



Improvement of lipid profile and hepatic oxidative stress in high-fat-diet- induced hyperlipidemic Swiss albino rats by *Piper betle* juice: evidences from *in vivo* and *in silico* studies

XiBo Jing¹, Md. Moklesur Rahman Sarker^{2,3*}, Md. Abu Jar Gifari², Md. Rifat Alam Maruf², Safaet Alam⁴, Farzana Khan⁵, Mahfuza Afroz Soma⁶, Md. Sanower Hossain⁷, Long Chiau Ming⁸, Shihab Uddin Ahmad⁶, Raquibul Hasan⁹

¹ Department of Structural Cardiology, Shandong Second Provincial General Hospital, Jinan, Shandong Province, 250000, China

² Department of Pharmacy, State University of Bangladesh, 77 Satmasjid Road, Dhanmandi, Dhaka 1205, Bangladesh

³ Pharmacology and Toxicology Research Division, Health Med Science Research Network, 3/1 Lalmatia, Block F, Dhaka 1207, Bangladesh

⁴ Drugs and Toxins Research Division, BCSIR Laboratories Rajshahi, Bangladesh Council of Scientific and Industrial Research, Rajshahi 6206, Bangladesh

⁵ Biochemistry and Molecular Biology, Upstate Medical University, New York, 132104, United States

⁶ Department of Pharmacy, University of Asia Pacific, 74/A, Green Road, Dhaka- 1205, Bangladesh

⁷ Centre for Sustainability of Ecosystem and Earth Resources (Pusat ALAM), Universiti Malaysia Pahang, 26300 Kuantan, Malaysia

⁸ School of Medical and Life Sciences, Sunway University, Sunway City 47500, Malaysia

⁹ Department of Pharmaceutical Sciences, College of Pharmacy, Mercer University, Atlanta, GA 30341, United States

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ABSTRACT

Piper betle L. leaves are very popular and traditionally used to chew with betel nut in many Asian countries. In this study, *P. betle* leaves juice (PBJ) was subjected to evaluation for its antihyperlipidemic activity in the high-fat-diet-induced hyperlipidemic rats model. Swiss albino rats were allowed to high-fat-diet for one month, followed by concurrent administration of PBJ for another month. The rats were then sacrificed and collected blood, tissues and organs. Pharmacokinetic, toxicological studies and molecular docking studies were performed using SwissADME, admetSAR and schrodinger suit-2017. Our investigation showed a promising effect of PBJ on body weight, lipid profile, oxidative and antioxidative enzymes, and the principle enzyme responsible for the synthesis of cholesterol. PBJ at 0.5 - 3.0 mL/rat significantly reduced body weight of hyperlipidemic rats compared to control. PBJ at the doses of 1.0, 1.5, 2.0, and 3.0 mL/rat significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) improved the levels of TC, LDL-c, TG, HDL-c and VLDL-c. Similarly, PBJ doses starting from 1.0 mL/rat to 3.0 mL/rat reduced the oxidative biomarkers AST, ALT, ALP, and creatinine. The level of HMG-CoA was significantly reduced by PBJ doses 1.5, 2, and 3 ml/rat. A number of compounds have been found to have good pharmacokinetic profile and safety and 4-coumaroylquinic acid exerted the best docking score among them. Thus our findings clearly demonstrated the potential lipid-lowering activities of PBJ both *in vivo* and *in silico* studies. PBJ can be a good candidate for the development of antihyperlipidemic medication or as an alternative medicine.

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Introduction

Hyperlipidemia is characterized by the increment of normal levels of plasma lipids such as cholesterol, triglycerides, cholesterol esters, phospholipids and sometimes of plasma lipoproteins including very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) along with a decrement of high-density lipoprotein (HDL) levels (1, 2). Currently, it has become a widespread disorder and a threatening cause of many metabolic dysfunctions which may contribute to several prevalent diseases like heart diseases, atherosclerosis, high blood pressure, diabetes mellitus, hypercholesterolemia, obesity and so on (3, 4) resulting in upraised rate of both morbidity and mortality incidence all over the world (5). The extravagant consumption of dietary lipids gives rise to hyperlipidemia leading to the elevation of plasma cholesterol levels, which in turn cause

more than four million deaths yearly (6, 7). Life style modification approaches like less intake of fatty foods, giving up smoking, performing aerobic exercise, and dietary therapies have been implemented to treat hyperlipidemic disease. Moreover, allopathic hypolipidemic drugs can also alleviate the risks of other associated life-threatening disease states (8, 9). Both monotherapy and combination therapy of anti-hyperlipidemic drugs have elucidated efficacy in the treatment of hyperlipidemia. Notably, there have been five major classes of anti-hyperlipidemic drugs available are- statins including lovastatin, pravastatin, simvastatin, atorvastatin, rosuvastatin; bile acid binding resins like, cholestyramine and colestipol; fibric acid derivatives including, gemfibrozil, bezafibrate, fenofibrate; nicotinic acid derivatives like, niacin; and cholesterol absorption inhibitors like, ezetimibe (6). However, these commercially available anti-hyperlipidemic therapies like statins, deliver

* Corresponding author. Email: moklesur2002@yahoo.com, prof.moklesur@sub.edu.bd

some major side effects including gastrointestinal discomfort, rhabdomyolysis, myalgia, myopathy and dizziness which abate their credibility (10). These side effects are more prevalent at higher doses of statins (6). In addition, these drugs can also cause kidney damage, cardiomyopathy along with an escalation in type 2 diabetes (11, 12). To compensate this, the usage of alternative treatments and therapies like herbal medicinal plants containing hypolipidemic constituents, have been increased and their popularity is rising day by day among both normal people and physicians across the world (3). The prospective therapeutic value and minimum or no-observable adverse-effects of nutraceuticals, functional foods and phytomedicines have created a lucrative option to the scientists and physicals around the world for the treatment of different diseases like type 2 diabetes mellitus (13-15), hyperlipidemia (16), obesity (17), cancer (18-19), immune impairment (20-21), inflammation (22-23), infections (24-26), neurological disorders (27), aging (28), etc. Thus, alternative medications such as consumption of lipid-lowering medicinal plants to attain antihyperlipidemic activity were observed in different localities. Furthermore, even in developed countries this approach has been adopted in a large scale, especially when conventional drugs have failed to alleviate the disease state notably (3).

Piper betel L., an evergreen and perennial creeper from the Piperaceae family, is renowned for its heart shaped leaves (29). It is native to Malaysia although cultivated in several other Asian countries including Bangladesh, India, Myanmar, Srilanka, Thailand, Vietnam etc. (30, 31). Leaf of *P. betel* is edible and known as “Paan” with a minimum hundreds of varieties (32). Betel leaf, having a pungent taste and distinct aroma due to the presence of phenols and terpenes is popularly used to chew with areca nut and slacked lime in many countries, especially taken after meals by local people as a mouth freshener. Its folkloric medicinal use includes wound healing and tonic properties. In China, this plant is believed as a cure of several disorders including detoxification (30). This leaf has profound importance in different social, cultural, and religious events in Hindu culture (33). Besides, oil extracted from betel leaf is utilized as a raw material of perfumes and food additives (34). Betel leaf comprises of several bioactive phytoconstituents which exert diverse pharmacological activities including antibacterial, anticancer, anthelmintic, antihypertensive, antiprotozoal, antifungal, antihistaminic, antifungal and antidiabetic properties (29). Moreover, the leaves are also popular in the treatment of alcoholism, asthma, leprosy, bronchitis and dyspepsia (35).

Phytochemical analysis of betel leaves provides an abundant source of carbohydrates, alkaloids, tannins, terpenoids, phenols, essential oils, and major other classes

and isolated compounds include 1, 8-cineole, cadinene, camphene, caryophyllene, limonene, quercetin, pinene, chavicol, allyl pyrocatechol, piperitol, carvacrol, safrole, eugenol, chavibetol, safrole, thymol, allyl eucalyptol, pyrocatechol monoacetate, eugenol, terpinen-4-ol, eugenyl acetate, quercetin etc. (36) However, the antihyperlipidemic potential of this popular plant leaf has not yet been explored extensively. Therefore, we have aimed to conduct this study for the evaluation of *P. betel* leaf juice (PBJ) extract on high-fat-diet induced hyperlipidemia and hepatic oxidative stress in Swiss albino rats.

A computational method is a reliable, currently used approach in drug discovery research to identify active phytochemical compounds from various databases (37). In the drug discovery process with the help of a computational approach, the selection of structural proteins is one of the major tasks. HMG-CoA reductase is an enzyme has been taken for this *in silico* study. It is one of the most common drug targets because of its prominent role in anti-hyperlipidemic activity (38). A total of 73 compounds were screened against HMG-CoA reductase by doing molecular docking study. Among them, the top binding affinity phyto-compounds: 1-phenylpropene-3,3-diol diacetate, 4-Chromanol, 4-*p*-coumaroylquinic acid, Benzeneacetic acid, 4-terpineol, β -sitosterol, stigmasterol, aristololactam A-II. The selected compounds were taken for further assessment using ADME/T, and drug likeness properties.

Materials and Methods

Materials

Betel (*Piper betel* L.) leaves were purchased from local sources in Bangladesh. The Cow's fat, dalda, cholesterol, ghee, coconut oil, and sodium cholate used in the high fat diet formulation were locally purchased. Ketamine HCl and Xylazine HCl were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other analytical grade reagents were locally procured and purchased. Simvastatin as a standard lipid lowering drug was purchased from a local pharmacy.

Preparation of *Piper betel* L. leaves juice (PBJ)

The betel leaves were washed with distilled water and twenty-five (25) medium sized whole betel leaves with the pod (weight 113 g) were masticated and pressed in mortar and pestle to collect the juice with pressure. A total of 25 mL juice was collected from 113 g leaves. The PBJ was collected in a glass jar and stored at 4-8°C temperature from where it was used for experiment purposes.

Preparation of high-fat diet

A high-fat diet formulation was used for the induction of hyperlipidemia in Swiss albino rats. The composition of high fat diet has been presented in Table 1. The cow's fat

Table 1. High fat diet formulation.

Sl. No.	Name of the ingredients	Quantity of ingredients	Percentage of ingredients
01	Cow's fat	600 g	20%
02	Dalda/Ghee	180 g	6%
03	Coconut oil	60 g	2%
04	Normal rats pellets diet	2.1 kg	70%
05	Cholesterol	60 g	2%
	Total	3 Kg	100%

and dalda were melted with heat and gradually added to the normal pellets diet in a bucket. The liquids were mixed well with the pellets and allowed to become semi-solid/solid mass. Similarly, cholesterol and coconut oil were poured into the blended pellets and again homogenously mixed. Finally, coconut oil was added to the pellets. Thus, high fat diet pellets were prepared and rats were provided with this high fat diet instead of the normal pellets diet.

Experimental animals

The experiments were conducted in Swiss albino rats, aged between 8-12 weeks. The rats having the age of 6-8 weeks old were purchased from Shanghai Laboratory Animal Center (SLAC, Shanghai, China). In Bangladesh, the Swiss albino rats (6-8 weeks) were purchased from Animal Breeding Laboratory of Jahangirnagar University. The rats were housed six per plastic cage provided with wood chip bedding maintaining a 12 h light/dark cycle and allowed to free access to standard rodent food and water ad libitum. Environmental changes were strictly controlled and prior to any experiment, the animals were kept for 1 week to adjust to the new housing environment.

Ethical approval of experimental protocol

The ethical approval was obtained from the Animal Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong Province, China (Approval Number: 2020-031101) and Animal Ethics Committee of State University of Bangladesh, Dharmondi, Dhaka, Bangladesh (Ethical approval No. 2020-02-03/SUB/A-AEC/0004). The experiments were conducted according to the approved Animal Use Protocol by the Ethics Committee and following the rules, regulations, and laws of Shandong prefecture of China, maintaining the animal use rules and laws of Bangladesh, and in accordance with the Guidelines for Care and Use of Laboratory Animals published by the US National Institutes of Health. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental animals. At the end of the experiments, the rats were sacrificed with anesthesia overdose: Ketamine HCl (100 mg/kg) and xylazine (10 mg/kg) through intraperitoneal route (39).

Acute toxicity study of PBJ

Oral acute toxicity study (LD50 determination) of *Piper betel* L. juice extract (PBJ) was performed following the OECD (Organization for Economic Cooperation and Development) by Fixed Dose Procedure (OECD protocol no. 420) as followed by Kifayatullah et al. (2015) (40). Briefly, Swiss Albino female (nulliparous and nonpregnant) rats, age: 8 weeks, were acclimatized to laboratory conditions 7 days prior to experiment. The rats were divided into five groups, each comprising 5 animals. Group-1 served as the untreated control (received water only), group-2, 3, and 4 received PBJ doses 1000 mg/kg, 3000 mg/kg, and 5000 mg/kg, respectively. The rats were overnight fasted for food (not fasted for water) before dosing and fasted for food 3-4 hours after the administration of doses. The animals were observed individually during the first 30 minutes after dosing, special attention was given during the first 4 hours, then to observe periodically during the first 24 hours to see any toxic effect in the animals. During the

entire period of observation for 14 days, the animals were observed and monitored for any changes in behavior, body weight, urinations, food intake, water intake, respiration, convulsions, tremor, temperature, constipation, changes in eye and skin colors and mortality of the animals.

Induction of hyperlipidemia

After the adaptation period, Swiss albino rats (7-9 weeks of age) were fed with a specially prepared high-fat diet (Table 1), consisting of 20% cow's fat, 6% dalda, 2% cholesterol, 2% coconut oil, 70% standard normal diet (w/w) for 1 month to induce hyperlipidemia. After 1 month, the rats were treated with PBJ along with the continuation of the high fat diet for another month.

Experiment design

The rats were divided into the following eight (08) groups; each group will contain 5 rats:

Group-I: Normal control (rats received normal pellet diet and water, no treatment)

Group-II: Hyperlipidemic control (hyperlipidemic rats, no treatment)

Group-III: PBJ 0.5 mL/rat

Group-IV: PBJ 1 mL/rat

Group-V: PBJ 1.5 mL/rat

Group-VI: PBJ 2.0 mL/rat

Group-VII: PBJ 3.0 mL/rat

Group-VIII: Standard lipid lowering drug - Simvastatin (4 mg/kg)

The Swiss Albino rats were administered with different doses of PBJ by oral gavaging every day for 30 days of the study period.

Measurement of body weight of rats

Body weights of rats were measured before starting the experiments, one month after the high fat diet, and at the end of the experiment in which the rats were treated with PBJ for another one month along with a high-fat-diet.

Determination of lipid profile and oxidative biomarkers in rats

At the end of the experiment period, blood was collected by cardiac puncture from the animals using anesthesia. The blood was then centrifuged at 3000 rpm for 15 min, and the serum was stored at -80 °C for analysis. The amounts of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c), and apolipoprotein B-48 (apoB48) were measured by using commercial assay kits according to the manufacturer's instructions (Roche, Basel, Switzerland). Other biomarkers which include aspartate transaminase (AST), alanine transaminase (ALP), alkaline phosphatase (ALP), and serum creatinine were determined following the standard protocol (41).

Determination of HMG-CoA reductase enzyme in liver tissue

The amount of HMG-CoA reductase enzyme was measured in the tissue homogenates of experimental rats by RT-PCR method as previously followed by other researchers (42). Briefly, TRIzol reagent (Invitrogen, CA, USA) was used for the total extraction of RNA from the liver tissues following the manufacturer's instructions. The tissues were homogenized and 1 mL of TRIzol reagent was added

to 100 mg of homogenized tissue samples. After this, the tissues were incubated at room temperature for 5 min to allow complete disassociation of nuclear proteins. The rest of the procedures were followed as described by Im et al (2014) (43).

Histopathological observation of liver

Histopathological experiments were performed as described by Song et al. (44). After sacrificing the rats, livers were collected and immediately fixed in 10% buffered formalin (pH 7.4) and embedded in paraffin. A portion of the liver was cut (4–5 μm), stained with haematoxylin-eosin (H&E), and the sections were examined with a computer-aided microscope for the determination of morphological and/or pathological changes ($\times 600$ magnification). The histological scores were assessed as mentioned by Ishak et al (45). Scoring systems were performed as followed by Yahya et al. (46) based on a sum of three parameters: inflammation grade, cell infiltration, and tissue disruption. H&E staining in the liver was scored using a scale of 0 to 4 (0 = no inflammation grade, cell infiltration, and tissue disruption; 1 = 0–25% inflammation grade, cell infiltration and tissue disruption; 2 = 25–50% inflammation grade, cell infiltration and tissue disruption; 3 = 50–75% inflammation grade, cell infiltration and tissue disruption; and 4 = 75–100% inflammation grade, cell infiltration and tissue disruption). Each tissue was evaluated for the sum of three parameters and by the degree of liver injury using a qualitative score that ranged from 0 to 4. A score of 0 was categorized as no damage, scores between 1 and 2 were categorized as light injury, and scores of 3 and 4 were categorized as serious injury. The assessed area has been indicated by arrow. At least three areas were analysed. Scoring had been done by blinded method.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) software (version 25.0) of IBM (International Business Machines) Corporation, USA, followed by Dunnett's-T3 and Tukey HSD tests to determine statistical significance between groups. The type of post-hoc test to be used was determined based on the value in 'significance' column of the Test of Homogeneity of Variances. In case the value in the significance column is higher than 0.05 then Dunette's-T3 post-hoc test is followed. Conversely, the Tukey HSD post hoc test is followed in case of significance value 0.05 or lower. The data are means \pm S.E.M. (standard error mean) of five animals with 95% confidence intervals (CI). The p-value, $p < 0.05$ was considered as statistically significant.

In silico study

Molecular Docking tools

Molecular docking of ligand-receptors has been performed by using Schrodinger suites 2017-1. Discovery studio (v 4.1) was used for the visualization.

Ligand and protein preparation

2D SDF structures of isolated compounds found from the literature review were imported and prepared by using LigPrep in Maestro 11.1. OPLS_2005 force field (47)

was implemented to study the conformational energy of the system.

Target proteins for antihyperlipidemic activity were selected according to the literature studies. The crystal structure PDB formats of HMG-CoA reductase (PDB ID: 1HW8) responsible for activity analysis was imported from the RCSB Protein Data Bank (PDB) an online database (<https://www.rcsb.org/>). In the Protein Preparation Wizard, preprocessing, optimization, and minimization processes were completed (48, 49). These processes are parts of Schrodinger suite-maestro (v11.1). The structures were optimized at pH 7.0 followed by removal of water molecules fewer than 3 H-bonds to non-waters. Restrained minimization was done where heavy atoms are converged to RMSD of 0.30 \AA with the implemented OPLS_2005 force field (47).

Glide ligand molecular docking

The prepared proteins and ligands are ready for molecular docking. The molecular docking was done to find the best receptors for the presumed activities of the ligands *in silico*, so that more *in vivo* investigations may be done to see how they compare to the standard medications that have been used as treatments for certain activities. Ligand docking option in Schrodinger suite-maestro (v11.1) was utilized to perform the docking. The spreadsheets and structures were then exported. For 3D visualization of receptor-ligand binding interaction Discovery Studio (v 4.1) software was used (50).

Determination of pharmacokinetic parameters by Swiss ADME

A predictive approach used for predicting the oral absorptivity and pharmacokinetic properties of selected compounds were performed by using SwissADME, an online platform (51). The pharmacokinetic parameters or drug-likeness properties (total molecular weight of the compounds, lipophilicity (LogP), the number of hydrogen-bond acceptors, and the number of hydrogen-bond donors based on the Lipinski's rule) of the selected compounds were determined(51). Toxicity was analyzed in an online site (<http://lmmd.ecust.edu.cn/admetar2>).

Protein-Ligand Interaction Visualization by Discovery Studio (v4.1)

For 3D visualization of receptor-ligand binding interaction Discovery Studio (v 4.1) software was used. Then the image files of interactions were downloaded.

Results

Effect of PBJ on the body weight of hyperlipidemic rats

Table 2 summarizes the effect of PBJ in hyperlipidemic rats model and showed a notable effect on the reduction of body weight. Administration of high fat diet for one month remarkably increased the body weight of experimental rats in the hyperlipidemic control group (HC) (from 161.8 gm to 288.4 gm; increased rate 78.25%) compared to the non-high fat diet control (NHC) group (174.2 gm to 183.8 gm; increased rate 5.51%). Rats were further provided with high-fat diet for another month along with the treatment with different doses of PBJ (0.1 mL/rat - 3.0 mL/rat) and the weights were redocumented. As presented in Table 2, it has been shown that the hyperlipidemic control rat weight

Table 2. Effect of PBJ on body weight of rats.

Treatment group	Body weight in gram		
	Body weight before high fat diet	Body weight 1 month after high fat diet	Body weight after 1 month treatment of hyperlipidemic rats
Non-hyperlipidemic Control (NHC)	174.2	183.8	186.00 ± 3.563
Hyperlipidemic control (HC)	161.8	288.4	319.00 ± 7.127 φφφ
<i>Piper betle</i> L. juice (PBJ) 0.5 mL/rat	178	291.2	197.00 ± 6.607 ***
<i>Piper betle</i> L. juice (PBJ) 1.0 mL/rat	177.8	291.4	220.00 ± 7.69 ***
<i>Piper betle</i> L. juice (PBJ) 1.5 mL/rat	163.8	271.6	200.00 ± 5.54 ***
<i>Piper betle</i> L. juice (PBJ) 2.0 mL/rat	182.6	294.6	210.00 ± 5.357 ***
<i>Piper betle</i> L. juice (PBJ) 3.0 mL/rat	170.2	295.8	218.6 ± 6.867 ***
Simvastatin (4 mg/kg)	184.2	302.8	206.4 ± 3.429 ***

Values are expressed as means ± S.E.M. of five rats in each group.

*Data differed significantly ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) when compared to hyperlipidemic control (HC) group.

φ Data differed significantly ($\phi p < 0.05$, φφ $p < 0.01$, φφφ $p < 0.001$) when compared to non-hyperlipidemic control (NHC) group.

Table 3. Food intake and estimated energy levels of experimental rats.

Rats group vs. food intake and energy levels	NHFD	HFDC	HFD+PBJ	HFD+Simvastatin
Food intake (g/ day/rat)	11.21 ± 1.25	10.53 ± 1.12	10.20 ± 1.03	10.34 ± 1.45
Energy levels (KJ/day/rat)	214.953 ± 21.182	300.621 ± 28.784	292.14 ± 26.471	293.738 ± 37.265

NHFD: Non high fat diet control group

HFDC: High fat diet control group

HFD +PBJ: High Fat Diet rats treated with *Piper betle* leaf juice

HFD + Simvastatin: High Fat Diet rats treated with Simvastatin

(319.00 ± 7.127) has significantly increased due to the exposure of rats with high fat diet compared to the weight (186.00 ± 3.563) of non-hyperlipidemic control group (NHC) (rats of normal diet) (φφφ $p < 0.001$). On the contrary, all five groups of rats treated with five different doses of PBJ (0.5, 1.0, 1.5, 2, and 3 mL/rat) exhibited prominent (** $p < 0.001$) abatement in body weight compared to the hyperlipidemic control rats. Simvastatin (4 mg/kg), used as a standard lipid lowering drug group also exhibited a potential reduction of body weight (206.4 ± 3.429 g) when compared with hyperlipidemic control (** $p < 0.001$). The amounts of daily food intake and estimated energy levels of different groups of experimental rats are presented in Table 3.

Effect of PBJ on the lipid profile in hyperlipidemic Swiss Albino rats

The effect of PBJ on lipid profile in hyperlipidemic rats has been presented in Fig. 1. The normal values of lipids in rats are TC 113.99±2.18 mg/dL, LDL-c 49.64±1.82 mg/dL, TG 76.13 ± 2.38 mg/dL, HDL-c 49.14±1.05 mg/dL, VLDL-c 15.22 ±0.48 mg/dL (44). Treatment of hyperlipidemic rats with PBJ at the doses of 1.0, 1.5, 2.0, and 3.0 mL/rat potentially reduced the total cholesterol levels (TC) (Fig.1A) and triglyceride (TG) (Fig. 1C) compared to the hyperlipidemic control (HC) rats group. PBJ at the dose of 1.5, 2.0, and 3.0 mL/rat significantly lowered the levels of low-density lipoprotein (LDL-c) (Fig. 1B). The level of very low density lipoprotein cholesterol (VLDL-c) were significantly reduced by PBJ 1.0, 2.0, and 3.0 mL/rat (Fig. 1E) comparing to that of hyperlipidemic control. Additionally, PBJ at the doses of 1.5, 2.0, and 3.0 mL/rat enhanced the high-density lipoprotein (HDL-c) in hyperlipidemic rats compared to untreated rats group (Fig. 1D).

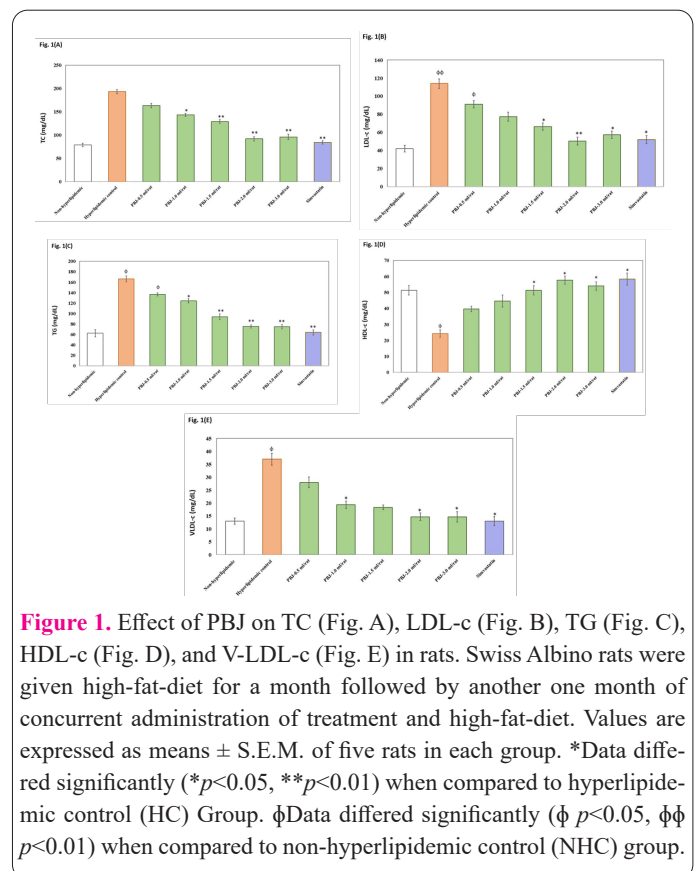


Figure 1. Effect of PBJ on TC (Fig. A), LDL-c (Fig. B), TG (Fig. C), HDL-c (Fig. D), and VLDL-c (Fig. E) in rats. Swiss Albino rats were given high-fat-diet for a month followed by another one month of concurrent administration of treatment and high-fat-diet. Values are expressed as means ± S.E.M. of five rats in each group. *Data differed significantly ($p < 0.05$, ** $p < 0.01$) when compared to hyperlipidemic control (HC) Group. φData differed significantly ($\phi p < 0.05$, φφ $p < 0.01$) when compared to non-hyperlipidemic control (NHC) group.

Effect of PBJ on the activities of oxidative and antioxidant enzymes

Figure 2 (A-D) demonstrates the positive effect of PBJ on biochemical markers in experimental hyperlipidemic rats model. The normal value of AST is 50-150 IU/L, ALT 10-40 IU/L, ALP 30-130 IU/L, and Creatinine 0.25 – 3.09 mg/dL in rats (52, 53). As shown in the Fig. 2, high-fat-

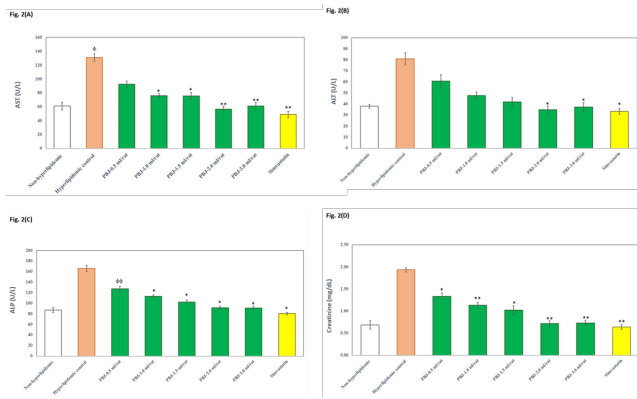


Figure 2. Effects of PBJ on AST (Fig. A), ALT (Fig. B), ALP (Fig. C), and creatinine (Fig. D) in rats. Swiss Albino rats were provided with high-fat-diet for a month followed by concurrent administration of treatment and high-fat diet for another month. Values are expressed as means \pm S.E.M. of five rats in each group. *Data differed significantly ($p < 0.05$, $**p < 0.01$) when compared to hyperlipidemic control (HC) group. ϕ Data differed significantly ($\phi p < 0.05$, $\phi\phi p < 0.01$) when compared to non-hyperlipidemic control (NHC) group.

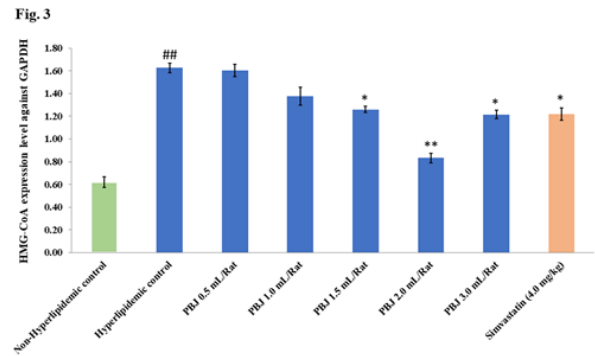


Figure 3. Effects of PBJ on HMG-CoA reductase enzyme levels in liver tissues of experimental rats. Swiss Albino rats were provided with high-fat-diet for a month followed by concurrent administration of treatment and high-fat diet for another month. The amount of HMG-CoA reductase enzyme was measured in the tissue homogenates of experimental rats by RT-PCR method. Values are expressed as means \pm S.E.M. of five rats in each group. *Data differed significantly ($p < 0.05$, and $**p < 0.01$) when compared to hyperlipidemic control (HC) group. # Data differed significantly ($##p < 0.01$) when compared to non-hyperlipidemic control group.

diet in rats drastically enhanced the levels of AST (131.0 ± 5.507 U/L vs. 61.0 ± 5.507 U/L), ALT (81.0 ± 5.507 U/L vs. 38.0 ± 1.732 U/L), ALP (165.67 ± 5.783 U/L vs. 87.0 ± 4.932 U/L) and creatinine (1.933 ± 0.049 mg/dL vs. 0.686 ± 0.095 mg/dL), respectively. However, treatment of hyperlipidemic rats with PBJ at the doses of 1.0, 1.5, 2.0, and 3.0 mL/rat significantly diminished the levels of AST (Fig. 2A) and ALP (Fig. 2C) biomarkers. While the levels of ALT and, creatinine were significantly reduced by PBJ 2.0 and 3.0 mL/rat (Fig. 2B), and PBJ 0.5, 1.0, 1.5, 2.0, and 3.0 mL/rat (Fig. 2D), respectively. Simvastatin (4 mg/kg), as a standard antihyperlipidemic drug, also significantly diminished the levels of AST ($**p < 0.01$), ALT ($*p < 0.05$), ALP ($*p < 0.05$), and creatinine ($**p < 0.01$).

Attenuation of the levels of HMG-CoA by PBJ in the liver tissues of high-fat-diet rats

Figure 3 shows the effect of different concentrations of PBJ (0.5 mL/rat to 3.0 mL/rat) administered orally. The level of HMG-CoA reductase enzyme has been tremendously increased in the high-fat-diet rats compared to non-hyperlipidemic animals. However, treatment of the high-fat-diet rats with PBJ at the doses of 1.5, 2, and 3 mL/rat significantly ($*p < 0.05$, $**p < 0.01$, $*p < 0.05$, respectively) reduced the levels of HMG-CoA reductase enzyme in liver tissues. Simvastatin (4 mg/kg) as a positive control has also significantly attenuated the level of HMG-CoA reductase enzyme in liver tissues. The highest reduction of HMG-CoA reductase enzyme was observed to by PBJ at the dose of 3.0 mL/rat which was even much lower than that of the level of HMG-CoA level reduced by standard lipid lowering agent simvastatin (4.0 mg/kg) (Fig. 3).

Effect of PBJ on histopathological assessment of high fat diet-induced liver damage

Histopathological observation of liver sections of non-hyperlipidemic control rats showed normal cellular architecture, distinct hepatic cells, sinusoidal spaces, central vein, and no accumulation of fat (Fig. 4A). In high-fat-diet group, hepatic cells were found to have hyperplasia, cellular degeneration, inflammation, and accumulation of

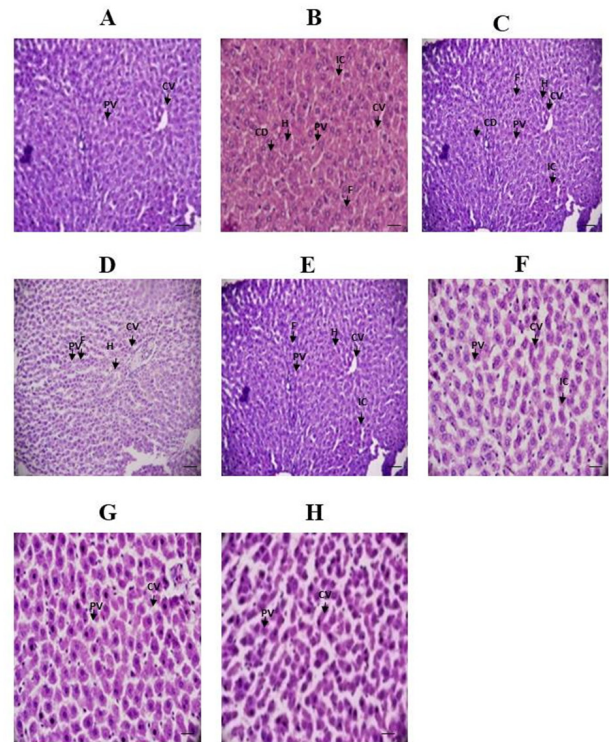


Figure 4. Representative photomicrographs of liver histopathology. Liver sections from Swiss Albino rats group of non-hyperlipidemic control (A), hyperlipidemic control (B), PBJ 0.5 mL/rat (C), PBJ 1.0 mL/rat (D), PBJ 1.5 mL/rat (E), PBJ 2.0 mL/rat (F), PBJ 3.0 mL/rat (G), and Simvastatin 4 mg/kg (H), respectively. H = hyperplasia, IC = Inflammatory cells, CD = cellular degeneration, F = fat deposition, PV = portal vein, CV = central vein. Pictures of hematoxylin and eosin (H&E) staining are at $10 \times$ magnification and Bar scale: 20μ .

fat (Fig. 4B). The rats treated with PBJ (0.5, 1.0, 1.5, and 2 mL/rat) (Fig. 4C, D, E, F, G) and Simvastatin 4 mg/kg (Fig. 4H) showed almost normal architecture of the hepatic cells having signs of protection with the reduced number/absence of inflammatory cells, vascular degeneration and significant reduction/absence of fatty cells accumulation.

Table 4. Scoring for Inflammation grade, Cell infiltration, and Tissue disruption.

Groups	Inflammation grade	Cell infiltration	Tissue disruption
Group-I	0.50	0.25	0.45
Group-II	3.90	3.20	3.80
Group-III	1.15	1.10	1.05
Group-IV	1.12	1.08	1.03
Group-V	0.90	0.85	0.95
Group-VI	0.86	0.87	0.85
Group-VII	0.56	0.54	0.65
Group-VIII	0.59	0.70	0.40

Inflammation grade, cell infiltration, and tissue disruptions scores in histopathological observations are presented in Table 4.

Oral acute toxicity study of PBJ

Oral acute toxicity study of PBJ was carried out following OECD guideline Fixed Dose Procedure (OECD protocol no. 420) as mentioned in the Methodology section. No case of mortality was observed during the 14 days of treatment with a limited dose of 5000 mg/kg BW of PBJ. All treated animals could tolerate the PBJ doses and there was no statistically significant difference in body weight between the treated and untreated groups. The animals did not exhibit any abnormalities or major behavioural changes such as respiratory distress, abnormal locomotion, tremors, salivation, diarrhoea, sleep, walking backwards, reactions to handling, catalepsy, coma or any toxic symptoms either immediately or during the post-treatment observational period of 14 days. Thus, we can say that the LD50 for oral administration of PBJ is higher than 5000 mg/kg B.W. and is apparently nontoxic. Therefore, the used doses of PBJ (0.5-3.0 mL/rat) were well tolerated by the animals (data not shown).

Isolated phytochemicals from PBJ

A total of 73 phytochemicals were found to be isolated from the leaves of Piper betle L. which include Hydroxychavicol (29, 52, 53), Eugenol (29, 52), Isoeugenol (29, 54); 4-allyl-1,2-diacetoxybenzene, 1-n-dodecanyloxy resorcinol, desmethylenesqualenyl deoxy-cepharadione-A, 4-Chromanol, 1-phenylpropene-3,3-diol diacetate, Neophytadiene, Elemicin, anethole, 3-p-coumaroylquinic acid, 4-p-coumaroylquinic acid, 4-chromanol, phenol 2 methoxy 4-(2-propenyl) acetate, Hexadecanoic acid, Octadecanoic acid, 2,3-bis(hydroxy)propyl ester, Benzeneacetic acid, estragole, β -cubebene, acetyleneugenol, amphenol, a-limonene, saffrole, 1,8-cineole, 4-terpineol, 4-allyl-2-methoxy-phenolacetate, and 4-allylphenyl acetate (29). Besides, Chavicol, carvacrol, Chavibetol, ursolic acid, cadinene, p-cymene, cepharadione A, dotriacontanoic acid, tritriacontane, adinene, hentriacontane, pentatriacontane, n-triacontanol, stearic acid, piperlonguminine, α -pinene, β -sitosterol palmitate, β -sitosterol, stigmasterol, 4-allyl resorcinol, aristololactam A-II, pellitorine, N-isobutyl-2E,4E-dodecadienamide, Dehydropiperonaline, piperdardine, piperolein-B, guineensine, syringaresinol-O- β -D-glucopyranoside, pinosresinol, cepharadione A, 6 β -hydroxystigmast-4-en-3-one, β -daucosterol, (2E,4E)-N-isobutyl-7-(3',4'-methylenedioxyphenyl)-2,4-heptadienamide, 23-hydroxyursan-12-en-28-oic acid, β -sitosterol-3-O- β -D-glucoside-6'-O-palmitate, (2S)-4'-hydroxy-2,3-dihydroflavonone-7-O- β -D-glucoside, and α -ethyl glucoside (54); caryophyllene, chavibetol acetate, 4-allylphenyl acetate, and β -pinene (29,54); Allylpyrocatechol monoacetate (29, 55); allylpyrocatechol 3,4-diacetate, and allylpyrocatechol piperbetol (56).

cetic acid, estragole, β -cubebene, acetyleneugenol, amphenol, a-limonene, saffrole, 1,8-cineole, 4-terpineol, 4-allyl-2-methoxy-phenolacetate, and 4-allylphenyl acetate (29). Besides, Chavicol, carvacrol, Chavibetol, ursolic acid, cadinene, p-cymene, cepharadione A, dotriacontanoic acid, tritriacontane, adinene, hentriacontane, pentatriacontane, n-triacontanol, stearic acid, piperlonguminine, α -pinene, β -sitosterol palmitate, β -sitosterol, stigmasterol, 4-allyl resorcinol, aristololactam A-II, pellitorine, N-isobutyl-2E,4E-dodecadienamide, Dehydropiperonaline, piperdardine, piperolein-B, guineensine, syringaresinol-O- β -D-glucopyranoside, pinosresinol, cepharadione A, 6 β -hydroxystigmast-4-en-3-one, β -daucosterol, (2E,4E)-N-isobutyl-7-(3',4'-methylenedioxyphenyl)-2,4-heptadienamide, 23-hydroxyursan-12-en-28-oic acid, β -sitosterol-3-O- β -D-glucoside-6'-O-palmitate, (2S)-4'-hydroxy-2,3-dihydroflavonone-7-O- β -D-glucoside, and α -ethyl glucoside (54); caryophyllene, chavibetol acetate, 4-allylphenyl acetate, and β -pinene (29,54); Allylpyrocatechol monoacetate (29, 55); allylpyrocatechol 3,4-diacetate, and allylpyrocatechol piperbetol (56).

Molecular docking results

By searching the research articles, 73 isolated compounds were found as mentioned earlier. Among these, compounds that are found in the Pubchem database have been performed molecular docking studies (Tables 5-7). A number of compounds: 1-phenylpropene-3,3-diol diacetate, 4-Chromanol, 4-p-coumaroylquinic acid, Benzeneacetic acid, 4-terpineol, β -sitosterol, stigmasterol, aristololactam A-II showed promising binding score with HMG-CoA reductase (PDB ID: 1HW8) (Table 5). 4-p-coumaroylquinic acid exerted best binding affinity is -6.915 Kcal/mol, whereas simvastatin showed -5.741Kcal/mol. The order of binding score is: 4-p-coumaroylquinic

Table 5. Isolated compounds that exerted promising docking scores of with HMG-CoA reductase- an enzyme responsible for cholesterol synthesis.

Compounds name	1HW8	
	Docking Score	Glide energy
1. Simvastatin	-5.741	-43.223
2. 1-phenylpropene-3,3-diol diacetate	-5.418	-37.666
3. 4-Chromanol	-5.313	-22.702
4. 4-p-coumaroylquinic acid	-6.915	-49.333
5. Benzeneacetic acid	-5.384	-21.919
6. 4-terpineol	-5.064	-22.652
7. β -sitosterol	-5.161	-30.523
8. stigmasterol	-5.237	-31.355
9. aristololactam A-II	-5.435	-30.889

Table 6. Pharmacokinetic and toxicological properties of the compounds with good oral bioavailability.

Compound name	Molecular weight	H-b on d acceptor	H-b on d donor	LogP	Lipinski's violation	Docking score IHW8	AMES toxicity	Carcinogens	Rat acute toxicityLD50, mol/kg
1-phenylpropene-3,3-diol diacetate	234.25	4	0	2.45	0	-5.418	No	No	2.1356
4-Chromanol	150.17	2	1	1.14	0	-5.313	Yes	No	1.8891
4-p-coumaroylquinic acid	486.51	9	4	1.75	0	-6.915	No	No	2.5758
Benzeneacetic acid	136.15	2	1	1.66	0	-5.384	No	No	1.8134
4-terpineol	154.25	1	1	2.30	0	-5.064	No	No	2.0424
β -sitosterol	409.56	3	2	4.28	1	-5.161	No	No	2.6561
stigmasterol	412.69	1	1	6.62	1	-5.237	No	No	2.6561
aristololactam A-II	265.26	3	2	2.34	0	-5.435	Yes	No	2.5785

Table 7. Interaction of ligands with the amino acid residues and bond categories.

Compound name	Bond category	Amino acid residue
1. Simvastatin	Hydrogen bond	C:ASN755, D:ARG590, D:ARG590, C:GLU559, D:ASP690
	Hydrophobic bond	C:CYS561, C:CYS561, C:LEU853, C:ALA856, C:LEU857, D:VAL683, C:LEU853, C:LEU857
	Hydrogen bond	D:ASP653, D:MET655, D:GLY656, D:ASN658, D:ASN658, D:ASP653, D:GLY807, D:GLY652, D:GLY656, D:GLY806, D:GLY806, D:GLY806, D:GLY806, D:SER651
2. 4-p-coumaroylquinic acid	Hydrophobic bond	D:PHE628, D:PHE628, D:VAL805, D:ALA826, D:ALA654, D:VAL805, D:ALA826
	Electrostatic bond	D:ARG627
	Hydrogen bond	D:ASP653, D:MET655, D:GLY656, D:ASN658, D:ASN658, D:MET659, D:GLY807, D:ASP653, D:GLY652, D:GLY656, D:GLY806, D:GLY806, D:GLY806, D:SER651
3. 4-terpineol	Hydrophobic bond	D:PHE628, D:PHE628, D:VAL805, D:ALA826, D:ALA654, D:VAL805, D:ALA826
	Electrostatic bond	D:ARG627
	Hydrogen bond	C:LYS735, C:ASN755, D:ARG590, D:SER684, D:LYS691
4. 1-phenylpropene-3,3-diol diacetate	Hydrophobic bond	C:CYS561, C:LEU853
	Hydrogen bond	D:ASP653, D:MET655, D:GLY656, D:ASN658, D:ASN658, D:MET659, D:GLY807, D:ADP102, D:ASP653, D:GLY652, D:GLY656, D:GLY806, D:GLY806, D:GLY806, D:SER651:O
	Hydrophobic bond	D:PHE628, D:PHE628, D:VAL805, D:ALA826, D:ALA654, D:VAL805, D:ALA826
5. 4-Chromanol	Electrostatic bond	D:ARG627
	Hydrogen bond	D:ASP653, D:MET655, D:GLY656, D:ASN658, D:ASN658, D:MET659, D:GLY807, D:ADP102, D:ASP653, D:GLY652, D:GLY656, D:GLY806, D:GLY806, D:GLY806
	Hydrophobic bond	D:ARG627, D:PHE628, D:PHE628, D:VAL805, D:ALA826, D:ALA654, D:VAL805, D:ALA826
6. Aristolactam-AII	Electrostatic bond	D:SER651
	Hydrogen bond	C:LYS735, D:LYS692, D:LYS692, D:SER684
	Hydrophobic bond	C:LEU853
7. Benzeneacetic acid	Electrostatic bond	C:LYS735, D:ARG590, D:ARG590, D:LYS692
	Hydrogen bond	C:ASN755, C:GLU559
	Hydrophobic bond	C:CYS561, C:LEU853, C:ALA856, C:ALA856, C:ALA859, C:ALA859, C:CYS561, C:LEU853
8. Beta-sitosterol	Hydrogen bond	C:ASN755 C, GLU559
	Hydrophobic bond	C:CYS561, C:LEU853, C:ALA856, C:ALA856, C:ALA859, C:ALA859, C:CYS561, C:LEU853
	Hydrophobic bond	C:ASN755 C, GLU559
9. Stigmasterol	Hydrophobic bond	C:CYS561, C:LEU853, C:ALA856, C:ALA856, C:ALA859, C:ALA859, C:CYS561, C:LEU853, C:CYS561, C:LEU853, C:LEU853, C:LEU853
	Hydrophobic bond	C:CYS561, C:LEU853, C:ALA856, C:ALA856, C:ALA859, C:ALA859, C:CYS561, C:LEU853, C:LEU853, C:LEU853

acid> simvastatin> aristololactam A-II> 1-phenylpropene-3,3-diol diacetate> Benzeneacetic acid> 4-Chromanol> stigmasterol> β -sitosterol> 4-terpineol.

Pharmacokinetic profile

The pharmacokinetic features of the substances set by Lipinski were determined using SwissADME, an online tool. Lipinski has here declared that if a drug/compound follows the following criteria such as molecular weight <500 amu, hydrogen bond donor site < 5, hydrogen bond acceptor site < 10, and lipophilicity value $\text{LogP} \leq 5$, then the compound would be orally bioavailable. The study showed that all compounds complied with the rules of Lipinski, suggesting the strong oral bioavailability of these compounds (Table 7).

Discussion

High-fat diet can increase cholesterol levels in susceptible individuals, which in turn results in hyperlipidaemia and obesity (57). Besides, hyperlipidaemia, a severe risk factor of coronary complications is considered as a prominent aetiology of early death throughout the world (58). Previous studies also reported that an elevated level of HDL-c and diminished levels of TG, TC, and LDL-c alleviate cardiac health by preventing ischemic condition (59). The action of antihyperlipidemic agents can be attributed to improving the cessation of intestinal cholesterol absorption, catabolism of body cholesterol, interfering with the production of lipoproteins along with enhanced expression of LDL-c receptors in the liver and their protection and elimination of LDL-c from the blood by promoting their degradation. However, all these episodes either collectively or independently abridged LDL-c levels resulting in mitigation of TC concentration in blood during the treatment with sample extracts (60, 61).

The present study was conducted to investigate the role of *P. betle* leaf juice in high-fat diet-induced hyperlipidaemic conditions. High-fat diet significantly increased TG, TC, LDL-c, and VLDL-c levels as expected in rat models along with a prominent reduction in HDL-c levels compared to the normal physiologic range. In this experiment, when the *P. betle* leaves juice was co-administered with the high-fat diet, it delivered very promising responses in all parameters which were very closely comparable to the standard drug, simvastatin. PBJ at the dose of 2.0 mL/rat has showed the highest level reduction of TC, LDL-c, TG, and VLDL-c (Fig. 1 A, B, C, and E); whereas the same dose of PBJ (2.0 mL/rat) exhibited the highest level of increment of HDL-c compared to hyperlipidemic control group (Fig. 1D). Similarly among all the used doses of PBJ (0.5-3.0 mL/rat), the highest level of effects for the reduction of AST, ALT, ALP, and creatinine (Fig. 2 A-D) have been observed by PBJ (2.0 mL/rat). Based on the results we can conclude that the optimum dose of PBJ is 2.0 mL/rat. Similar to our findings, Venkadeswaran et al. (2014) reported that seven days treatment of Triton WR-1339-induced hypercholesterolemic rats with *Piper betle* extract (500 mg/kg b.wt) significantly lowered the levels of TC, TG, LDL-c, VLDL-c and glucose in blood (62). They also showed that *Piper betle* extract increased HDL-c and, enzymatic and non-enzymatic antioxidants in hepatic tissues. Additionally, they also reported that treatment of the hyperlipidemic rats with eugenol (5 mg/kg), the phyto-

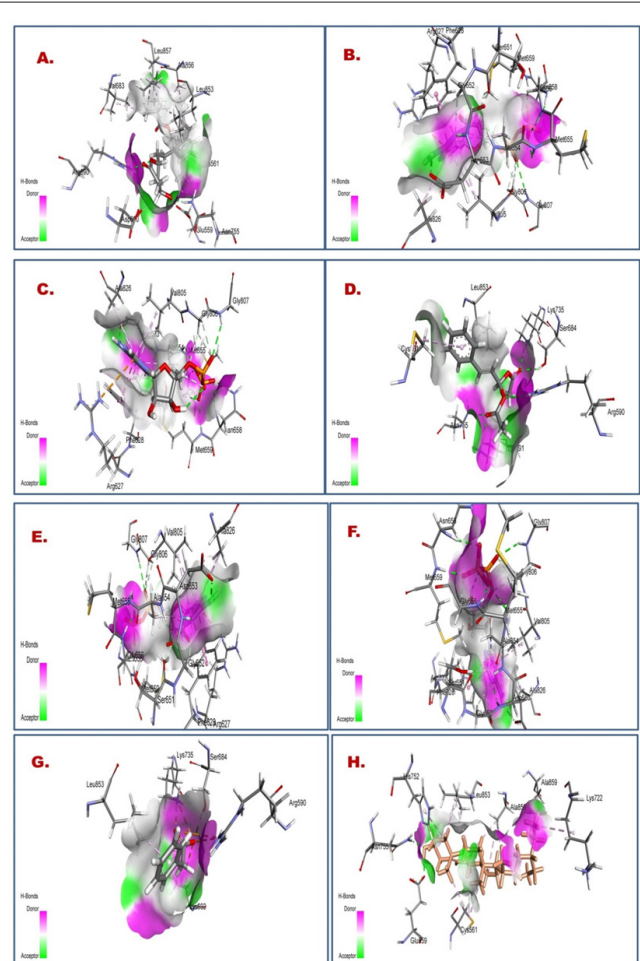


Figure 5. Ligand receptor interactions: A) Simvastatin+HMG-CoA reductase; B) 4-p-coumaroylquinic acid+HMG-CoA reductase; C) 4-Terpineol+HMG-CoA reductase; D) 1-phenylpropene-3,3-diol diacetate+HMG-CoA reductase; E) 4-Chromanol+HMG-CoA reductase; F) Aristolactam-AII+HMG-CoA reductase; G) Benzeneacetic acid+HMG-CoA reductase; H) Beta-sitosterol+HMG-CoA reductase; I) Stigmasterol+HMG-CoA reductase.

compound of *Piper betle*, in the same study exerted more stronger effect to reduce TC, TG, LDL-c, VLDL-c, and blood glucose levels as well as improvement of HDL-c and antioxidants (62). Their study concluded that eugenol from *Piper betle* is the bioactive compound or one of the bioactive compounds responsible for the hyperlipidemic activity of *Piper betle*. Another study conducted by Saravanan and Pugalendi (2004) reported that co-administration of *P. betle* leaf ethanol extract (100-300 mg/kg) for 30 days along with the daily dose of alcohol (alcohol induced hyperlipidemia) significantly lowered lipid levels in plasma and hepatic tissues compared to ethanol-treated control rats, and the highest hypolipidemic effect was observed by 300 mg/kg of the extract (63). In contrast to the finding of Venkadeswaran et al (2014), Saravanan and Pugalendi (2004) also reported that the leaf ethanol extract of *P. betle* significantly increased blood glucose levels in hyperlipidemic rats. Another study conducted by Thirumalai et al (2014) also reported the hypolipidemic activity of methanol extract of *P. betle* in high fat diet induced hyperlipidemic rats with the significant lowering of the levels of TC, TG, LDL, and VLDL (64).

HMG-CoA reductase (3-Hydroxy-3-methyl glutaryl-CoA reductase) is a polytopic transmembrane protein that plays critical role in the synthesis of cholesterol by cata-

lyzing the rate-limiting step in cholesterol synthesis (65). The cholesterol synthesis reaction is mediated by sterols and non-sterol metabolites derived from mevalonate, in which HMG-CoA is converted to mevalonate (66). HMG-CoA reductase inhibitory mechanism is extensively focused in the discovery of novel anticholesterolemic drugs based on cholesterol-synthesis-inhibiting mechanism. Although statins (such as atorvastatin, simvastatin, rosuvastatin, etc.) of synthetic origin have been shown to display antihyperlipidemic action by inhibiting HMG-CoA reductase via the mevalonate pathway. and though statins are well tolerated, these drugs are reported to exhibit potential side-effects including renal failure, hepatotoxicity, muscle wastage, etc. (56, 67). Hence, it is very important to search for novel drug molecule(s) and/or alternative medicines that would be used for the treatment of hyperlipidemia with minimal or no side-effects and its associated co-morbidities. In this respect, the present finding is significant to deliver important data for the progress of the development of alternative medicaments or novel medicines from *P. betle* is it can be used directly for the amelioration of hyperlipidemia.

Our findings observed that the lipid lowering activity of PBJ was increased in a dose dependent manner starting from 0.5 mL/rat upto the 2.0 mL/rat and then started to decline. Thus the optimum dose for the lipid lowering activity of PBJ was reported to be 2.0 mL/rat. Although PBJ 3.0 mL/rat also showed significant activities to decrease bad cholesterols and inhibited HMG-reductase enzymes, its effect was less than the dose of 2.0 mL/rat. It is assumed that the doses of PBJ higher than 3.0 mL/rat would exhibit lesser activities than that of its respective lower doses although we have not applied higher doses than BPJ 3.0 mL/rat. It is not clear why the higher doses of drugs/bioactive compounds exhibit less activity with higher doses beyond the optimum dose. One hypothesis is that every drug molecule/bioactive compound works either by binding its specific cellular receptors or by binding with endogenous biomolecules. When such kind of available binding sites are saturated with the increased dose of drugs (by the optimum dose), the higher dose may have drug/molecule that cannot bind to increase its therapeutic response. Rather the additional molecules/drugs in some way interrupt to the affinity, efficacy, and ultimately the therapeutic response of the drug. In the case of PBJ, the maximum affinity and efficacy was exhibited by PBJ 2.0 mL/rat, and higher dose PBJ 3.0 mL/rat exhibited lower activity than that its previous dose because of the saturation of specific receptors of PBJ bioactive compound(s) that is responsible for its therapeutic action.

Histopathological observation of liver sections was also documented the efficacious role of *P. betle* juice in experimental rat models. In addition, previously conducted phytochemical analysis of *P. betle* revealed several phytochemicals including polyphenols, by which it may exert its noteworthy benevolent activity by alleviating the hyperlipidaemia state (68). Along with this, further molecular docking study corroborates this finding: many compounds showed promising results, yet 4- ρ -coumaroylquinic acid manifested a much better score than that of simvastatin, an antihyperlipidemic drug that blocks HMG-CoA reductase, thereby may be the responsible mechanism of inhibiting cholesterol synthesis (Figure 4). Venkadeswaran et al. (2014) showed the potential antihyperlipidemic activ-

ity of eugenol, the bioactive compound of *P. betle* (62). Thus, the bioactive compound responsible for hyperlipidemic activities in our study would be eugenol and/or 4- ρ -coumaroylquinic acid among other phytochemicals. Interactions of ligands with amino acid residues will be boon for synthesizing new drug candidates for the treatment of hyperlipidemia. This observation also ascertains the claim of considering *P. betle* as a promising candidate for drug discovery or using betel leaf juice preparations for the treatment of hyperlipidaemia. In our study, we could not investigate the phytochemical profiling and identification of responsible bioactive phytochemical(s) and also evaluation of the bioactive compounds for its antihyperlipidemic activities. However, further studies are warranted to identify and determination of the bioactive compound(s) responsible for antihyperlipidemic activities as well as to elucidate its underlying mechanism of actions.

The conducted study on a high fat diet-induced hyperlipidemic rat model provides new insights into the antihyperlipidemic activity of *P. betle* L. leaf juice by evaluating biochemical and histopathological parameters. Reduction of TG, TC, LDL-c, and VLDL-c levels as well as promotion of HDL-c concentrations by PBJ clearly demonstrated a potential lipid lowering activity of *P. betle* which were very close to the commercially available drug, simvastatin (used as a reference drug) and gives a ray of hope to consider it as a possible wellspring of hypolipidemic drug agents or the PBJ can be directly used as an alternative medicine for the treatment of hyperlipidemia. Additionally, in molecular docking analysis, several bioactive promising biomolecules exhibited optimistic binding affinity to specific proteins, and the ADME study displayed their drug-like characters. However, further studies are requested to establish the responsible active compounds and their possible mode of action as well as a complete safety profile.

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Conflict of interest

The authors declare that they do not have any conflict of interests on the research work and to publish the article.

Consent of publication

All authors of this manuscript have consented to publish the article and they don't have any conflict of interest with this article.

Authors' contributions

MMRS conceptualized, designed, interpreted the results, and supervised the whole project. XJ, AJG, and MRAM carried out experiments. XJ, MRAM, and SA analyzed and interpreted data and presented the data in tables and figures. XJ, MRAM, SA, FK, and MAS wrote the manuscript. MSH, and LCM critically reviewed the manuscript. MMRS revised the final manuscript. SUA and RH significantly contributed to address the reviewers' comments and revising the manuscript. All authors read the manuscript and agreed to be accountable for all aspects of the work and approved the final manuscript.

Data availability

All data generated or analyzed during this study have been included in the article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethical approval

The ethical approval for the experimental protocol of animal care and use in this study was obtained from the Animal Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong Province, China (Approval Number: 2020-031101) and Animal Ethics Committee of State University of Bangladesh, Dhanmondi, Dhaka, Bangladesh (Ethical approval No. 2020-02-03/SUB/A-AEC/0004).

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