1 *Dillenia indica* fruit extract has Glucose and Cholesterol Lowering
2 effects

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26 Abstract

27 **Background:** *Dillenia indica* (*D. indica*) can suppress carbohydrates
28 hydrolysis by inhibiting α -amylase and α -glucosidase. However, there is a lack
29 of understanding of its therapeutic potential as an antidiabetic and anti-
30 hyperlipidemic agent.

31 **Methods and findings:** Type 2 diabetes (T2D) was induced by a single
32 intraperitoneal injection of Streptozotocin (STZ; 90mg/kg) and hyperlipidemia
33 by feeding with 1% cholesterol, 5% coconut oil and 5% cow fat diet.
34 Administration of *D. indica* extracts in water for four weeks triggered a
35 significant ($p \leq 0.05$) reduction in fasting serum glucose (FSG) levels with
36 concomitant improvement in serum insulin levels. Both the water- and
37 ethanol-extract of *D. indica* treated groups showed significant ($p \leq 0.01$)
38 reduction in total cholesterol levels by 25% and 19%, respectively. HDL-
39 cholesterol was also augmented (by 14%) in ethanol-extract treated group.
40 Liver glycogen content was higher in the water-extract treated group.
41 Histopathological examination revealed that there was no tubular epithelial
42 cell degeneration or necrosis in the renal tissues or hepatocyte degeneration
43 and sinusoidal dilation in liver tissues in animals that received the water-
44 extract. On the other hand, consumption of *D. indica* extract with 1%
45 cholesterol, 5% coconut oil diet or with a 5% cow fat diet for 14 days
46 significantly reduced serum cholesterol levels in group-III (60→45 mg/dl;
47 $p \geq 0.05$) and -IV (85→66 mg/dl; $p \geq 0.05$) hypercholesterolemic model rats. *D.*
48 *indica* fruit extract also reduced serum TG levels (Group-III: 87→65 mg/dl;
49 Group-IV: 40→90 mg/dl; $p \geq 0.05$). Interestingly, treatment with *D. indica*

prevented a reduction in serum HDL levels in those hypercholesterolemic model rats. Serum LDL levels were significantly lower in group-III (47→39 mg/dl; $p \geq 0.05$) and group-IV (57→44 mg/dl; $p \geq 0.05$) hypercholesterolemic model rats after *D. indica* treatment.

Conclusion: *D. indica* fruit ameliorates FSG, insulin secretion, glycogen synthesis, and serum lipid profile. Therefore, *D. indica* fruit can be a potential therapeutic agent for diabetic and hyperlipidemia.

Keywords: *D. indica* fruit; Diabetes; Insulin; Lipid-Profile; Histopathology.

74 Introduction

75 Diabetes mellitus is a chronic metabolic disorder characterized by on-going
 76 hyperglycemia. Insulin resistance and decreased insulin secretion are the two
 77 cardinal features of type 2 diabetes mellitus (T2DM). T2DM is typically
 78 diagnosed based on glucose levels, either a fasting plasma glucose (FPG) $\geq$
 79 7.0 or a 2-hour 75 g oral glucose tolerance test (OGTT) $\geq$ 11.1 mmol/L levels.
 80 Pre-diabetic classifications include a fasting blood glucose (FBG) of 5.6–
 81 6.9 mmol/L or a 2-hour blood glucose level of $\geq$ 7.8 and $<$ 11.1 mmol/L [1].
 82 T2DM is often accompanied by cardiovascular disease, diabetic neuropathy,
 83 nephropathy, and retinopathy. An altered lipid profile is common in T2DM
 84 patients. Furthermore, reduced hepatic glycogen storage is also observed in
 85 diabetes [2-4]. Advancements in the modern lifestyle over the last century
 86 have contributed to a dramatic escalation in the incidence of T2DM and
 87 hyperlipidemia worldwide [5, 6]. Diabetes mellitus (DM) ranks seventh among
 88 the leading causes of death and ranks third when its complications are taken
 89 into account [7]. One of the potential complications of T2DM is coronary heart
 90 disease due to hyperlipidemia. Hyperlipidemia is a metabolic disorder
 91 characterized by successive accumulation of lipids and leukocytes in the
 92 arterial wall. This can contribute to many forms of diseases, especially
 93 cardiovascular ones such as myocardial infarction and stroke. Moreover,
 94 systemic hypercholesterolemia is associated with massive neutrophilia and
 95 monocytosis [8-11]. Peripheral leukocyte amount is proportional to the level of
 96 cardiovascular complications [12]. Moreover, hypercholesterolemia is
 97 positively associated with systemic neutrophil as well as monocyte expansion,
 98 suggesting that abnormalities in circulating lipids can influence myeloid cell

99 expansion [13]. Thus the pathophysiological mechanism underlying
 100 hypercholesterolemia is the enrichment and accumulation of systemic
 101 neutrophils and monocytes which subsequently increase atherosclerosis.
 102 Atherosclerosis due to hyperlipidemia (elevated levels of cholesterol, TG,
 103 LDL) is the principal cause of mortality affecting people worldwide [14].
 104 According to the World Health Organization (1999), it was estimated that high
 105 cholesterol level causes around one-third of all cardiovascular disease
 106 worldwide and that there are 10,000,000 people with familial
 107 hypercholesterolemia worldwide [15, 16]. Thus, overall prevention and
 108 amelioration of T2DM and hyperlipidemia require an integrated, international
 109 approach to combat the rapid increase in the number of patients in the
 110 forthcoming years [5, 17].

111 Dietary cholesterol has a direct effect on plasma levels of cholesterol [18-20].
 112 Dietary cholesterol increases serum cholesterol in all common species if high
 113 enough loads are given. The extent of increase depends on the compensatory
 114 mechanisms such as enhanced excretion of bile acids and neutral sterols and
 115 regulation of cholesterol synthesis and absorption [21]. Lipid-lowering agents
 116 have been responsible for a 30% reduction of cardiovascular diseases,
 117 therefore validating the search for new therapeutic drugs to reduce
 118 hyperlipidemia [22]. In addition, natural products have the potential to prevent
 119 T2DM or to keep the disease under control [23-25]. The World Health
 120 Organization (WHO) has also recommended evaluating the effectiveness of
 121 natural products when there is a lack of safe modern drugs [26, 27]. Moreover,
 122 it is believed that natural products may have fewer side effects than
 123 conventional drugs. One such natural product is *Dillenia indica* (*D. indica*),

locally known as chalta or “elephant apple”. The fruit of *D. indica* is a 5-12 cm diameter aggregate of 15 carpels, with each carpel containing five seeds embedded in an edible pulp. *D. indica* fruit is widely used in many indigenous medicinal preparations against several diseases [28] (Supplemental Figure 1).

The enzyme inhibition capacity of the active constituents in *D. indica* has been reported in several studies. For example, betulinic acid in *D. indica* fruits can inhibit tyrosinase [29], and sterols in *D. indica* leaves can inhibit α -amylase and α -glucosidase [30]. Nevertheless, despite its traditional claims [31], limited data is available on the hypoglycemic and anti-hyperlipidemic activities exerted by *D. indica* fruit. Here we hypothesized that *D. indica* fruit may reduce post-prandial hyperglycemia and hyperlipidemia by suppressing carbohydrate hydrolysis and lipid absorption in the gut respectively, which may be beneficial for diabetic and hyperlipidemic control. Thus, the aim of this present study was to investigate the antidiabetic and anti-hyperlipidemic effect of *D. indica* fruit in Streptozotocin (STZ)-induced type 2 diabetic model rats and high fat diet (1% cholesterol, 5% coconut oil and 5% cow fat) induced hyperlipidemic model rats.

Here we showed that oral administration of both *D. indica* extract in water or ethanol in Long-Evans female rats for 28 consecutive days significantly ameliorates serum fasting glucose with concomitant enhancement in insulin secretion and liver glycogen content. Moreover, no noticeable change was observed in the serum creatinine and ALT levels in the extract in water and extract in ethanol treated mice, which is reflected in the histopathological analysis, indicating that the extract is safe for kidney and liver functions. In

addition, consumption of *D. indica* extract with 1% cholesterol and 5% coconut oil or a 5% cow fat diet for 14 days significantly reduced serum cholesterol, TG, LDL levels with concomitant enhancement in HDL in the hyperlipidemic model rats.

Methods

Plant material

Cultivated matured fruits of *D. indica* (20 kg) were purchased from the local market. Bangladesh National Herbarium ascertained the fruit to be the correct specimen, and a voucher (DACB-35371) was deposited. The fruits were rinsed with fresh sterilized water and sliced into small pieces with a clean knife. *D. indica* slices were naturally dried under the sun. The dried slices (7 kg) were then ground with a blender to yield 1.8 kg of fine powder.

Preparation of Extract in Ethanol

D. indica powder (900 g) was mixed with 5.4 L of 80% ethanol (1:6), before being kept frozen overnight. On a subsequent day, the suspension was filtered with a sterile cloth, followed by filter paper. Approximately 3.3 L of the filtrate was then collected. The main constituents of *D. indica* were extracted by using a BUCHI Rotavapor R-114 followed by incubation in a water bath (55°C) to remove the ethanol. The process yielded approximately 420 ml of filtrate. The semi-dried extract was further dehydrated in a freeze dryer (HETOSICC, Heto Lab Equipment Denmark) at -55°C followed by storage in an amber bottle (-8°C). The dried extract was weighed using a digital balance

170 (GIBERTINI E 42-B). Approximately 150 g (yield: 16.67% w/w) of extract of *D.*
171 *indica* fruits was produced.

172 ***Preparation of Extract in Water***

173 The *D. indica* fine powder (900 g) was dissolved in 9 L water (1:10) and was
174 kept frozen overnight. The suspension was filtered using sterile cloth. The
175 solution was then re-filtered using filter paper to produce approximately 5.5 L
176 of filtrate. The suspension was dried by a BUCHI Rota vapor R-114 and was
177 incubated in a water bath (70°C) to evaporate the water, yielding
178 approximately 310 mL of filtrate. The semi-dried aqueous extract was further
179 dried in a freeze dryer (HETOSICC, Heto Lab Equipment Denmark) at -55°C.
180 Subsequently, it was stored in an amber bottle (-8°C). Ultimately, 90 g of *D*
181 *.indica* fruit extract in water was acquired (10% w/w).

182 ***Animal Model for Anti-Diabetic Study***

183 Adult Long-Evans female rats weighing between 170 and 230 g were used.
184 The animals were bred at the Bangladesh Institute of Research and
185 Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) in
186 the Animal House, Dhaka, Bangladesh. The animals were kept at constant
187 room temperature (22 ± 5°C), with a humidity of 40-70%, and in a natural 12-
188 hour day-night cycle. The rats were sustained with a standard laboratory
189 pellet diet while water was allowed *ad libitum*. All animal procedures were
190 performed according to the National Institute of Health (NIH) guidelines under
191 the protocol as approved by the Institutional Animal Care and Use Committee
192 of BIRDEM.

Preparation of Type 2 Diabetes Model Rats

STZ (2-deoxy-2-(3-methyl-3-nitrosurea) 1-d-glucopyranose) is a potent alkylating agent that is highly genotoxic and responsible for generating mutations in the cells. STZ contains a highly reactive nitrosurea side chain which is responsible for the initiation of cytotoxic and genotoxic action on pancreatic β -cells. Moreover, STZ is transported into pancreatic β -cells through GLUT2, the glucose transporter, which becomes non-functional in T2DM. Thus, STZ is widely used for the induction of T2DM [32]. Before use, a fresh STZ solution was prepared by dissolving 100 mg STZ in 10 ml (0.1 M) citrate buffer, pH 4-5. The molecular weight for STZ is 265.222, so the molarity for STZ was 3.77 μ M. A T2DM condition was created by a single intraperitoneal injection (90 mg/kg) of STZ in rat pups (48 hours old; mean weight 7 g) as previously described [33, 34]. The experiments were done three months after the STZ injection. T2DM can onset at a young age, but most patients are diagnosed at middle age or later. In our study, we focused on adult-onset diabetes by using 3-month-old rats which are an appropriate model for adult-onset. Rats with blood glucose levels of 8–12 mmol/L under fasting conditions were included in the experiments to ensure that STZ had induced type 2 diabetes in all subjects.

Grouping of Diabetes Model Rats

The rats (n=32) were divided into four groups (n=8). Depending on their group, they were given one treatment per day for 28 consecutive days of one of the following:

216 1) Type 2 diabetic control group (T2 Control) was given deionized water (10
217 ml/kg).

218 2) Type 2 positive control group (Glibenclamide) was treated with
219 glibenclamide (5 mg/10 ml) (9.9 ml H₂O + 0.1 ml Twin 20)/kg.

220 3) Type 2 diabetic extract in water treated group was administered aqueous
221 extract of *D. indica* (1.25 g/10 ml/kg), which was standardized to 138.89 g
222 fresh fruit of *D. indica*.

223 4) Type 2 diabetic extract in ethanol treated group was treated with ethanol
224 extract of *D. indica* at a dose of 1.25 g/10 ml/kg, which was standardized to
225 83.34 g fresh fruit of *D. indica*.

226 ***Animal Model for Anti-hyperlipidemic Study***

227 The rats (n=24) were divided into four dietary groups (I, II, III and IV) of six
228 rats each. The rats of all groups were fed on a standard pellet diet (diet I) and
229 water ad libitum. Experimental diets were supplied each day through a
230 metallic, smooth stomach tube, along with pellet diet. Rats in group-I were fed
231 with lab pellet diet for 24 consecutive days until the end of the experiment. In
232 order to make the rats hyperlipidemic, the rats from groups-II and -III were fed
233 with 1% cholesterol and 5% coconut oil (diet II) for the first ten days of the
234 experiment. Subsequently, group-II was continued on diet II (1% cholesterol
235 and 5% coconut oil) for an additional 14 days. Rats of group-III were fed with
236 1% cholesterol and 5% coconut oil (for 10 days) in addition to ethanol extract
237 of *D. indica* for the next 14 days. On the other hand, rats from group-IV
238 received a 5% cow fat diet for the first ten days of experiment and

subsequently were continued with the feeding of 5% cow fat diet along with ethanol extract of *D. indica* for an additional 14 days.

Dose for D. indica Extracts

For the oral toxicity study, the Organization for Economic Co-operation and Development (OECD) guideline 425 was followed. Moreover, the histopathological studies on kidney and liver tissues for both extract-treated groups serve as a marker of the safety of *D. indica* extracts. Treatment with the selected doses for the extract of *D. indica* in ethanol at 1.25 g/10 ml/kg did not lead to any mortality; all the animals were found to be alive, healthy and active during the experimental periods.

Blood Collection Procedure for Biochemical Analysis

The animals were anesthetized using isoflurane before blood collection (400 µl) following amputation of the tail tip. The samples were centrifuged (2500 rpm X 15 minutes), and the serum was transferred to fresh Eppendorf tubes. Serum was refrigerated (-20°C) until further analysis. All the biochemical experiments were performed within two weeks of serum collection.

Collection and Preservation of Liver and Kidney Tissue for Histology

Following sacrificed by cervical dislocation, the liver and kidney tissues were collected, washed in normal saline, and fixed by using 40% formaldehyde for 24 hours. Subsequently, the tissues were subjected to alcohol dehydration. All tissue samples were washed and embedded by using paraffin and xylene. The tissues were then double-stained.

261 ***Determination of serum glucose levels***

262 Glucose concentration was estimated by glucose oxidase (GOD-PAP,
263 Boheringer Mannheim GmbH) as described previously [35].

264 ***Determination of serum insulin levels***

265 Serum insulin was analyzed by an enzyme-linked immunosorbent assay
266 (ELISA) kit for rat insulin (Crystal Chem Inc., Downers Grove, IL, USA) [36].

267 ***Measurement of Liver Glycogen Content***

268 The glycogen content in the rat liver was measured by using the anthrone-
269 sulfuric acid method as described previously [37].

270 ***Determination of serum ALT and creatinine levels***

271 Alanine aminotransferase (ALT) levels as well as serum creatinine were
272 measured using a Clinical Biochemistry Analyzer (BioMajesty® JCA-
273 BM6010/C).

274 ***Haematoxylin & eosin (H & E) staining***

275 H & E staining was performed as described previously [38]. In order to
276 demonstrate the difference between the nucleus and the cytoplasm, acid
277 (eosin) and basic (haematoxylin) dyes were used. The slides were placed in
278 Harri's haematoxylin for 10 minutes and rinsed with tap water until the water
279 was colorless. Then they were counterstained with 1% eosin solution for 1
280 minute. Photomicrographs were acquired by using a transmission microscope
281 (Nikon, Minako, Tokyo) at Dhaka Medical College Hospital, Bangladesh.

282 ***Measurement of serum cholesterol levels***

283 Serum total cholesterol level was measured by an enzymatic colorimetric
284 method (Cholesterol Oxidase /Peroxidase, CHOD-PAP, Randox Laboratories
285 Ltd., UK), as described previously [39].

286 ***Determination of serum TG levels***

287 Serum triglyceride (TG) was measured by an enzymatic colorimetric glycerol-
288 3-phosphate oxidase phenol aminophenazone (GPO-PAP) method (Randox
289 Laboratories Ltd., UK), as described previously [40].

290 ***Determination of serum HDL levels***

291 Serum HDL level was determined by using an enzymatic colorimetric assay
292 (HDL cholesterol E kit, WAKO Diagnostics), as described previously [41].

293 ***Determination of serum LDL levels***

294 Serum LDL level was calculated either indirectly by using the Friedewald
295 Formula [50] or directly by using the Equal LDL Direct Select Cholesterol
296 Reagent as described previously [41]

297 ***Statistical Analysis***

298 Data were analyzed using a Statistical Package for Social Science software
299 version 12 (SPSS Inc., Chicago, Illinois, USA). The data were reported as
300 mean $\pm$ SD or as median (range) where appropriate. Statistical analysis was
301 accomplished by using student t-tests (paired and unpaired) or ANOVA

(analysis of variance) followed by a Bonferroni post hoc test. A p-value of ≤ 0.05 was considered statistically significant.

Results

D. indica decreased fasting glucose levels

After oral administration of the respective treatments for 28 days, there was a decrease in the fasting serum glucose (FSG) levels of animals in all the groups (Figure 1). Only type 2 diabetic rats treated with extract of *D. indica* in water showed a significant reduction ($p \leq 0.05$) in FSG, although the extract in ethanol treated group showed a reduction of FSG level (by 11%) when compared to the baseline (Day 0). As expected, glibenclamide significantly ($p \leq 0.01$) ameliorated the diabetic condition on day 28 by a 23% reduction as compared to the baseline (Day 0). These data suggest that *D. indica* fruit can lower fasting serum glucose levels.

Figure 1. Chronic effect of fruit extracts on fasting serum glucose (FSG) level in STZ-induced type 2 diabetic model rats. Water-extract of *D. indica* significantly decreased FSG levels in diabetic rats. Results are expressed as mean $\pm$ standard deviation (SD). Statistical analysis within groups was conducted using a paired t-test while the comparison between groups was done using a one-way ANOVA with post-hoc Bonferroni correction. * $p \leq 0.05$; ** $p \leq 0.01$.

D. indica increased serum insulin levels

After 28 days, the extract in water treated group showed a significant ($p \leq 0.01$) increase (208%) in serum insulin level, while the type 2 control group showed

a 44% reduction compared to baseline (Day 0). Moreover, the insulin level was significantly ($p \leq 0.05$) higher in the extract in water treated group when compared with the type 2 control group (Figure 2). On the other hand, the glibenclamide treated group showed a 30% increase and the extract in ethanol treated group showed a 19% increase in serum insulin levels compared to baseline (Day 0) (Figure 2). These data indicate that *D. indica* fruit positively modulates pancreatic β -cells to release insulin into the blood.

Figure 2. Effect of *D. indica* fruit extracts in serum insulin levels. Water-extract of *D. indica* significantly increased serum insulin levels in STZ-induced type 2 diabetic model rats. Results are expressed as mean $\pm$ SD. Statistical analysis within the groups was done using a one-way ANOVA with *post-hoc* Bonferroni correction. * $p \leq 0.05$, ** $p \leq 0.01$.

***D. indica* did not affect body weight**

The effect of both *D. indica* extracts (in water and ethanol) on the body weight of type 2 diabetic rats during 28 days of chronic administration was observed. The body weight of each rat was taken at seven days' intervals and was found to increase on average by 2-7% in all groups. However, there was no significant difference among the groups (Figure 3), suggesting that *D. indica* fruit extracts did not affect body weight.

Figure 3. A consequence of *D. indica* fruit extracts on rat body weight.

No significant change was observed in rat body weight among the groups after chronic treatment with *D. indica* fruit extracts. Statistical analysis between the groups was done using a one-way ANOVA with *post-hoc* Bonferroni correction.

***D. indica* improved liver glycogen content**

The extract in water treated group showed the highest amount (20.39 mg/g) of liver glycogen content among all the groups, whereas liver glycogen content was lowest (7.54 mg/g) in the type 2 control group. There was no significant difference in the liver glycogen content when the glibenclamide and extract-treated groups were compared (Figure 4). Thus, the fruit of *D. indica* enhances liver glycogen content in diabetic rats.

Figure 4. Effect of *D. indica* fruit extracts on liver glycogen content. *D. indica* extract in water showed the highest amount of liver glycogen content among all the groups including the glibenclamide treated group. The results are expressed as mean $\pm$ SD.

***Effect of D. indica* on serum lipid profile in diabetic model rats**

D. indica extract in water significantly decreased ($p \leq 0.01$) serum cholesterol on Day 28 [serum cholesterol (mean $\pm$ SD) mg/dl: Day 0 (75.00 $\pm$ 6.37) vs Day 28 (56.00 $\pm$ 4.84)] when compared with the glibenclamide-treated group (Figure 5A). Extract of *D. indica* in ethanol caused a significant ($p \leq 0.01$) reduction in the total cholesterol level on day 28 [serum cholesterol (mean $\pm$ SD) mg/dl: Day 0 (75.00 $\pm$ 6.30) vs Day 28 (61.00 $\pm$ 2.78)] when compared with type 2 control (Figure 5A). Both the extract in water and the extract in ethanol treated groups showed a reduction in serum TG by Day 28, about 29% (62.00 to 44.00 mg/dl; $p \leq 0.05$) and 32% (57.00 to 39.00 mg/dl; $p \leq 0.05$), respectively (Figure 5B).

The extract of *D. indica* in ethanol significantly increased serum HDL by 14% (36 to 41 mg/dl; $p \leq 0.05$), while it reduced LDL by 24% (34 to 26 mg/dl) by Day 28 as compared to the baseline (Day 0). In addition, enhanced HDL cholesterol in the extract in ethanol treated group was statistically significant ($p \leq 0.05$) when compared with the type 2 control group. Moreover, both the glibenclamide and extract in water treated groups showed an increase in serum HDL levels by 6% (34 to 36 mg/dl) (Figure 5C). Atherogenic LDL-cholesterol levels were decreased by 24% (34 to 26 mg/dl), 19% (36 to 29 mg/dl), and 23% (30 to 23 mg/dl) for the extract in ethanol, extract in water, and glibenclamide treated groups, respectively (Figure 5D). These data suggest that *D. indica* fruit can ameliorate the lipid profile in diabetic rats.

Figure 5. Effect of *D. indica* on serum lipid profile. (A) Both extract in water and in ethanol significantly decreased serum cholesterol levels. (B) Both extracts in water and in ethanol showed a significant reduction in serum TG levels. (C) Extract in ethanol significantly increased serum HDL levels. (D) Reduction in serum LDL levels was also observed by *D. indica* fruit extracts but was not statistically significant. Results are expressed as mean $\pm$ SD. Statistical analysis within the groups was done using a one-way ANOVA with *post-hoc* Bonferroni correction. * $p \leq 0.05$, ** $p \leq 0.01$.

Effects of D. indica on serum ALT and creatinine levels

The effects of the extract of *D. indica* in water or in ethanol on kidney and liver functions were also investigated. There was an increase in the serum creatinine levels only in the type 2 control group, although serum creatinine was decreased in glibenclamide (9%), extract in water (13%), and extract in

ethanol (6%) treated groups (Figure 6A). In addition, there was no significant change in serum ALT levels in any of the groups (Figure 6B). These data indicate that *D. indica* extracts are safe for kidney and liver functions.

Figure 6. Evaluation of *D. indica* extracts on kidney and liver functions.

D. indica fruit extracts led to no significant change in the serum creatinine (A) and the serum ALT (B) levels. Results are expressed as mean $\pm$ SD. Statistical analysis within the groups was done using a one-way ANOVA with *post-hoc* Bonferroni correction.

Histopathology

To further confirm the safety of *D. indica* (1.25 g/10 ml/kg) on kidney and liver functions, histopathological examination was performed. Tubular epithelial cell degeneration, necrosis, and hyperemic vessels in the interstitium were examined in the kidney samples. Histological examination revealed that kidney tissues of the type 2 control group were more affected as compared to the extract in water, extract ethanol, and glibenclamide treated groups. Tubular epithelial cell degeneration and tubular epithelial cell necrosis were observed in the kidney tissue of type 2 control group but were absent in the extract in water treated group. The glibenclamide treated group showed well-arranged cells and there was only mild necrosis observed in the extract in ethanol treated group (Figure 7; Table 1), indicating that the aqueous extract of *D. indica* has some renal protective effects.

Figure 7. Histological examination of kidney samples after treatment with *D. indica* fruit extracts. (A) The normal control group showed well-arranged kidney cells. (B) The type 2 control group (diabetic) showed mild

tubular epithelial cell degeneration and moderate tubular epithelial cell necrosis. (C) The glibenclamide-treated group showed the presence of well-arranged cells. (D) The extract in water treated group showed no toxic effect on kidney cells. (E) The extract in ethanol treated group showed mild necrosis. All figures are observed under a 40x magnification.

Table 1: Histopathological changes in kidney samples

Parameters	Normal group	Type 2 Control group	Glibenclamide group	Water extract group	Ethanol extract group
Tubular epithelial cell degeneration	-	+	-	-	-
Tubular epithelial cell necrosis	-	++	-	-	+
Hyperemic vessels in the interstitium	-	-	-	-	-

**Histopathologic assessments of the experimental parameters were graded as follows: (-) showing no change and (+), (++) indicating mild and moderate changes respectively.*

In the liver samples, hepatocyte degeneration, sinusoidal dilation, and pleomorphism of the hepatocytes were investigated. Histological examination

revealed that the liver tissue of the glibenclamide treated group was more affected as compared to type 2 control, extract in water, and extract in ethanol treated groups. Mild hepatocyte degeneration and sinusoidal dilation were observed in the glibenclamide treated group. Notably, there was no hepatocyte degeneration, sinusoidal dilation, or pleomorphism of the hepatocytes in the extract in water treated group, although mild sinusoidal dilation was observed in the extract in ethanol treated group, suggesting that *D. indica* aqueous extract is safe for liver function (Figure 8; Table 2).

Figure 8. Histological consequences in liver samples after treatment with *D. indica* fruit extracts. (A) The normal control group showed well-arranged liver cells. (B) The type 2 control group (diabetic) showed no significant changes. (C) The glibenclamide-treated group showed mild hepatocyte degeneration and sinusoidal dilation. (D) The extract in water treated group showed no significant pathological changes in liver cells. (E) The extract in ethanol treated group showed mild sinusoidal dilation. All figures are observed under a 40x magnification.

Table 2: Histopathological changes in liver samples

Parameters	Normal group	Type 2 Control group	Glibenclamide group	Water extract group	Ethanol extract group
Hepatocyte degeneration	-	-	+	-	-

Sinusoidal dilation	-	-	+	-	+
Pleomorphism of the hepatocyte	-	-	-	-	-

**Histopathologic assessments of the experimental parameters were graded as follows: (-) showing no change and (+), (++) indicating mild and moderate changes respectively.*

Treatment with D. indica fruit extract significantly reduced serum cholesterol levels in hyperlipidemic model rats

Measurement of serum cholesterol is crucial since an altered serum metabolic profile is a potential indicator of many pathological conditions including cardiovascular diseases. In this experiment, we showed that consumption of pellet diet did not enhance serum cholesterol levels in control rats (group I). Moreover, there was also no noticeable change in the control rats among all the groups. In contrast, treatment with 1% cholesterol and 5% coconut oil significantly enhanced serum cholesterol levels at day 10 (41 to 66 mg/dl; $p \geq 0.05$), and at day 24 from 66 to 71 mg/dl. Moreover, ANOVA analysis showed that enhancement of serum cholesterol levels in group-II was significant ($p \geq 0.05$) when compared with the normal pellet diet group. These data suggest that consumption of 1% cholesterol and 5% coconut oil has an acute effect on the enhancement of serum cholesterol levels as it was

observed at day 10 and further consumption for another 14 days did not lead to any significant enhancement at day 24 (Figure 9).

Figure 9: Effect of *D. indica* fruit on serum cholesterol levels in hyperlipidemic model rats. After 14 days treatment with *D. indica* fruit extract in ethanol, significantly reduce serum cholesterol levels in 1% cholesterol and 5% coconut oil diet group and in 5% cow fat treated group. Results are expressed as mean $\pm$ standard deviation (SD). Statistical analysis within groups was conducted using a paired t-test while the comparison between the groups was done using a one-way ANOVA with post-hoc Bonferroni correction.* $p \leq 0.05$; ** $p \leq 0.01$.

Interestingly, when *D. indica* fruits extract in ethanol (1.25 mg/kg) was supplemented to the 1% cholesterol and 5% coconut oil treatment in group-III, those hypercholesterolemic rats showed significant reduction in serum cholesterol levels (60 to 45 mg/dl; $p \geq 0.05$), suggesting that *D. indica* fruit extract has potential to reduce serum cholesterol level (Figure 9).

Cow fat (tallow) is primarily made up of triglycerides. In this experiment, a 5% cow fat diet significantly enhanced serum cholesterol levels (38 to 85 mg/dl) in group-IV rats. Moreover, enhancement in serum cholesterol levels was higher (224%) when fed the cow fat diet, than when rats were fed the 1% cholesterol and 5% coconut oil diet (162%), suggesting that cow fat is more potent than 1% cholesterol and 5% coconut oil in inducing serum cholesterol levels. As expected, group-IV rats given the cow fat diet with *D. indica* extract showed a significant reduction (85 to 66 mg/dl; $p \geq 0.05$) in cholesterol levels. Although, the reduction was significant, the value (66 mg/dl) was higher than levels (45

mg/dl) in group-III rats (Figure 9). Taken together, these data suggest that extract of *D. indica* in ethanol possesses an anti-cholesterolemic effect with a saturation capacity depending on the fat content and quality of the diet.

Treatment with D. indica fruit extract significantly reduced serum TG levels in hyperlipidemic model rats

Like was seen with cholesterol levels, the pellet diet did not enhance TG levels in control rats (Group-I). However, treatment with 1% cholesterol and 5% coconut oil for 10 days, enhanced serum TG levels (53 to 80 mg/dl) in group-II rats. Further treatment for an additional 14 days, led to no additional enhancement in serum TG levels, indicating a saturation effect of the extract in lowering TG levels, as was observed with cholesterol levels (Figure 10).

Figure 10: Effect of *D. indica* fruit on serum TG levels in hyper-lipidemic model rats. Consecutive treatment for 14 days with *D. indica* fruit extract significantly reduced serum TG level in the hypercholesterolemia model rats consumed with 1% cholesterol and 5% coconut oil diet group and in 5% cow fat. Results are expressed as mean $\pm$ standard deviation (SD). Statistical analysis within groups was conducted using a paired t-test while the comparison between the groups was done using a one-way ANOVA with post-hoc Bonferroni correction.* $p \leq 0.05$.

Interestingly, in group-III rats, treatment with 1% cholesterol and 5% coconut oil enhanced serum TG levels at day 10, however, the *D. indica* extract for an additional 14 days significantly reduced serum TG levels (87 to 65 mg/dl; $p \geq 0.05$). Treatment with cow fat diet for 10 days significantly enhanced serum TG levels (40 to 90 mg/dl; $p \geq 0.05$) in group-IV. Moreover, ANOVA analysis

showed that enhancement of serum TG levels in group-II and -III by cow fat diet was statistically significant ($p \geq 0.05$) compared with group-I rats. Consecutive treatment with *D. indica* extract for 14 days with the cow fat diet significantly decreased serum TG levels (90 to 66 mg/dl; $p \geq 0.05$). Noticeably, the enhancement in serum TG levels by 1% cholesterol, 5% coconut oil and a 5% cow fat diet in the group-II and group-III rats was statistically significant ($p \geq 0.05$) when compared to group-I rats as revealed by ANOVA analysis (Figure 10). Moreover, reduction in serum TG levels in group-III and group-IV rats by *D. indica* treatment were similar 75% (87 to 65 mg/dl) and 73.3% (90 to 66 mg/dl), suggesting that *D. indica* extract reduced serum TG levels beyond the fat quality and composition of the diet. Taken together, these data suggest that *D. indica* fruit extract in ethanol has a strong serum TG lowering effect in the hyper-cholesterolemic rats beyond the quality of the fat diet.

Treatment with D. indica fruit extract showed no change in serum HDL levels in hyperlipidemic model rats

In this study, the mean serum HDL level was 38 mg/dl in the pellet diet group (Group-I), with no significant change throughout the 24 days. In group-II rats, there no significant change in serum HDL levels for 10 days, however, treatment with 1% cholesterol and 5% coconut oil significantly reduced serum HDL levels (33 mg/dl to 25 mg/dl; $p \geq 0.05$) at 24 days, suggesting that the 1% cholesterol and 5% coconut oil diet is sufficient to reduce the amount of HDL in a chronic consumption strategy (24 days) (Figure 11). This was unlike pattern seen with the diets in groups-I and -II which were sufficient to enhance cholesterol and TG within 10 days and plateaued by the end of 24 days.

Moreover, ANOVA analysis showed that the reduction of HDL levels in group-II rats was statistically significant ($p \geq 0.05$) when compared to group-I (Figure 11).

Figure 11: Effect of *D. indica* fruit on serum HDL levels in hyperlipidemic model rats. *D. indica* fruit extract maintains normal HDL levels in the hypercholesterolemic model rats. 1% cholesterol and 5% coconut oil diet significantly reduced serum HDL levels. Treatment with *D. indica* fruit extract prevented reduction in serum HDL levels in group III and IV rats. Results are expressed as mean $\pm$ standard deviation (SD). Statistical analysis within groups was conducted using a paired t-test while the comparison between the groups was done using a one-way ANOVA with post-hoc Bonferroni correction. $*p \leq 0.05$.

Interestingly, in group-III rats, treatment with *D. indica* fruit extract with the 1% cholesterol and 5% coconut oil diet for 14 consecutive days suppressed a reduction in serum HDL levels (33 mg/dl to 34 mg/dl) as seen in group-II rats. In group-IV rats, the cow fat diet reduced serum HDL levels by 26% (35 mg/dl to 26 mg/dl) at 10 days, and this reduction was higher than the reduction seen in the 1% cholesterol and 5% coconut oil diet in group-III rats (13%; 38 mg/dl to 33 mg/dl). As expected, treatment with *D. indica* fruit extract with the cow fat diet suppressed the reduction of HDL levels (Figure 11). Taken together, these data suggest that *D. indica* extract has the potential to maintain normal serum HDL levels in hypercholesterolemic rats.

Treatment with *D. indica* fruit extract significantly reduced serum LDL levels in hyperlipidemic model rats

The relationship between HDL and LDL is inversely proportional; a high level of HDL is beneficial, while a high level of LDL is bad for health. In this experiment, consumption of the pellet diet for 24 days did not lead to any significant change in serum LDL levels in group-I rats. On the contrary, chronic consumption of 1% cholesterol and 5% coconut oil diet for 24 days in group-II rats significantly enhanced serum LDL levels (27 mg/dl to 42 mg/dl; $p \geq 0.05$). Although the 1% cholesterol and 5% coconut oil diet for 10 days were not sufficient for significant enhancement of LDL levels, consumption for 24 days was sufficient to do so (Figure 12).

Figure 12: Effect of *D. indica* fruit extract on serum LDL levels in hyperlipidaemic model rats. Chronic consumption of *D. indica* fruit extract for 14 days with 1% cholesterol+5% coconut oil and 5% cow fat diet significantly reduced serum LDL levels in group III and IV rats. Results are expressed as mean $\pm$ standard deviation (SD). Statistical analysis within groups was conducted using a paired t-test while the comparison between the groups was done using a one-way ANOVA with post-hoc Bonferroni correction. * $p \leq 0.05$.

Interestingly in group-III rats, chronic consumption of 1% cholesterol and 5% coconut oil with *D. indica* fruit extract in ethanol for 14 days significantly lowered serum LDL levels (47 mg/dl to 39 mg/dl; $p \geq 0.05$), suggesting that *D. indica* fruit simultaneously reduces cholesterol and LDL levels. As expected, chronic consumption of 5% cow fat diet significantly enhanced serum LDL levels (29 mg/dl to 57 mg/dl, $p \geq 0.05$) in the group-IV rats. Moreover, treatment with 5% cow fat diet with *D. indica* fruit extract significantly suppressed serum

LDL levels (57 mg/dl to 44 mg/dl; $p \geq 0.05$) in group-IV rats (Figure 12). Taken together, these data suggest that a 5% cow fat diet is a potent enhancer of serum LDL levels, while *D. indica* fruit extract ameliorated LDL levels in hypercholesterolemic model rats.

Discussion

To our knowledge, this is the first study to report on the potential of *D. indica* extracts in ameliorating diabetes and cholesterol levels. In this study, there was a significant reduction of FSG level in the extract of *D. indica* in water and glibenclamide treated groups and a non-significant FSG reduction in the extract in ethanol treated group. Our finding confirms that extracts of *D. indica* in both water and ethanol improved glycemic status in T2DM rats.

Alterations in carbohydrates and lipid metabolism are associated with insulin-resistant states, ultimately causing diabetes [42, 43]. In our study, to determine the probable mechanism(s) of the hypoglycemic effect following chronic treatment with *D. indica* extracts, serum insulin levels of type 2 diabetic rats were measured at baseline on Day 0 and compared to levels on Day 28. Four weeks of treatment with *D. indica* extract in water significantly improved serum insulin levels in type 2 diabetic rats. Thus, it is possible that *D. indica* aqueous extract may enhance cytoplasmic calcium (Ca^{2+}) responsible for changes in electrical activity in pancreatic β -cells, leading to enhanced insulin secretion. Moreover, it is plausible that *D. indica* acts on the pancreas to cause a hypoglycemic effect similar to the effects seen with Aloe barbadensis and Litsea glutinosa extracts, both of which ameliorated diabetic conditions [40, 44].

Multiple regulatory mechanisms are involved in glucose homeostasis, and the glucose transporter GLUT2 plays a key role. GLUT2 is expressed in different types of tissues including the liver, kidney, intestine, the pancreatic β -cells, neurons, and astrocytes. Insulin secretion is crucial for GLUT2 activation in pancreatic β -cells, and diabetes causes a significant reduction of GLUT2 expression. A known mutation in GLUT2 is responsible for transient juvenile diabetes [45, 46]. Orexin neurons and GABAergic cells in the CNS express GLUT2 [47]. It has been reported that, in a hypoglycemic condition, closure of K^+ leak channels and increased activity of AMP-activated protein kinase in GABAergic cells are involved in the activation of GLUT2 to maintain glucose homeostasis [48]. Thus, in contrast, *D. indica* treatment in hyperglycemic conditions may be responsible for the opening of K^+ leak channels and decreased activity of AMP-activated protein kinase, an alternative mechanism to maintain glucose homeostasis in the CNS.

It has been reported that *D. indica* fruits, which are rich in proanthocyanidins, contain a high amount of B-type procyanidins but a lower amount of B-type prodelphinidins [49]. Proanthocyanidin is a class of polyphenols, which are water-soluble in nature, are found mostly in a variety of fruits. In our study, *D. indica* extract in water conferred stronger glucose-lowering effect than the extract in ethanol. This may be explained by water's high polarity, leading to an uneven distribution of electron density as compared to ethanol. Moreover, most polyphenols are more soluble in water than in ethanol. However, the extract in ethanol also showed anti-diabetic and significant lipid-lowering activities, so other components present in the extract in ethanol must be playing a role.

At the end of the experimental period, the liver glycogen content was increased in the extract in water treated group. Therefore, it may be hypothesized that the hypoglycemic activity of *D. indica* in T2DM rats occurs due to the increased uptake of glucose for the formation of glycogen due to enhanced glycogenesis. Additionally, it is also possible that suppression of hydrolysis of carbohydrates by inhibiting α -amylase and α -glucosidase may be another underlying mechanism for the antidiabetic effect of *D. indica*, as was seen in other studies [50-52]. Glucagon is a peptide hormone secreted from the pancreatic α -cells [53]. It is elevated under stress conditions and helps to increase energy expenditure [54]. The effect of glucagon is opposite that of insulin, i.e. producing an enhanced glucose level in response to hypoglycemia, which may responsible for the development of diabetes [55]. Thus, *D. indica* fruit extracts may decrease glucagon levels, which may be another possible underlying mechanism for reducing serum glucose levels.

Additionally, T2DM is associated with a marked imbalance in lipid metabolism [56]. Both the extract in water and the extract in ethanol treated groups had a significant ($p \leq 0.01$) decrease in serum cholesterol level. There was also a decrease in serum TG (31%) and LDL (24%) levels, while serum HDL cholesterol (14%) was increased in the extract in ethanol treated group. Thus, extract of *D. indica* in ethanol has cholesterol-lowering effects in type 2 diabetic rats, which is comparative with another study [57].

In hyper-cholesterolemic study, consumption of a pellet diet (2.5 Kcal/g) for 24 days resulted in no significant changes in serum cholesterol, TG, HDL and LDL levels (Fig. 1-4; 1st panel), consistent with a previous report that said that mice fed with a pellet diet did not become obese [58], while a high fat diet-

induced obesity in rats [59]. Moreover, a hypercaloric pellet diet (6 Kcal/g) led to an increase in body fat, arterial pressure, and high serum glucose, insulin, and leptin, levels, a while normal pellet diet (3.5 Kcal/g) did not lead to an increase in any of these [60]. Thus, we may conclude that the lab pellet diet in this experiment was safe for the rats.

A high-fat diet is key step in making a hypercholesterolemic/hyperlipidemic rat [61]. In our experiment, we used a 1% cholesterol and 5% coconut oil, or a 5% cow fat diet to make the rat hypercholesterolemic. It is well established that dietary cholesterol is a potent enhancer of systemic circulating lipids. Moreover, data from population studies reported that dietary cholesterol is atherogenic beyond LDL concentrations in the blood, although other studies reported that high cholesterol consumption causes moderate increases in serum cholesterol levels [62]. In this experiment, we not only added 1% cholesterol but also 5% coconut oil in the diet because coconut oil normally enhances cholesterol and LDL to a greater extent than cholesterol alone, as reported previously [63]. Moreover, coconut oil decreases myocardial capillary density and aggravates cardiomyopathy [64]. Thus 1% cholesterol with 5% coconut oil diet was sufficient to make the rats hypercholesterolemic in this study. Indeed, chronic consumption of a 1% cholesterol with 5% coconut oil diet for 24 days significantly enhanced serum cholesterol levels and serum TG levels in addition to serum LDL levels. Moreover, we also observed a significant reduction in serum HDL levels in rats after 1% cholesterol with 5% coconut oil treatment (Fig. 1-4; 2nd panel). It has been reported that cows fed with tallow showed higher total cholesterol in plasma than cows fed a low-fat diet [65]. Moreover, 21% tallow with 1.25% cholesterol consumption for six

weeks led to renal dysfunction and atherosclerosis in the rats [66]. In addition, a beef tallow diet promoted body fat accumulation and reduced norepinephrine turnover rate in brown adipose tissue by reducing sympathetic activity [67]. Thus, cow fat can be a good atherogenic diet to make rats hypercholesterolemic. In this experiment, a 5% cow fat diet significantly enhanced serum cholesterol and LDL levels after 10 days' treatment (Fig. 1 and 4; 4th panel). Moreover, 5% cow fat also enhanced serum TG levels (Fig. 2; 4th panel). A further extension of the cow fat effect was a reduction in serum HDL levels (Fig. 4; 4th panel). Taken together, 1% cholesterol with 5% coconut oil or a 5% cow fat diet is enough to make the rat hypercholesterolemic.

The results of *D. indica* on hypercholesterolemic model rats showed that there was a significant decrease in serum cholesterol level in the group treated with 1% cholesterol and 5% coconut oil diet and in the group treated with a 5% cow fat diet after 14 days of *D. indica* extract supplementation (Fig. 1; 3rd and 4th panels). Thus *D. indica* fruit possesses therapeutic potential to reduce serum cholesterol levels. The possible mechanisms behind the reduction in serum cholesterol levels may include proanthocyanidin (plant sterols) which is a class of polyphenols found in a variety of fruits including *D. indica* [49]. It is well established that plant sterols are a potent therapeutic target for cardiovascular health [68]. In addition, other components present in the *D. indica* extract besides proanthocyanidins may contribute to the lipid-lowering activity. To maintain lipid homeostasis, cholesterol absorption in the intestine is a significant physiological process [69]. In fact, targeting the cholesterol absorption pathway is one of the most important pharmacological

interventions for hyperlipidemia [70]. A carbohydrate diet is positively correlated with serum lipid levels [71]. Chromatographic separation of *D. indica* leaves extract revealed the existence of betulinic acid, quercetin, β sitosterol, and stigmasterol palmitate [72], all of which significantly inhibited the activities of α -amylase and α -glucosidase, which may contribute to the lipid-lowering effects. Phytosterols (Proanthocyanidins) can cross the blood-brain barrier (BBB) [73]. Therefore, it may possible that proanthocyanidins in *D. indica* inhibit HMG-CoA reductase and reduce cholesterol biosynthesis. It has been reported that bioactive palmitoleate can prevent atherosclerosis by suppressing organelle stress and inflammasome activation [74]. Moreover, it has also been reported that phytosterols in *D. indica* possess antioxidant [49] and anti-inflammatory activities by inhibiting the production of tumor necrosis factor-alpha [75] and may be the crucial underlying mechanism to prevent atherosclerosis. Microscopy and phytochemical analysis indicated the presence of xylem fibers, phytosterols, pro-anthocyanidins, terpenoids, glycosides, fatty acids, flavonoids, and phenolic compounds in *D. indica* [49, 76]. *D. indica* is also rich in different types of soluble and insoluble dietary fibers. Phytosterols reduce cholesterol levels by different mechanisms, such as by competing with cholesterol absorption in the gut and in dietary mixed micelles. Additionally, sterols compete with cholesterol for solubilization, co-crystallize with cholesterol to form insoluble mixed crystals and inhibit cholesterol hydrolysis by lipases [77-79]. Therefore, phytosterols in *D. indica* extracts may inhibit cholesterol absorption and hydrolysis in the gut.

Hypercholesterolemic model rats treated with *D. indica* extract for 14 days showed a significant reduction in serum TG levels in the 1% cholesterol and

734 5% coconut oil or the 5% cow fat diet group (Fig. 2; 3rd and 4th panels). *D.*
735 *indica* extract may interfere with TG biosynthesis and absorption. Overall,
736 lipase is a critical enzyme to digest dietary TGs. Thus the sterols in *D. indica*
737 fruit extract in ethanol may inhibit lipase, thereby ameliorating fat
738 malabsorption in the intestinal lumen by forming an irreversible bond with fat
739 molecules and ultimately leading to fecal excretion without degradation.
740 Moreover, it has been reported that polyphenols in boysenberry and okra
741 extract suppressed enhancement of TG in plasma [80, 81]; this is consistent
742 with our study, where polyphenol in *D. indica* fruit may lower TG levels in
743 hypercholesterolemic rats. Diacylglycerol acyltransferase 2 (DGAT2)
744 catalyzes the last step of TG biosynthesis in rodents [82]. Thus, we do not
745 exclude the possibility that the *D. indica* extract may inhibit DGAT2 and
746 reduce hepatic TG synthesis.

747 Chronic consumption of a 1% cholesterol and 5% coconut oil diet or a 5% cow
748 fat diet for 24 days significantly reduced serum HDL levels but treatment with
749 *D. indica* fruit extract suppressed reduction and restored normal HDL levels
750 (Fig. 3). ATP-binding cassette protein A1 (ABCA1), lecithin-cholesterol
751 acyltransferase, and apolipoprotein (apo) A-I are the key components for HDL
752 biosynthesis [83]. ABCA1 exports cholesterol from the membranes to the
753 nascent HDL, while ABCG1 (G Member sub-family of ATP-binding Cassette
754 transporter) transports cholesterol to mature HDL; the key features of
755 cholesterol homeostasis [84]. One possible mechanism to reduce HDL levels
756 with chronic consumption of 1% cholesterol and 5% coconut oil is by
757 downregulation of the genes associated with ABCA1, LCAT, and APOAI

molecules. On the contrary, *D. indica* fruit extract could restore and/or up-regulate these gene functions to maintain HDL homeostasis

Both a 1% cholesterol and 5% coconut oil diet, and a 5% cow fat diet significantly enhanced serum LDL levels, and, more importantly, *D. indica* fruit extract significantly reduced serum LDL levels in both groups (Fig. 4). To investigate the underlying mechanism of such reduction, it is important to understand the pathophysiology of LDL. After entering into the artery wall endothelium, LDL initiates atherosclerotic plaque formation by binding to glycosaminoglycans [85]. LDL is oxidized, thus producing modified apoB by altering lysine residues [86]. The modified LDL is recognized by the macrophages and internalized by endocytosis, forming foam cells [87]. These foam cells release cytokines, initiating inflammatory reactions [88]. As a consequence, the cells of the arterial wall proliferate and produce collagen. The plaque enlarges, eventually leading to blood clot formation and blockage of the vessel. Several studies have reported that HDL prevents LDL oxidation, a crucial initial step in LDL pathophysiology, and, thus, reducing LDL levels [89]. In this experiment, *D. indica* fruit extract maintained normal HDL levels in hypercholesterolemic model rats, which may contribute to reducing LDL levels by inhibiting LDL oxidation. In addition, LDL enhances CXCR2 (cytokine) expression, facilitating neutrophils accumulation [90]. On the other hand, monocytes use CCR2 for entry into the atherogenic lesion [91, 92]. Therefore, it may possible that *D. indica* fruit extract may reduce CXCR2 and CCR2 expression and reduce atherosclerosis.

The muscles use creatine to make energy and produce creatinine as a waste product. High levels of creatinine in the blood may indicate diabetic nephropathy. Hyperglycemia enhances the level of reactive oxygen species, which then facilitate the formation of glycation end products as implicated in the pathogenesis of diabetic nephropathy [93, 94]. In our study, treatment with *D. indica* extracts suppressed enhancement of creatinine levels in diabetic rats. On the other hand, ALT is an indicator of liver function. It has been reported that elevated serum levels of ALT are significantly associated with increased diabetic risk [95, 96]. In this study, there was no significant change in serum ALT levels in the *D. indica* extract-treated groups, indicating that *D. indica* does not affect liver function. These observations are correlated with the histological changes as extract of *D. indica* in water showed no tubular epithelial cell degeneration, necrosis, and hyperemic vessels in the interstitium of the kidney tissues and no hepatocyte degeneration, sinusoidal dilation, and pleomorphism of the hepatocytes in liver tissues. Interestingly, the glibenclamide-treated group showed mild hepatocyte degeneration and sinusoidal dilation in our study. Moreover, it has been reported that glibenclamide reduced pro-inflammatory cytokine production and reduced glutathione levels in diabetic patients [97], which be the underlying mechanism of observed hepatocyte degeneration and sinusoidal dilation in our study. Thus, our study not only defines an emerging role of *D. indica* fruit as an anti-diabetic and anti-hyperlipidemic agent but also opens a new window on the safety concerns of glibenclamide treatment.

Conclusions

D. indica fruit has a glucose-lowering effect and enhances insulin secretion as well as glycogen synthesis. It decreases total cholesterol levels and increases HDL-cholesterol. It does not affect renal and liver functions in terms of creatinine and ALT, and, therefore, has the potential to be an anti-diabetic and cholesterol-lowering agent.

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1137 **Supplementary Materials:**

1138 **Supplemental Figure 1: *D. indica* fruit**

1139 **Graphical Abstract:** Extract of *D. indica* in water reduces FSG, serum insulin
1140 levels, and ameliorates the serum lipid profile in diabetic model rats without
1141 any adverse effects on kidney and liver tissues.

1142 Extract of *D. indica* in ethanol significantly reduces serum cholesterol, TG,
1143 LDL with no reduction in HDL levels in hyper-lipidemic model rats.

1144 **Highlights**

1145 •*D. indica* fruit extracts diminished fasting serum glucose (FSG) levels in STZ-
1146 induced type 2 diabetic model rats

1147 •*D. indica* fruit extracts boosted insulin secretion

1148 •*D. indica* fruit extracts showed no toxic effects on the kidney and the liver
1149 functions

1150 •Extract in water was more effective in reducing FSG levels than extract in
1151 ethanol

1152 •Chronic consumption of 1% cholesterol, 5% coconut oil and 5% cow fat diet
1153 was sufficient to make the rat hypercholesterolemic

1154 •*D. indica* fruit extract has the potential to reduce serum cholesterol, TG, LDL
1155 with prevention in reduction in serum HDL levels.

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Cover Letter

28 day

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Cover Letter

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Groups	Treatments	Serum Cholesterol (mg/dl)		
		0 day	10 days	24 days
I (n=6)	Pellet diet	43±5.56 (100%)	46±8.84 (107%)	48±7.33 (112%)
II (n=6)	Pellet diet + 1% cholesterol + 5% coconut oil	41±7.60 (100%)	66±11.89 (161%)	71±2.69 (173%)
III (n=6)	Pellet diet + 1% cholesterol + 5% coconut oil + ethanol extract (after 10 days)	37±3.29 (100%)	60±2.88 (162%)	45±3.27 (122%)
IV (n=5)	Pellet diet + 5% cow fat + ethanol extract (after 10 days)	38±2.43 (100%)	85±11.31 (224%)	66±4.95 (174%)

Groups	Treatments	Serum TG (mg/dl)		
		0 day	10 days	24 days
I (n=6)	Pellet diet	66±25.3 (100%)	65±5.56 (98.4%)	70±17.5 (106%)
II (n=6)	Pellet diet + 1% cholesterol + 5% coconut oil	53±24.9 (100%)	80±11.26 (151%)	82±13.3 (155%)
III (n=6)	Pellet diet + 1% cholesterol + 5% coconut oil + ethanol extract (after 10 days)	54±22.3 (100%)	87±7.83 (161%)	65±12.9 (120%)
IV (n=5)	Pellet diet + 5% cow fat + ethanol extract (after 10 days)	40±12.0 (100%)	90±3.70 (225%)	66±4.95 (165%)

Groups	Treatments	Serum HDL (mg/dl)		
		0 day	10 days	24 days
I (n=6)	Pellet diet	38±2.08 (100%)	34±7.56 (89%)	34±5.00 (89%)
II (n=6)	Pellet diet + 1% cholesterol + 5% coconut oil	37±5.50 (100%)	33±3.62 (89%)	25±3.43 (68%)
III (n=6)	Pellet diet + 1% cholesterol + 5% coconut oil + ethanol extract (after 10 days)	38±5.45 (100%)	33±3.84 (87%)	34±4.09 (90%)
IV (n=5)	Pellet diet + 5% cow fat + ethanol extract (after 10 days)	35±1.41 (100%)	26±2.14 (74%)	31±2.82 (86%)

Groups	Treatments	Serum LDL (mg/dl)		
		0 day	10 days	24 days
I (n=6)	Pellet diet	29±3.10 (100%)	31±3.10 (100%)	32±7.56 (100%)
II (n=6)	Pellet diet + 1% cholesterol + 5% coconut oil	27±4.23 (100%)	37±5.96 (100%)	42±3.43 (100%)
III (n=6)	Pellet diet + 1% cholesterol + 5% coconut oil + ethanol extract (after 10 days)	32±7.73 (100%)	47±8.08 (100%)	39±4.09 (100%)
IV (n=5)	Pellet diet + 5% cow fat + ethanol extract (after 10 days)	29±5.34 (100%)	57±3.12 (100%)	44±2.82 (100%)