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Pharmacology Section

Hepatoprotective and Hypolipidaemic Effects of *Bacopa monnieri* in High-fructose Corn Syrup-induced Non Alcoholic Fatty Liver Disease in Male Wistar Rats: An Experimental Study

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ABSTRACT

Introduction: Non Alcoholic Fatty Liver Disease (NAFLD) is a global health concern strongly linked to excessive dietary fructose intake. High-Fructose Corn Syrup (HFCS) contributes significantly to metabolic dysregulation, hepatic steatosis and inflammation. *Bacopa monnieri*, a traditional Ayurvedic herb with antioxidant properties, was investigated for its potential protective effects against HFCS-induced hepatic injury and dyslipidaemia.

Aim: To evaluate the hepatoprotective and hypolipidaemic potential of an alcoholic extract of *Bacopa monnieri* against HFCS-induced NAFLD in Wistar albino rats.

Materials and Methods: This 12-week experimental study was conducted at the Department of Pharmacology, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India, from March 2024 to February 2025 and included 18 Wistar albino rats divided into three groups (n = 6): Group I (Normal Control), Group II (HFCS Control) and Group III (HFCS + *Bacopa monnieri* 40 mg/kg, per os). Hepatic injury was assessed using serum markers: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Gamma-Glutamyl Transferase (GGT). Lipid profile parameters such as Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C) were analysed. Liver sections were subjected to histopathological examination {Haematoxylin and Eosin (H&E) staining}. Statistical analysis was performed using One-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test ($p<0.05$).

Results: Chronic HFCS administration caused significant hepatocellular injury, evidenced by elevated serum AST, ALT and GGT levels compared to the normal control group ($p<0.05$). The HFCS group also exhibited profound dyslipidaemia, characterised by increased TC, TG and LDL-C levels, along with reduced HDL-C levels. Treatment with *Bacopa monnieri* significantly attenuated these enzymatic elevations and reversed lipid profile alterations ($p<0.05$). Histopathological findings corroborated the biochemical results, showing reduced macrovesicular steatosis, hepatocyte ballooning and inflammatory infiltration, with restoration of near-normal hepatic architecture.

Conclusion: *Bacopa monnieri* demonstrates significant hepatoprotective and hypolipidaemic activity against HFCS-induced liver injury, as evidenced by attenuation of serum liver enzymes, improvement in lipid profile parameters and reduction in histopathological alterations. These findings suggest its potential utility as a therapeutic adjunct in the management of NAFLD.

Keywords: Fructose, Inflammation, Metabolic Syndrome, Plant extracts

INTRODUCTION

The NAFLD has emerged as a major global health challenge and is now recognised as the most prevalent chronic liver disease globally. Recent systematic reviews and meta-analyses estimate that NAFLD affects approximately 30% of the global adult population, with prevalence continuing to rise across both developed and developing regions [1-3]. NAFLD is strongly associated with obesity, type 2 diabetes mellitus and other components of the metabolic syndrome; its prevalence reaches 70-75% among individuals with obesity and exceeds 50% in those with type 2 diabetes [4,5]. With the global escalation of metabolic disorders, NAFLD is projected to become the leading cause of liver-related morbidity and mortality in the coming decades.

The rising prevalence of NAFLD is closely associated with increased dietary consumption of fructose, particularly through sugar-sweetened beverages and processed foods. Controlled trial evidence demonstrates that high fructose intake significantly elevates hepatic fat content, serum ALT and TG levels, indicating hepatic lipid accumulation and metabolic disturbance [6]. Experimental studies employing HFCS further confirm that fructose-enriched or HFCS-containing diets promote hepatic steatosis, dyslipidaemia and insulin resistance in rodent models [7,8]. Together, these findings indicate that dietary fructose, particularly in the form of HFCS, contributes to the development and progression of NAFLD.

Sustained fructose exposure is associated with hepatocellular injury, as evidenced by elevation of serum liver enzymes, inflammatory infiltration and progressive architectural distortion of the liver parenchyma [6,8]. Histopathologically, NAFLD is characterised by macrovesicular steatosis, hepatocyte ballooning and lobular inflammation, which constitute the hallmark features evaluated by the NAFLD Activity Score (NAS) [9]. In advanced stages, these changes progress to fibrosis and cirrhosis, underpinning the transition from simple steatosis to Non Alcoholic Steatohepatitis (NASH) and its associated complications.

Although resmetirom was recently approved for the treatment of non cirrhotic Metabolic Dysfunction-associated Steatohepatitis (MASH) with moderate to advanced liver fibrosis (stages F2–F3) [10], lifestyle modification remains the primary management strategy for the broad spectrum of NAFLD [11,12]. Long-term adherence to lifestyle interventions is often poor and therapeutic responses are variable, underscoring the need for complementary pharmacological strategies [10].

Traditional herbal medicines have attracted considerable attention due to their multi-targeted pharmacological profiles and favourable safety margins. Among these, *Bacopa monnieri* (Brahmi), a classical Ayurvedic herb widely used in traditional medicine, has demonstrated hepatoprotective, hypolipidaemic and metabolic regulatory effects in experimental models, including significant improvements in liver enzyme profiles and histopathological parameters [13,14]. These findings suggest that *Bacopa monnieri* may provide hepatoprotective benefits in diet-induced NAFLD. Despite growing evidence suggesting hepatoprotective effects of *Bacopa monnieri* [13,14], its potential role in HFCS-induced NAFLD models remains insufficiently explored. Therefore, the present study was designed to evaluate the protective effect of *Bacopa monnieri* against HFCS-induced hepatic injury and dyslipidaemia in a rat model of NAFLD.

MATERIALS AND METHODS

The present experimental animal study was conducted in the Department of Pharmacology, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India from March 2024 to February 2025. The study was designed to evaluate the hepatoprotective effect of *Bacopa monnieri* against hepatic injury induced by chronic exposure to HFCS in male Wistar albino rats. The study duration was 12 weeks [8], which is sufficient for the development of sustained metabolic and hepatic alterations following prolonged HFCS intake.

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Jawaharlal Nehru Medical College (Reg. No.: 627/PO/Re/S/02/CPCSEA dated 23.02.2024). All procedures were performed in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. Throughout the study period, animals were handled in a manner that ensured welfare and minimised pain and distress.

Inclusion and Exclusion criteria: Healthy adult male Wistar albino rats (body weight 180-200 g) were procured from the Central Animal Facility of Jawaharlal Nehru Medical College. Animals showing signs of disease, injury, or body weight outside the specified range were excluded from the study.

Study Procedure

Experimental animals: Animals were housed in standard polypropylene cages with paddy husk bedding under controlled laboratory conditions (12-hour light–dark cycle; temperature 22 ± 2°C; relative humidity 50-60%). Standard laboratory pellet diet and drinking water were provided ad libitum. Animals were acclimatised to laboratory conditions for seven days prior to commencement of the experiment.

Test substance and chemicals: The alcoholic extract of *Bacopa monnieri* was obtained from a certified commercial supplier (Natural Remedies, Bengaluru) in standardised powdered form. The required quantity of extract was freshly suspended in distilled water each day and administered orally. HFCS-55 was procured from IAMPURE INGREDIENTS, Chennai, Tamil Nadu. It was diluted with potable water to prepare a 20% (w/v) solution, which was provided as drinking fluid to the designated groups as described in established rodent models of fructose-induced NAFLD [15]. Thiopentone sodium was obtained through institutional procurement channels. Commercially available diagnostic kits and reagents were used for biochemical estimations.

Induction of hepatic injury: Hepatic injury was induced by providing 20% HFCS-55 ad libitum in drinking water for 12 consecutive weeks, consistent with previously reported rodent models of fructose-induced metabolic derangements, including dyslipidaemia and elevation of hepatic transaminases [8,15].

Experimental grouping and drug administration: Following acclimatisation, animals were randomly allocated to experimental groups using a simple randomisation method. Rats were divided into three groups (n = 6 per group). The sample size was determined using the resource equation method, where E= Total number of animals − Number of groups= 18-3= 15, which falls within the acceptable range of 10–20, ensuring adequate statistical power while minimising animal use, in accordance with CCSEA guidelines [16]. [Table/Fig-1] shows the details of grouping and treatment regimen.

Group	Treatment	Dose and route
Group-I- normal control	Regular drinking water (ad libitum) + Normal saline	0.5 mL, per orally, once daily for 12 weeks
Group-II- HFCS control	20% HFCS (ad libitum) + Normal saline	0.5 mL, per orally, once daily for 12 weeks
Group-III- *Bacopa monnieri*	20% HFCS (ad libitum) + Alcoholic extract of *Bacopa monnieri*	40 mg/kg body weight [13], per orally, once daily for 12 weeks

[Table/Fig-1]: Details of experimental groups, treatment regimen and dosing protocol.
HFCS: High-Fructose corn syrup; ad libitum: Free access; *Bacopa monnieri* extract was suspended in distilled water and administered orally. HFCS was administered as a 20% w/v solution in drinking water; n=6 animals per group

Group-I (Normal Control): Animals received normal saline (0.5 mL) orally once daily. They had access to regular drinking water.

Group-II (HFCS Control): Animals received normal saline (0.5 mL) orally once daily. They had access to 20% HFCS dissolved in regular drinking water.

Group-III (*Bacopa monnieri*): Animals received alcoholic extract of *Bacopa monnieri* at a dose of 40 mg/kg body weight [13], administered orally once daily. They had access to 20% HFCS dissolved in regular drinking water.

All treatments were continued for 12 consecutive weeks. Oral administration was performed using a gavage needle to ensure accurate and consistent dosing. The experimental protocol followed in the present study [8,13,15] as shown in [Table/Fig-2].

Day	Procedure
Day 1	Animals were housed under standard laboratory conditions and acclimatised for 7 days under a 12-hour light/dark cycle with free access to standard chow and water.
Day 8	Rats were housed individually. High-Fructose Corn Syrup (HFCS-55) was administered as a 20% (w/v) solution in drinking water. Alcoholic extract of *Bacopa monnieri* (40 mg/kg) [13] was administered according to the assigned treatment groups.
Day 8-day 91	Treatments were continued for a duration of 12 weeks [8,15].
Day 92	Rats were sacrificed in accordance with CCSEA guidelines. Blood and liver tissues were collected for biochemical and histopathological investigations.

[Table/Fig-2]: Chronological outline of the experimental protocol showing the progression from acclimatisation to sacrifice [8,13,15].
HFCS: High-fructose corn syrup; CCSEA: Committee for control and supervision of experiments on animals; w/v: weight by volume; All animals had free access to water and standard pellet diet throughout the study period unless otherwise specified; n=6 animals per group

Sample collection and organ harvesting: At the end of the experimental period, animals were fasted overnight and anaesthetised with thiopentone sodium (40 mg/kg, intraperitoneal) [17]. Blood samples were collected by cardiac puncture and allowed to clot. Serum was separated by centrifugation and stored under appropriate conditions until biochemical analysis. Following blood collection, animals were humanely sacrificed using an overdose of thiopentone sodium in accordance with CCSEA guidelines [18]. The liver was excised, rinsed in ice-cold normal saline and blotted dry. Representative portions of liver tissue were fixed in 10% neutral buffered formalin for histopathological examination.

Biochemical assessment: Serum levels of ALT, AST and GGT were estimated using commercially available diagnostic kits obtained through a certified clinical pathology laboratory (Belagavi, Karnataka, India). Lipid profile parameters including TC, TGs, LDL and HDL were measured using standard enzymatic methods according to the manufacturers' instructions.

Histopathological evaluation: Formalin-fixed liver tissues were processed by routine paraffin embedding. Sections of 4–5 µm thickness were cut and stained with Haematoxylin and Eosin (H&E). Histological evaluation was performed to assess hepatocellular architecture, steatosis, lobular inflammation, hepatocyte ballooning degeneration and overall tissue integrity. Histological grading was performed using the NAS as defined by the NASH Clinical Research Network (NASH-CRN) [19]. Although the original histological scoring system described multiple features, including fibrosis and other architectural changes, the NAS is specifically derived from three components-steatosis (0-3), lobular inflammation (0-3) and hepatocyte ballooning (0-2)-with a composite score ranging from 0 to 8. Accordingly, only these three components were evaluated for NAS computation in the present study. The NAS scoring system is a validated and widely used semi-quantitative method for assessing histological severity in NAFLD.

STATISTICAL ANALYSIS

All results were expressed as Mean±Standard Deviation (SD). Data were analysed using GraphPad Prism software version 10 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at p <0.05. Intergroup comparisons were performed using One-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test wherever appropriate.

RESULTS

Effect of *Bacopa monnieri* on serum liver enzymes: Chronic administration of HFCS for 12 weeks resulted in significant hepatocellular injury, evidenced by elevated serum AST, ALT and GGT levels in the HFCS Control group compared to the Normal Control group (p <0.001). Treatment with *Bacopa monnieri* (40 mg/kg, p.o.) significantly attenuated these elevations, with AST, ALT and GGT levels being significantly lower in the treated group compared to the HFCS control group (p<0.001), as shown in [Table/Fig-3]. When compared to the normal control group, AST levels in the *Bacopa monnieri*-treated group remained statistically significantly higher (p=0.0337), whereas ALT (p>0.9999) and GGT (p=0.9902) levels were not significantly different. These findings indicate near-complete restoration of hepatic enzyme levels, with a residual elevation in AST.

Parameters (U/L)	Normal control	HFCS control	*Bacopa monnieri*
AST	132.8±16.9	404.7±9.7* (p<0.001)	159.8±21.3*# (*p=0.0337) (#p<0.001)
ALT	63.2±7.2	178.5±10.3* (p<0.001)	63±6.4# (p <0.001) ns (p>0.9999)
GGT	2.8±0.8	9.5±1.5* (p<0.001)	3.2±1.0# (p <0.001) ns (p=0.9902)

[Table/Fig-3]: Effect of *Bacopa monnieri* on serum liver enzymes in HFCS-induced hepatic injury.
Data are expressed as Mean±SD (n=6); HFCS: High-fructose corn syrup; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; *p <0.05 compared to normal control; #p <0.05 compared to HFCS control; ns= not significant compared to normal control

Effect of *Bacopa monnieri* on serum lipid profile: The HFCS control group exhibited profound dyslipidaemia, characterised by a significant increase in serum TC (p<0.001), TGs (p<0.001) and LDL-C levels (p=0.0026), along with a marked reduction in HDL-C levels (p=0.0004), when compared to the normal control group.

Co-administration of *Bacopa monnieri* (40 mg/kg, p.o.) significantly reversed these alterations, with a reduction in TC (p<0.001), TGs (p<0.001) and LDL-C levels (p=0.0015) and a significant improvement in HDL-C levels (p<0.001), compared to the HFCS control group [Table/Fig-4]. Compared to the normal control group, the *Bacopa monnieri*-treated group showed a significant reduction in TC (p=0.0257), whereas serum LDL-C (p=0.9995), TGs (p=0.9151) and HDL-C (p=0.7069) levels were not significantly different, indicating near-complete restoration of lipid homeostasis.

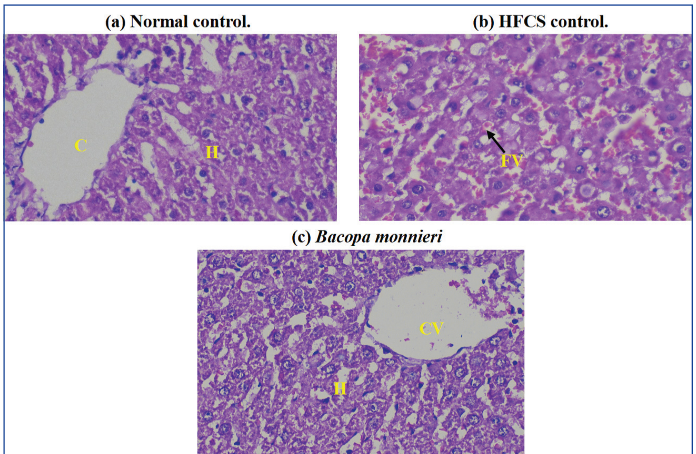
Parameters (mg/dL)	Normal control	HFCS control	*Bacopa monnieri*
Total Cholesterol (TC)	48.2±2.1	86.7±2.1* (p<0.001)	42.2±1.9*# (*p=0.0257) (#p<0.001)
HDL-C	23.3±3.7	13.8±3.0* (p=0.0004)	24.8±3.0# (p<0.001) ns (p=0.7069)
LDL-C	13.8±2.7	23.7±3.1* (p=0.0026)	13.3±2.6 # (p=0.0015) ns (p=0.9995)
Triglycerides (TG)	108.5±10.11	205.2±7.4* (p<0.001)	113.5±13.0# (p<0.001) ns (p=0.9151)

[Table/Fig-4]: Effect of *Bacopa monnieri* on serum lipid profile in HFCS-induced hepatic injury.
Data are expressed as Mean±SD (n=6); HFCS: High-fructose corn syrup; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol. * p <0.05 compared to normal control. #p <0.05 compared to HFCS control; ns= not significant compared to normal control

Histopathological studies: Histopathological examination of liver sections from the Normal Control group revealed normal hepatic architecture with intact hepatocytes and clearly defined Central Veins (CV), with no evidence of steatosis, inflammation or hepatocyte ballooning (NAS=0) [Table/Fig-5a].

In contrast, the HFCS Control group demonstrated histological features consistent with moderate NAFLD. Liver sections showed macrovesicular steatosis affecting 33–66% of hepatocytes (steatosis score: 2), mild lobular inflammatory cell infiltration (inflammation score: 1) and occasional hepatocyte ballooning (ballooning score: 1), yielding a composite NAS of 4 and indicative of moderate histological activity [Table/Fig-5b].

Treatment with *Bacopa monnieri* (40 mg/kg) resulted in marked attenuation of HFCS-induced hepatic injury. Liver sections demonstrated a reduction in steatosis (steatosis score: 1), absence of significant lobular inflammation (inflammation score: 0) and no hepatocyte ballooning (ballooning score: 0), yielding a composite NAS of 1, consistent with near-normal histological appearance [Table/Fig-5c].



[Table/Fig-5]: Photomicrographs showing liver sections from experimental rats. (a) Normal Control: Shows normal hepatic architecture with intact hepatocytes (H) and Central Vein (CV); (b) HFCS Control: Shows moderate NAFLD changes with steatosis, Hepatocellular Vacuolisation (HV) and mild inflammatory cell infiltration; (c) *Bacopa monnieri*: Shows relatively near normal hepatocytes with significant reduction in steatosis. Sections stained with haematoxylin and eosin (H&E, 40x)

DISCUSSION

The present study evaluated the hepatoprotective and hypolipidaemic effects of *Bacopa monnieri* in a preclinical model of HFCS- induced NAFLD. Chronic HFCS administration produced significant hepatocellular injury and dyslipidaemia in experimental rats, which were effectively attenuated following treatment with *Bacopa monnieri*. These findings support the potential of *Bacopa monnieri* as a protective agent against fructose-induced hepatic and metabolic derangements.

Excessive dietary fructose intake is a recognised contributor to metabolic dysfunction and NAFLD. Unlike glucose, fructose is phosphorylated to fructose-1-phosphate by fructokinase, bypassing the phosphofructokinase regulatory checkpoint, resulting in unregulated hepatic fructose metabolism, enhanced de novo lipogenesis and ATP depletion, thereby promoting lipid accumulation and hepatocellular injury [20]. In the present study, rats in the HFCS control group exhibited significantly elevated serum hepatic marker enzymes (AST, ALT and GGT), reflecting hepatocellular damage in fructose-induced NAFLD, consistent with the established role of fructose in hepatic injury [21]. These findings are in agreement with experimental observations reported by Alwahsh SM and Gebhardt R who highlighted dietary fructose as a major risk factor for the progression of hepatic steatosis in experimental models [22]. Variations in baseline AST levels among rat colonies are well-recognised and may reflect strain-specific, environmental and assay-related differences, as variability in biochemical reference intervals across rat populations has been previously reported [23].

Administration of *Bacopa monnieri* (40 mg/kg) resulted in a marked reduction of serum AST, ALT and GGT levels, consistent with previous reports of *Bacopa monnieri*-mediated reduction in hepatic marker enzymes in experimental models indicating attenuation of hepatocellular injury and restoration of hepatic functional integrity [13]. Although AST levels remained marginally elevated compared to the normal control group, the overall reduction across all hepatic enzymes indicates substantial hepatoprotection. These findings are also consistent with Mengesha T et al., who reported significant reduction in serum liver enzymes following treatment in a similar fructose-induced NAFLD model in Wistar rats, further validating the hepatoprotective utility of plant-derived compounds in this model [8]. Although oxidative stress parameters were not directly assessed in the present study, the observed hepatoprotective effect may be consistent with previously reported properties of *Bacopa monnieri*. However, the underlying mechanisms require further investigation, including evaluation of oxidative stress markers such as Malondialdehyde (MDA), Superoxide Dismutase (SOD) and catalase in future studies.

Dyslipidaemia is a hallmark feature of fructose-induced NAFLD. In the present study, HFCS consumption significantly increased serum TC, TGs and LDL-C levels, reflecting fructose-induced disturbances in lipid metabolism. These findings align with the "fructose overload" hypothesis, which implicates dysregulated fructose metabolism in the development of hepatic steatosis and systemic lipid abnormalities [24]. Treatment with *Bacopa monnieri* significantly improved the lipid profile, indicating its hypolipidaemic potential. The hypolipidaemic effect observed following *Bacopa monnieri* treatment may be consistent with its previously reported lipid-regulatory properties in experimental metabolic disorder models [25], though validation in fructose-specific models warrants further study. However, as molecular pathway analysis was not performed in the present study, the precise mechanisms underlying the observed reduction in serum lipid parameters remain to be elucidated and warrant further investigation. Histopathological evaluation further substantiated the biochemical findings. Rats in the HFCS Control group exhibited macrovesicular steatosis and lobular inflammatory infiltration, characteristic histological features of NAFLD, with a composite NAS of 4, consistent with moderate histological activity [19]. Treatment with *Bacopa monnieri* (40 mg/kg) resulted in marked attenuation of HFCS-induced hepatic injury, with reduced steatosis, absence of significant lobular inflammation and no hepatocyte ballooning, yielding a composite NAS of 1, indicative of near-normal hepatic architecture. These histological findings correspond with the biochemical improvements observed in serum liver enzymes and lipid profile parameters, collectively supporting the hepatoprotective potential of *Bacopa monnieri* in fructose-induced NAFLD. Whether this structural improvement involves modulation of inflammatory pathways warrants further investigation through assessment of hepatic inflammatory markers in future studies.

Importantly, no signs of overt toxicity were observed in the treated group. This finding supports the favourable safety profile of *Bacopa monnieri* at the therapeutic dose employed in the present study and is consistent with available clinical and preclinical evidence indicating that this herb has not been associated with significant elevations in liver enzymes or clinically apparent liver injury [26]. Collectively, these findings suggest that *Bacopa monnieri* exerts hepatoprotective and hypolipidaemic effects in HFCS-induced NAFLD, highlighting its potential as a complementary therapeutic agent in the management of fructose-associated metabolic liver disease.

Limitation(s)

The present study has certain limitations. Firstly, the absence of a *Bacopa monnieri*-only group (without HFCS) is acknowledged as a limitation of the present study. Consequently, the independent effects of *Bacopa monnieri* on lipid metabolism cannot be entirely excluded and the observed improvements should be interpreted as protective effects in the context of fructose-induced metabolic derangement rather than isolated pharmacological actions. Secondly, while the present study observed significant hepatoprotection, the molecular mechanisms involving specific inflammatory cytokines {e.g., Tumour Necrosis Factor (TNF-α), Interleukin (IL-6)} and gene expression profiles were not quantified. Thirdly, the study was conducted on a rodent model and the translational potential to human clinical practice requires further validation through clinical trials. Finally, the duration of the study was limited to 12 weeks; long-term effects of *Bacopa monnieri* on chronic liver disease warrant further investigation.

CONCLUSION(S)

In conclusion, the present study demonstrates that chronic HFCS administration successfully induced NAFLD-like hepatic injury and dyslipidaemia in male Wistar albino rats, confirmed by elevated serum liver enzymes, lipid profile derangements and histopathological findings with a composite NAS of 4. Treatment with alcoholic extract of *Bacopa monnieri* (40 mg/kg, p.o.) for 12 weeks significantly attenuated HFCS-induced hepatocellular injury, as evidenced by reduction of serum AST, ALT and GGT levels, with near-normalisation of ALT and GGT levels. The herb also demonstrated significant hypolipidaemic activity, reversing HFCS-induced dyslipidaemia by reducing TC, TGs and LDL-C while improving HDL-C levels. Histopathological examination corroborated the biochemical findings, showing marked reduction in hepatic steatosis and restoration of near-normal hepatic architecture (NAS=1). No signs of overt toxicity were observed, supporting the favourable safety profile of *Bacopa monnieri* at the therapeutic dose employed. These findings collectively suggest that *Bacopa monnieri* holds promise as a hepatoprotective and hypolipidaemic agent in fructose-associated metabolic liver disease, warranting further clinical evaluation.

Future studies should focus on elucidating the molecular mechanisms underlying the hepatoprotective effects of *Bacopa monnieri*, including evaluation of oxidative stress markers (MDA, SOD and catalase), inflammatory cytokines (tumour necrosis factor-α, interleukin-6) and lipid regulatory pathways. Additionally, well-designed clinical trials are warranted to validate these preclinical findings in patients with NAFLD.

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