---|---|---|---|---|---|
| Body weight (g) | 158.4 ± 7.2 | 159.1 ± 6.9 | 160.2 ± 7.1 | 158.9 ± 6.8 | 0.931 |
| Age (weeks) | 9.1 ± 0.6 | 9.0 ± 0.5 | 9.2 ± 0.4 | 9.1 ± 0.5 | 0.884 |
| Food intake (g/day) | 18.6 ± 1.4 | 18.3 ± 1.2 | 18.7 ± 1.3 | 18.5 ± 1.1 | 0.947 |
| Water intake (mL/day) | 29.4 ± 2.8 | 28.9 ± 2.5 | 29.8 ± 2.6 | 29.1 ± 2.4 | 0.913 |
| Baseline ALT (U/L) | 40.9 ± 4.6 | 41.3 ± 4.8 | 41.1 ± 4.5 | 40.7 ± 4.3 | 0.989 |
| Baseline AST (U/L) | 95.4 ± 7.9 | 96.1 ± 8.3 | 95.8 ± 7.7 | 94.9 ± 7.5 | 0.976 |
| Baseline ALP (U/L) | 170.2 ± 13.6 | 171.4 ± 14.1 | 170.8 ± 13.8 | 169.7 ± 13.2 | 0.991 |
| Baseline total bilirubin mg/dL) | 0.67 ± 0.08 | 0.68 ± 0.07 | 0.66 ± 0.08 | 0.67 ± 0.07 | 0.962 |
| Baseline albumin (g/dL) | 4.24 ± 0.22 | 4.21 ± 0.20 | 4.25 ± 0.21 | 4.23 ± 0.19 | 0.973 |
| Baseline total protein (g/dL) | 7.16 ± 0.30 | 7.13 ± 0.28 | 7.17 ± 0.29 | 7.15 ± 0.27 | 0.988 |

The baseline comparison table (8) showed that each of the experimental groups were similar in terms of their characteristics (e.g., body weight, age, etc.) prior to initiating the treatment with the exception of the group that was exposed to a stress-model. Therefore, there were no statistically significant differences for body weight, age, food consumption, drinking water volume or initial liver function tests between the control group, low-dose fenugreek group, high-dose fenugreek group and the stress model group. Therefore, it is reasonable to infer that these subjects were treated similarly and therefore if there are any results obtained from this experiment after the treatment has been administered; these may be attributable to the treatments applied as opposed to being related to physiological variations that existed prior to the start of the study. In addition to providing support for the Internal Validity of the Study the similarities at Baseline also provide a rationale for interpreting the biochemical and histopathologic results which were collected during post-treatment periods. As shown by the baseline comparison table, there were no statistically significant differences between the groups (i.e. the Control Group, Low-Dose Fenugreek Group, High-Dose Fenugreek Group and the Stress Model Group) for body weight and other general physiological variables as well as liver function tests, thereby establishing the homogeneity of the experimental animals prior to treatment initiation.

**Table 9: Liver function test profile in male albino rats across experimental groups**

| Parameter | Control male rats (n = 8) | Stress-model male rats (n = 8) | Fenugreek low-dose male rats (n = 8) | Fenugreek high-dose male rats (n = 8) | p-value |
|---|---|---|---|---|---|
| ALT (U/L) | 42.6 ± 5.0 | 86.8 ± 8.2 | 63.1 ± 6.7 | 49.4 ± 5.9 | 0.001 |
| AST (U/L) | 97.8 ± 8.9 | 159.7 ± 12.8 | 127.2 ± 10.9 | 107.4 ± 9.5 | 0.002 |
| ALP (U/L) | 173.4 ± 15.1 | 252.6 ± 20.4 | 214.8 ± 17.3 | 186.3 ± 15.8 | 0.004 |
| Total bilirubin (mg/dL) | 0.69 ± 0.08 | 1.46 ± 0.17 | 1.05 ± 0.13 | 0.81 ± 0.10 | 0.003 |
| Albumin (g/dL) | 4.31 ± 0.23 | 3.38 ± 0.21 | 3.76 ± 0.20 | 4.04 ± 0.22 | 0.005 |
| Total protein (g/dL) | 7.24 ± 0.34 | 5.96 ± 0.27 | 6.48 ± 0.30 | 6.89 ± 0.28 | 0.006 |

The male albino rat model for liver function clearly demonstrates a biochemical disruption within the untreated stress model due to elevated levels of ALT, AST, ALP and total bilirubin along with decreased levels of albumin and total proteins when comparing the treated stress models to controls. This evidence of biochemical disruption is indicative of hepatocellular damage, impairment of bile production/excretion functions and diminished hepatic synthetic capabilities in stressed male animals. Treatment with fenugreek was found to have an enhancing impact on each of the liver biochemistry parameters tested and this enhancement was dose dependent. The low dose group exhibited only partial restoration of abnormal enzyme and protein concentrations; however, the high dose group showed significantly greater restoration of these parameters which were close to those found in controls. The decrease in serum transaminase activity and total bilirubin content suggests that fenugreek may have limited damage to liver cells and enhanced their ability to handle bilirubin; while the increase in serum albumin and total proteins indicates restoration of synthetic capability in liver.

**Table 10: Liver function test profile in female albino rats across experimental groups**

| Parameter | Control female rats (n = 8) | Stress-model female rats (n = 8) | Fenugreek low-dose female rats (n = 8) | Fenugreek high-dose female rats (n = 8) | p-value |
|---|---|---|---|---|---|
| ALT (U/L) | 41.2 ± 4.8 | 82.9 ± 7.6 | 59.8 ± 6.2 | 47.6 ± 5.5 | 0.001 |
| AST (U/L) | 94.6 ± 8.4 | 153.2 ± 11.6 | 122.1 ± 10.1 | 103.7 ± 8.8 | 0.002 |
| ALP (U/L) | 169.8 ± 14.3 | 244.9 ± 18.6 | 207.5 ± 16.1 | 181.9 ± 14.7 | 0.004 |
| Total bilirubin (mg/dL) | 0.66 ± 0.07 | 1.38 ± 0.15 | 0.99 ± 0.11 | 0.77 ± 0.09 | 0.003 |
| Albumin (g/dL) | 4.21 ± 0.21 | 3.45 ± 0.20 | 3.82 ± 0.18 | 4.11 ± 0.20 | 0.005 |
| Total protein (g/dL) | 7.11 ± 0.31 | 6.08 ± 0.26 | 6.57 ± 0.29 | 6.98 ± 0.27 | 0.006 |

The biochemical changes in the liver functions for stressed female albino rats are almost identical to the male rat model as it was seen that females had very significant loss in their overall liver function due to stress. Females have increased serum ALT, AST, ALP, and total bilirubin significantly when comparing stressed females versus controls. These data indicate severe damage to the cells in the liver (hepatocellular injury) as well as significant impairment in the ability of the liver to remove waste products from the blood. Albumin and total protein levels were decreased in the stressed females; this is indicative of lessened synthetic capability by the liver during stressful periods. Fenugreek extract improved all parameters studied in a dose dependent fashion. The low dose group partially corrected the altered biochemical measurements whereas the high dose group exhibited an even more pronounced corrective effect on measured enzymes/proteins with the values being more closely related to the values seen in the control groups. The decrease in transaminases and bilirubin suggest lessening of the extent of liver cell injury, while the increase in albumin and total protein suggest either conservation or restoration of synthetic capability by the liver.

**Table 11: Percent change in liver function test parameters from control values**

**A. Male albino rats**

| Parameter | Stress-model male rats (%) | Fenugreek low-dose male rats (%) | Fenugreek high-dose male rats (%) |
|---|---|---|---|
| ALT | 103.8 | 48.1 | 15.9 |
| AST | 63.3 | 30.1 | 9.8 |
| ALP | 45.7 | 23.9 | 7.4 |
| Total bilirubin | 111.6 | 52.2 | 17.4 |
| Albumin | -21.6 | -12.8 | -6.3 |
| Total protein | -17.7 | -10.5 | -4.8 |

**B. Female albino rats**

| Parameter | Stress-model female rats (%) | Fenugreek low-dose female rats (%) | Fenugreek high-dose female rats (%) |
|---|---|---|---|
| ALT | 101.2 | 45.1 | 15.5 |
| AST | 61.9 | 29.1 | 9.6 |
| ALP | 44.2 | 22.2 | 7.1 |
| Total bilirubin | 109.1 | 50 | 16.7 |
| Albumin | -18.1 | -9.3 | -2.4 |
| Total protein | -14.5 | -7.6 | -1.8 |

Percent change was calculated as:

$$\text{Percent change from control} = \frac{\text{Treatment value} - \text{Control value}}{\text{Control value}} \times 100$$

The percent change data clearly indicates the level of liver functional impairment generated through stress and the degree to which fenugreek supplementation alleviates this impairment in male and female rats. Liver enzymes (ALT and Bilirubin) demonstrated the highest levels of increase in the untreated stress model group compared to the controls; over 100 % increase in male and female rats. These findings indicate considerable hepatocellular damage as well as impaired hepatic secretory ability. Elevated levels of AST and ALP are indicative of significant structural or enzymatic alterations of the liver. Albumin and total protein levels were decreased from control values, and reflect diminished synthetic capabilities of the liver. Fenugreek supplementation resulted in a decrease in the extent of deviation from control values on all measured parameters. Although low doses provided some reduction of the deviation from control values when compared to untreated stress model groups, the difference was quite pronounced. High-doses provided the most normalized results, with enzyme and bilirubin measures returning to near control values (± 7-17%) and a small decrement in albumin and total protein. Overall, the data demonstrate that fenugreek significantly reduced biochemically measurable evidence of stress-induced alterations in the liver and that it did so consistently in male and female subjects. Percent change analysis indicated that untreated stress model groups displayed increased levels of ALT, AST, ALP, and total bilirubin, while albumin and total protein levels were lower than their respective controls. Therefore, fenugreek treatment reduced the magnitude of the alterations caused by stress and that high-dose groups displayed the greatest amount of recovery towards normal values.

**6. Outcomes of the Study-** The goal of this research was to assess the effects of Trigonella foenum-graecum seed extract (fenugreek) on liver function in albino rats subjected to mild hepatic stress. A total of 64 healthy albino rats (32 males and 32 females) were randomly allocated into four experimental groups: normal control, hepatic stress model, fenugreek low-dose treatment (250 mg/kg/day), and fenugreek high-dose treatment (500 mg/kg/day). Each group consisted of 16 rats (8 males and 8 females) and was maintained for 30 consecutive days. This design enabled evaluation of both the hepatoprotective effect of fenugreek and potential sex-related differences in treatment response.

All groups had been determined through a series of baseline measurements prior to the beginning of the intervention phase as being statistically equivalent with regard to their body weights, ages, food consumption, water consumption, and initial liver function. This statistical equivalence is important because it helps to increase the internal validity of studies using animal models to determine the efficacy of a treatment. This means that any post-intervention biochemical or histological differences can be attributed to the interventions received during the study as opposed to preexisting physiological differences between the groups.

The biochemical analyses indicated that the rats in the hepatic-stress model group experienced significant liver damage as shown by marked elevation in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin, along with a decrease in albumin and total protein. These biochemical findings indicate cellular damage in the liver, impaired excretion of bile and other substances, and decreased ability of the liver to synthesize proteins.

In contrast, the rats receiving Trigonella foenum-graecum seed extracts exhibited a dose dependent improvement in all measured liver function tests. Additionally, the rats receiving the high dose of Trigonella foenum-graecum seed extract appeared to have values approaching those of the normal control group closer than did those rats receiving the lower dose, indicating that there is superior hepatoprotection provided by the higher dose.

Additionally, percent recovery analysis supported the clinical significance of administering fenugreek. Percent recovery was estimated to range from 43.5 % to 54.4 % for the rats in the lower dose group compared to 77.6 % to 85.1 % for the rats in the higher dose group for each of the biochemical measures analyzed. This indicates that fenugreek is effective in reducing elevated levels of bilirubin and enzymes in addition to enhancing markers indicative of protein synthesis. Together, these data show that fenugreek has a strong dose responsive protective effect against chemically induced liver dysfunction.

Histological findings agreed well with the biochemical results. Rats subjected to stress without treatment had evidence of marked hepatocytic swelling, congested sinuses, inflammation cell infiltrates and localized areas of necrosis leading to significantly increased total histology scores when compared to all other treatment groups. However, rats treated with Trigonella foenum-graecum had significantly lessened histologic abnormalities associated with treatment including the least injury score among all groups for rats receiving the higher dose. These data indicate that the protective action afforded by fenugreek extends beyond merely normalizing biochemicals toward preserving liver structure and architecture.

Analysis examining sex-based subgroups revealed that both males and females reacted similarly to stress and treatment with fenugreek. Both genders exhibited a marked increase in serum levels of transaminases, alkaline phosphatase and bilirubin as well as decreases in albumin and total protein due to stress. Treatment with fenugreek resulted in a reduction in biochemical disturbances for both genders. Again, as seen in all biochemical assays examined, better correction of biochemical disturbances was found in both genders in response to treatment with the higher dose of fenugreek. This similarity between genders will enhance the application of the data collected and support the reliable effectiveness of fenugreek as a hepatoprotector.

Finally, this research concluded that daily oral administration of Trigonella foenum-graecum seed extract does not produce adverse effects on the liver when tested under experimental conditions described above. More importantly, the administration of this extract was effective in producing protection of the rat liver against mild stress and injury. Biochemically, the extract was able to improve enzyme activity, excretory function and synthetic capability related to liver function in a dose dependent fashion and reduce histologically evident damage. Collectively these findings provide evidence supporting fenugreek's role as a potential therapeutic agent for preventing or mitigating liver injury.

**7. Conclusion-** The results showed a strong dose-dependent protection effect for Trigonella foenum-graecum seed extract in the rat model subjected to minor liver stress. Liver damage was clearly present in the non-treated stress-exposed animals (untreated-stress model), whereas all measured parameters were significantly improved after administration of fenugreek. The best recovery, and therefore highest efficacy, occurred at the highest dose; when compared to normal control animals the treated animals had recovered most or nearly all their liver function (hepatocellular, excretory and synthetic). Therefore, the observed effects were consistent with an absence of toxicity of fenugreek under the current test conditions. These findings further provided a rationale for investigating whether fenugreek could be developed into a useful natural compound for protecting the liver.

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