# *Dillenia indica* fruit extract has Glucose and Cholesterol Lowering effects

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## Abstract

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

**Methods and findings:** Type 2 diabetes (T2D) was induced by a single intraperitoneal injection of Streptozotocin (STZ; 90mg/kg) and hyperlipidemia by feeding with 1% cholesterol, 5% coconut oil and 5% cow fat diet. Administration of *D. indica* extracts in water for four weeks triggered a significant ($p \leq 0.05$) reduction in fasting serum glucose (FSG) levels with concomitant improvement in serum insulin levels. Both the water- and ethanol-extract of *D. indica* treated groups showed significant ($p \leq 0.01$) reduction in total cholesterol levels by 25% and 19%, respectively. HDL-cholesterol was also augmented (by 14%) in ethanol-extract treated group. Liver glycogen content was higher in the water-extract treated group. Histopathological examination revealed that there was no tubular epithelial cell degeneration or necrosis in the renal tissues or hepatocyte degeneration and sinusoidal dilation in liver tissues in animals that received the water-extract. On the other hand, consumption of *D. indica* extract with 1% cholesterol, 5% coconut oil diet or with a 5% cow fat diet for 14 days significantly reduced serum cholesterol levels in group-lll (60→45 mg/dl; $p \geq 0.05$) and -IV (85→66 mg/dl; $p \geq 0.05$) hypercholesterolemic model rats. *D. indica* fruit extract also reduced serum TG levels (Group-III: 87→65 mg/dl; 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≥0.05) and group-IV (57→44 mg/dl; p≥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.

## Introduction

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

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

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

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

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

141 Here we showed that oral administration of both *D. indica* extract in water or
142 ethanol in Long-Evans female rats for 28 consecutive days significantly
143 ameliorates serum fasting glucose with concomitant enhancement in insulin
144 secretion and liver glycogen content. Moreover, no noticeable change was
145 observed in the serum creatinine and ALT levels in the extract in water and
146 extract in ethanol treated mice, which is reflected in the histopathological
147 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

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

### *Preparation of Extract in Water*

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

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

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

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

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

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

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

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

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

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

241 ***Dose for D. indica Extracts***

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

249 ***Blood Collection Procedure for Biochemical Analysis***

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

255 ***Collection and Preservation of Liver and Kidney Tissue for Histology***

256 Following sacrificed by cervical dislocation, the liver and kidney tissues were
257 collected, washed in normal saline, and fixed by using 40% formaldehyde for
258 24 hours. Subsequently, the tissues were subjected to alcohol dehydration. All
259 tissue samples were washed and embedded by using paraffin and xylene.
260 The tissues were then double-stained.

### *Determination of serum glucose levels*

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

### *Determination of serum insulin levels*

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

### *Measurement of Liver Glycogen Content*

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

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

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

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

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

### *Measurement of serum cholesterol levels*

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

### *Determination of serum TG levels*

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

### *Determination of serum HDL levels*

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

### *Determination of serum LDL levels*

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

### *Statistical Analysis*

Data were analyzed using a Statistical Package for Social Science software version 12 (SPSS Inc., Chicago, Illinois, USA). The data were reported as mean ± SD or as median (range) where appropriate. Statistical analysis was 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 ± 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 ± 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 ± 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 ± SD) mg/dl: Day 0 (75.00 ± 6.37) vs Day 28 (56.00 ± 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 ± SD) mg/dl: Day 0 (75.00 ± 6.30) vs Day 28 (61.00 ± 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).

371 The extract of *D. indica* in ethanol significantly increased serum HDL by 14%
372 (36 to 41 mg/dl; p≤0.05), while it reduced LDL by 24% (34 to 26 mg/dl) by Day
373 28 as compared to the baseline (Day 0). In addition, enhanced HDL
374 cholesterol in the extract in ethanol treated group was statistically significant
375 (p≤0.05) when compared with the type 2 control group. Moreover, both the
376 glibenclamide and extract in water treated groups showed an increase in
377 serum HDL levels by 6% (34 to 36 mg/dl) (Figure 5C). Atherogenic LDL-
378 cholesterol levels were decreased by 24% (34 to 26 mg/dl), 19% (36 to 29
379 mg/dl), and 23% (30 to 23 mg/dl) for the extract in ethanol, extract in water,
380 and glibenclamide treated groups, respectively (Figure 5D). These data
381 suggest that *D. indica* fruit can ameliorate the lipid profile in diabetic rats.

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

390 ***Effects of D. indica on serum ALT and creatinine levels***

391 The effects of the extract of *D. indica* in water or in ethanol on kidney and liver
392 functions were also investigated. There was an increase in the serum
393 creatinine levels only in the type 2 control group, although serum creatinine
394 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 ± 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

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

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

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

479 Cow fat (tallow) is primarily made up of triglycerides. In this experiment, a 5%
480 cow fat diet significantly enhanced serum cholesterol levels (38 to 85 mg/dl) in
481 group-IV rats. Moreover, enhancement in serum cholesterol levels was higher
482 (224%) when fed the cow fat diet, than when rats were fed the 1% cholesterol
483 and 5% coconut oil diet (162%), suggesting that cow fat is more potent than
484 1% cholesterol and 5% coconut oil in inducing serum cholesterol levels. As
485 expected, group-IV rats given the cow fat diet with *D. indica* extract showed a
486 significant reduction (85 to 66 mg/dl; p≥0.05) in cholesterol levels. Although,
487 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 ± 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 (Grpup-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 hyper-lipidemic 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 ± 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***

560 The relationship between HDL and LDL is inversely proportional; a high level
561 of HDL is beneficial, while a high level of LDL is bad for health. In this
562 experiment, consumption of the pellet diet for 24 days did not lead to any
563 significant change in serum LDL levels in group-I rats. On the contrary,
564 chronic consumption of 1% cholesterol and 5% coconut oil diet for 24 days in
565 group-II rats significantly enhanced serum LDL levels (27 mg/dl to 42 mg/dl;
566 $p \geq 0.05$). Although the 1% cholesterol and 5% coconut oil diet for 10 days
567 were not sufficient for significant enhancement of LDL levels, consumption for
568 24 days was sufficient to do so (Figure 12).

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

577

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

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

589 **Discussion**

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

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

609 Multiple regulatory mechanisms are involved in glucose homeostasis, and the
610 glucose transporter GLUT2 plays a key role. GLUT2 is expressed in different
611 types of tissues including the liver, kidney, intestine, the pancreatic β-cells,
612 neurons, and astrocytes. Insulin secretion is crucial for GLUT2 activation in
613 pancreatic β-cells, and diabetes causes a significant reduction of GLUT2
614 expression. A known mutation in GLUT2 is responsible for transient juvenile
615 diabetes [45, 46]. Orexin neurons and GABAergic cells in the CNS express
616 GLUT2 [47]. It has been reported that, in a hypoglycemic condition, closure of
617 $K^+$ leak channels and increased activity of AMP-activated protein kinase in
618 GABAergic cells are involved in the activation of GLUT2 to maintain glucose
619 homeostasis [48]. Thus, in contrast, *D. indica* treatment in hyperglycemic
620 conditions may be responsible for the opening of $K^+$ leak channels and
621 decreased activity of AMP-activated protein kinase, an alternative mechanism
622 to maintain glucose homeostasis in the CNS.

623 It has been reported that *D. indica* fruits, which are rich in proanthocyanidins,
624 contain a high amount of B-type procyanidins but a lower amount of B-type
625 prodelphinidins [49]. Proanthocyanidin is a class of polyphenols, which are
626 water-soluble in nature, are found mostly in a variety of fruits. In our study, *D.*
627 *indica* extract in water conferred stronger glucose-lowering effect than the
628 extract in ethanol. This may be explained by water's high polarity, leading to
629 an uneven distribution of electron density as compared to ethanol. Moreover,
630 most polyphenols are more soluble in water than in ethanol. However, the
631 extract in ethanol also showed anti-diabetic and significant lipid-lowering
632 activities, so other components present in the extract in ethanol must be
633 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; 1$^{st}$ 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-

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

664 A high-fat diet is key step in making a hypercholesterolemic/hyperlipidemic rat
665 [61]. In our experiment, we used a 1% cholesterol and 5% coconut oil, or a 5%
666 cow fat diet to make the rat hypercholesterolemic. It is well established that
667 dietary cholesterol is a potent enhancer of systemic circulating lipids.
668 Moreover, data from population studies reported that dietary cholesterol is
669 atherogenic beyond LDL concentrations in the blood, although other studies
670 reported that high cholesterol consumption causes moderate increases in
671 serum cholesterol levels [62]. In this experiment, we not only added 1%
672 cholesterol but also 5% coconut oil in the diet because coconut oil normally
673 enhances cholesterol and LDL to a greater extent than cholesterol alone, as
674 reported previously [63]. Moreover, coconut oil decreases myocardial capillary
675 density and aggravates cardiomyopathy [64]. Thus 1% cholesterol with 5%
676 coconut oil diet was sufficient to make the rats hypercholesterolemic in this
677 study. Indeed, chronic consumption of a 1% cholesterol with 5% coconut oil
678 diet for 24 days significantly enhanced serum cholesterol levels and serum
679 TG levels in addition to serum LDL levels. Moreover, we also observed a
680 significant reduction in serum HDL levels in rats after 1% cholesterol with 5%
681 coconut oil treatment (Fig. 1-4; 2$^{nd}$ panel). It has been reported that cows fed
682 with tallow showed higher total cholesterol in plasma than cows fed a low-fat
683 diet [65]. Moreover, 21% tallow with 1.25% cholesterol consumption for six

684 weeks led to renal dysfunction and atherosclerosis in the rats [66]. In addition,
685 a beef tallow diet promoted body fat accumulation and reduced
686 norepinephrine turnover rate in brown adipose tissue by reducing sympathetic
687 activity [67]. Thus, cow fat can be a good atherogenic diet to make rats
688 hypercholesterolemic. In this experiment, a 5% cow fat diet significantly
689 enhanced serum cholesterol and LDL levels after 10 days' treatment (Fig. 1
690 and 4; 4$^{th}$ panel). Moreover, 5% cow fat also enhanced serum TG levels (Fig
691 2; 4$^{th}$ panel). A further extension of the cow fat effect was a reduction in serum
692 HDL levels (Fig. 4; 4$^{th}$ panel). Taken together, 1% cholesterol with 5%
693 coconut oil or a 5% cow fat diet is enough to make the rat
694 hypercholesterolemic.
695 The results of *D. indica* on hypercholesterolemic model rats showed that there
696 was a significant decrease in serum cholesterol level in the group treated with
697 1% cholesterol and 5% coconut oil diet and in the group treated with a 5%
698 cow fat diet after 14 days of *D. indica* extract supplementation (Fig. 1; 3$^{rd}$ and
699 4$^{th}$ panels). Thus *D. indica* fruit possesses therapeutic potential to reduce
700 serum cholesterol levels. The possible mechanisms behind the reduction in
701 serum cholesterol levels may include proanthocyanidin (plant sterols) which is
702 a class of polyphenols found in a variety of fruits including *D. indica* [49]. It is
703 well established that plant sterols are a potent therapeutic target for
704 cardiovascular health [68]. In addition, other components present in the *D.*
705 *indica* extract besides proanthocyanidins may contribute to the lipid-lowering
706 activity. To maintain lipid homeostasis, cholesterol absorption in the intestine
707 is a significant physiological process [69]. In fact, targeting the cholesterol
708 absorption pathway is one of the most important pharmacological

709 interventions for hyperlipidemia [70]. A carbohydrate diet is positively
710 correlated with serum lipid levels [71]. Chromatographic separation of *D.*
711 *indica* leaves extract revealed the existence of betulinic acid, quercetin, β
712 sitosterol, and stigmasterol palmitate [72], all of which significantly inhibited
713 the activities of α-amylase and α-glucosidase, which may contribute to the
714 lipid-lowering effects. Phytosterols (Proanthocyanidins) can cross the blood-
715 brain barrier (BBB) [73]. Therefore, it may possible that proanthocyanidins in
716 *D. indica* inhibit HMG-CoA reductase and reduce cholesterol biosynthesis. It
717 has been reported that bioactive palmitoleate can prevent atherosclerosis by
718 suppressing organelle stress and inflammasome activation [74]. Moreover, it
719 has also been reported that phytosterols in *D. indica* possess antioxidant [49]
720 and anti-inflammatory activities by inhibiting the production of tumor necrosis
721 factor-alpha [75] and may be the crucial underlying mechanism to prevent
722 atherosclerosis. Microscopy and phytochemical analysis indicated the
723 presence of xylem fibers, phytosterols, pro-anthocyanidins, terpenoids,
724 glycosides, fatty acids, flavonoids, and phenolic compounds in *D. indica* [49,
725 76]. *D. indica* is also rich in different types of soluble and insoluble dietary
726 fibers. Phytosterols reduce cholesterol levels by different mechanisms, such
727 as by competing with cholesterol absorption in the gut and in dietary mixed
728 micelles. Additionally, sterols compete with cholesterol for solubilization, co-
729 crystallize with cholesterol to form insoluble mixed crystals and inhibit
730 cholesterol hydrolysis by lipases [77-79]. Therefore, phytosterols in *D. indica*
731 extracts may inhibit cholesterol absorption and hydrolysis in the gut.

732 Hypercholesterolemic model rats treated with *D. indica* extract for 14 days
733 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

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

760 Both a 1% cholesterol and 5% coconut oil diet, and a 5% cow fat diet
761 significantly enhanced serum LDL levels, and, more importantly, *D. indica* fruit
762 extract significantly reduced serum LDL levels in both groups (Fig. 4). To
763 investigate the underlying mechanism of such reduction, it is important to
764 understand the pathophysiology of LDL. After entering into the artery wall
765 endothelium, LDL initiates atherosclerotic plaque formation by binding to
766 glycosaminoglycans [85]. LDL is oxidized, thus producing modified apoB by
767 altering lysine residues [86]. The modified LDL is recognized by the
768 macrophages and internalized by endocytosis, forming foam cells [87]. These
769 foam cells release cytokines, initiating inflammatory reactions [88]. As a
770 consequence, the cells of the arterial wall proliferate and produce collagen.
771 The plaque enlarges, eventually leading to blood clot formation and blockage
772 of the vessel. Several studies have reported that HDL prevents LDL oxidation,
773 a crucial initial step in LDL pathophysiology, and, thus, reducing LDL levels
774 [89]. In this experiment, *D. indica* fruit extract maintained normal HDL levels in
775 hypercholesterolemic model rats, which may contribute to reducing LDL levels
776 by inhibiting LDL oxidation. In addition, LDL enhances CXCR2 (cytokine)
777 expression, facilitating neutrophils accumulation [90]. On the other hand,
778 monocytes use CCR2 for entry into the atherogenic lesion [91, 92]. Therefore,
779 it may possible that *D. indica* fruit extract may reduce CXCR2 and CCR2
780 expression and reduce atherosclerosis.

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

804 **Conclusions**

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

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**Supplementary Materials:**

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

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

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

**Highlights**

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

•*D. indica* fruit extracts boosted insulin secretion

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

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

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

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

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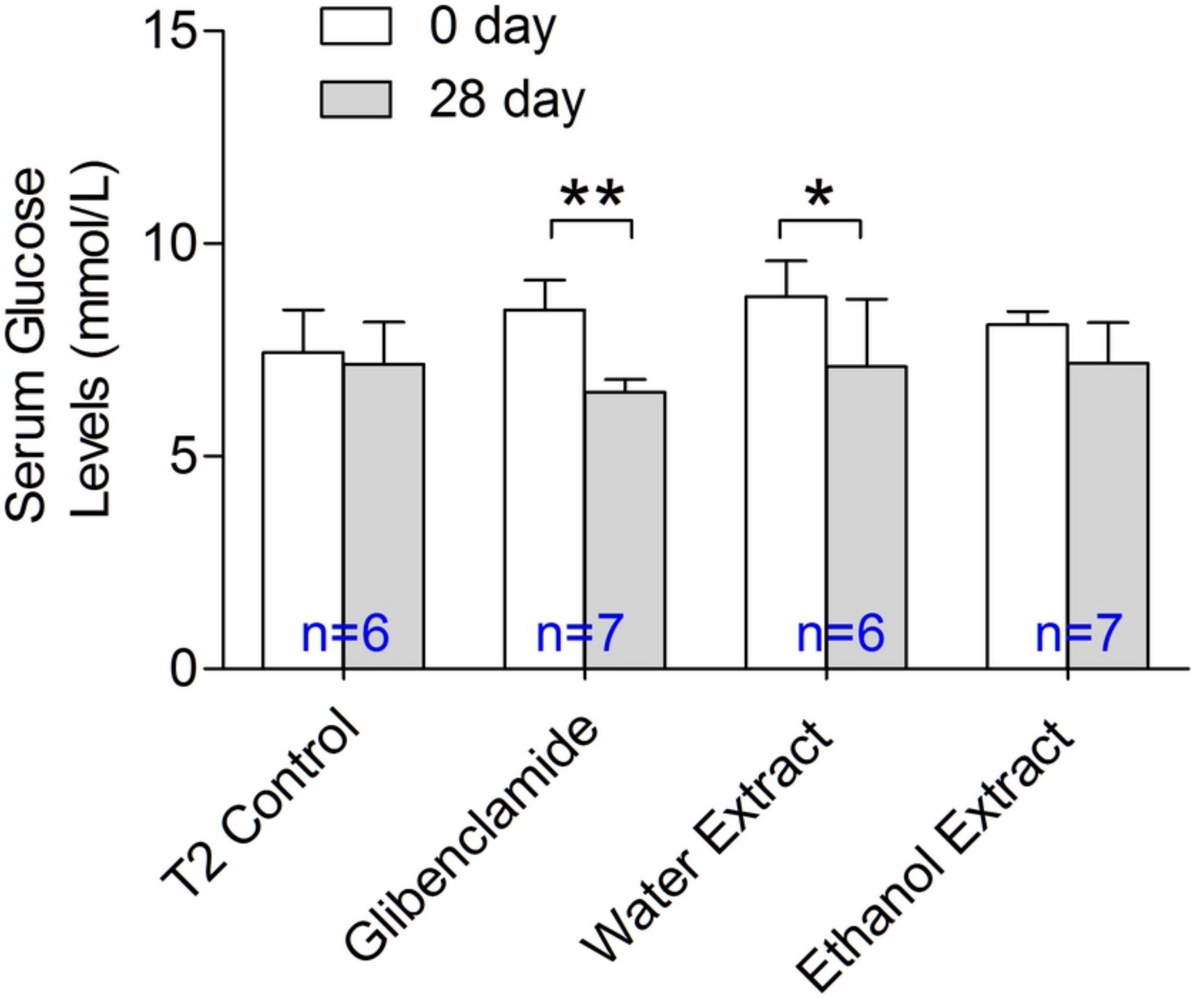


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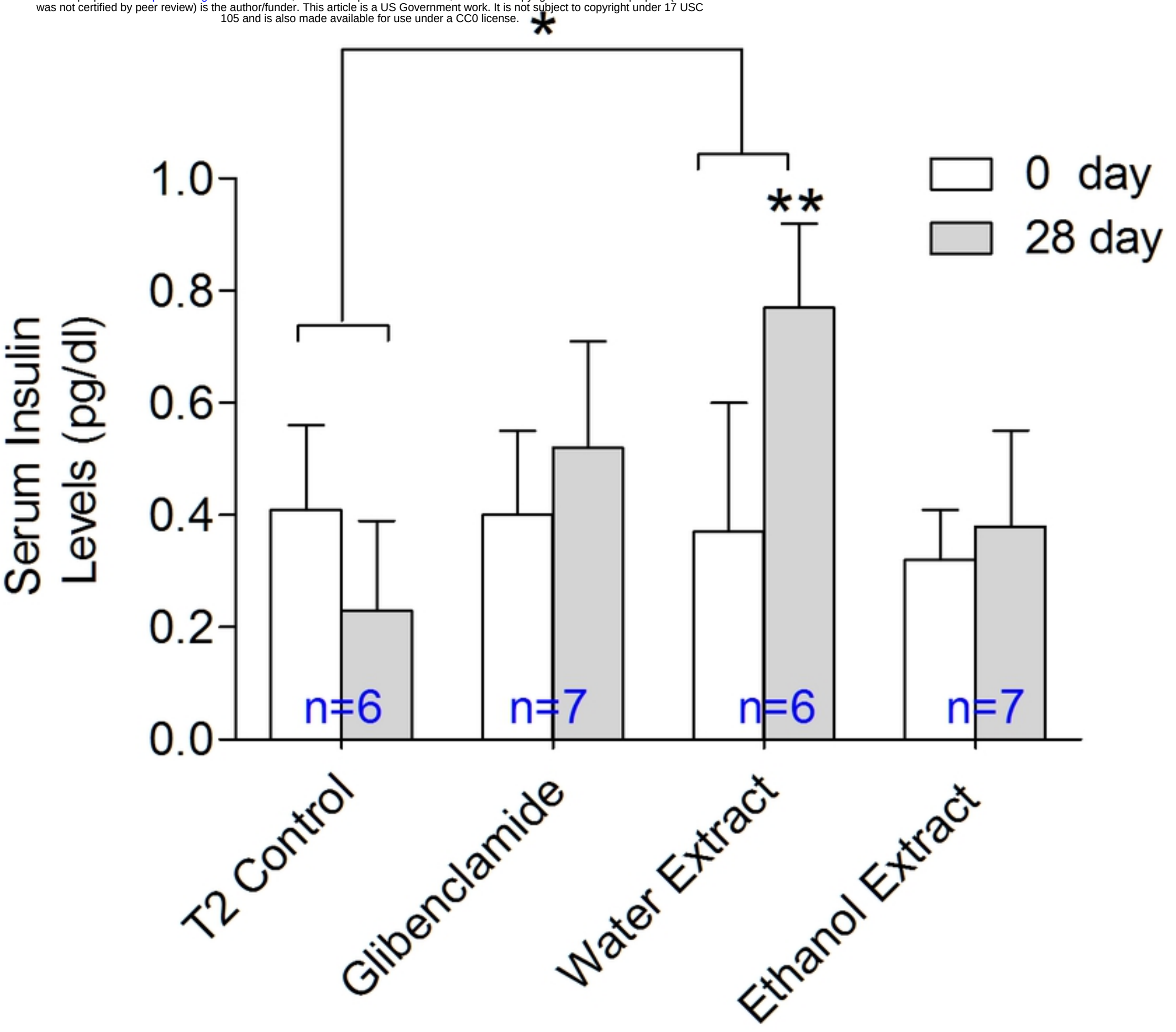


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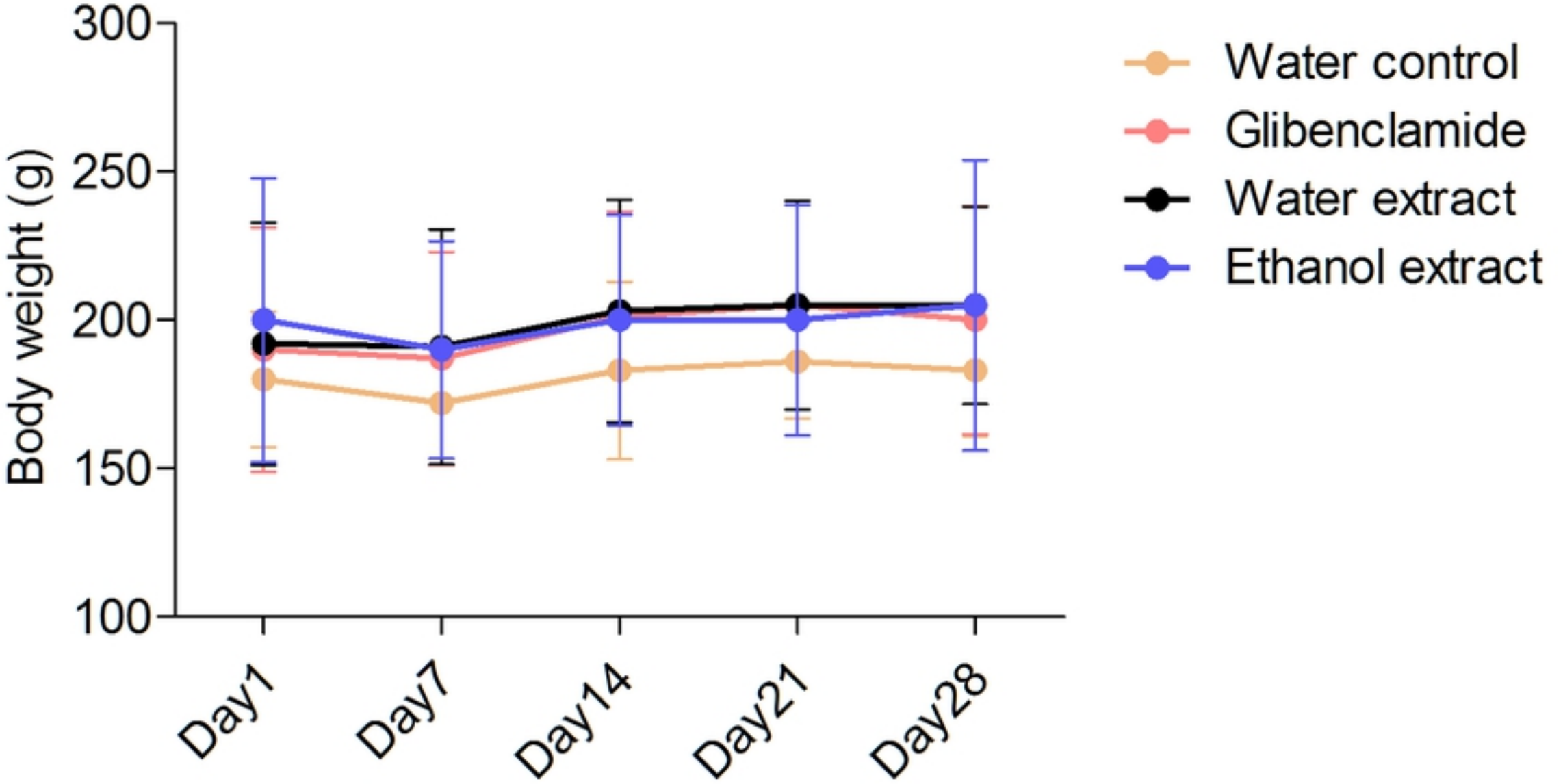


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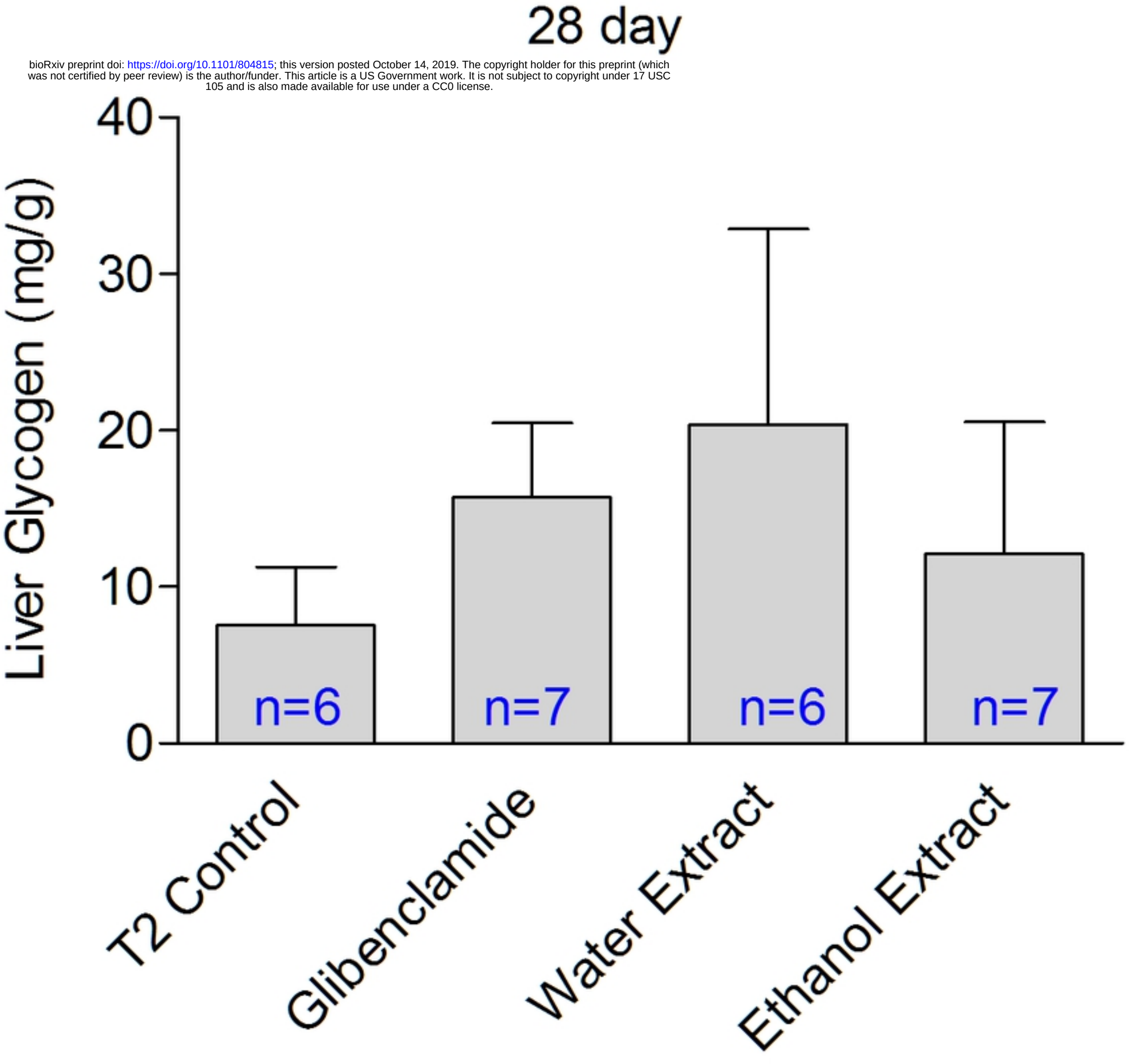


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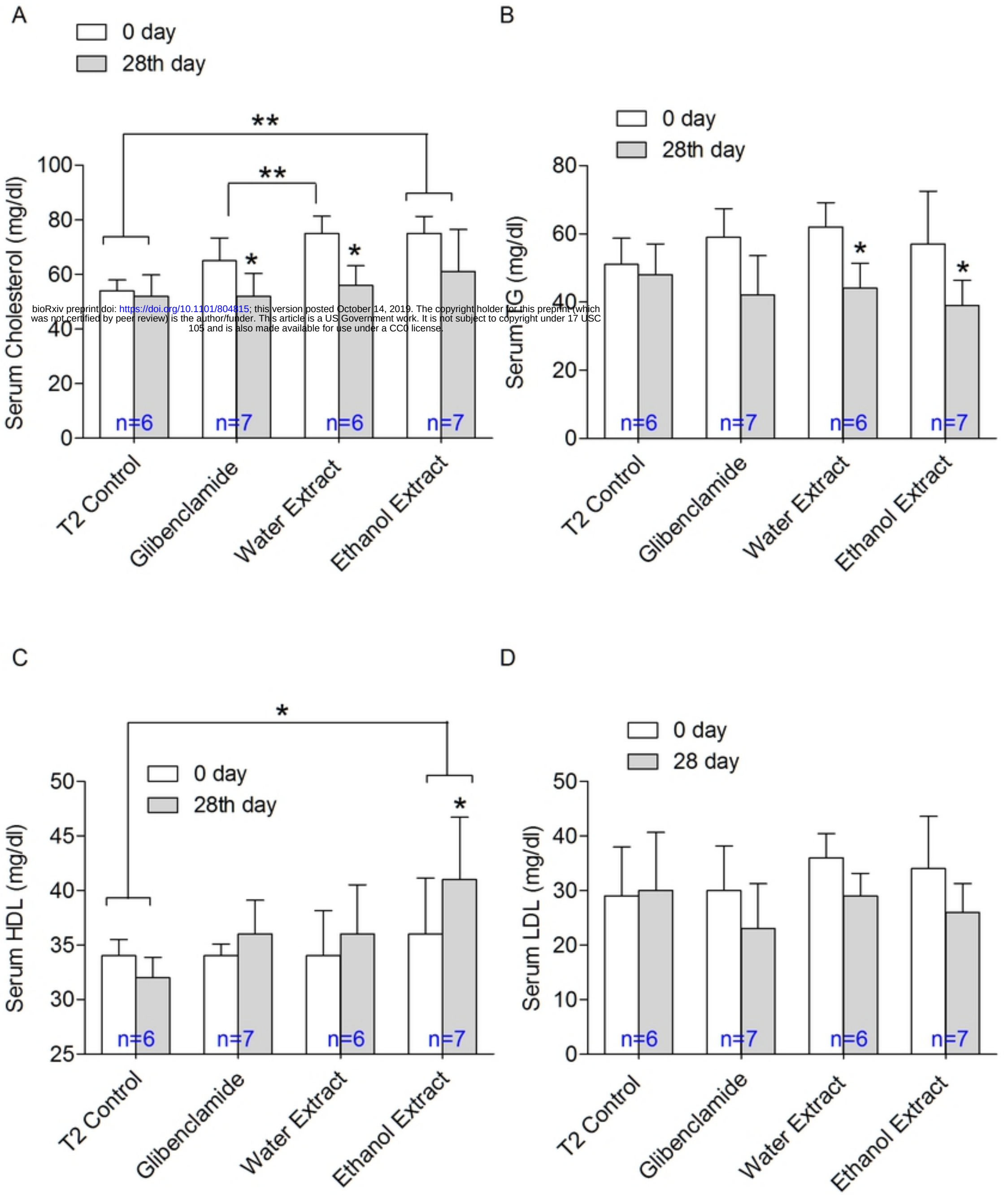


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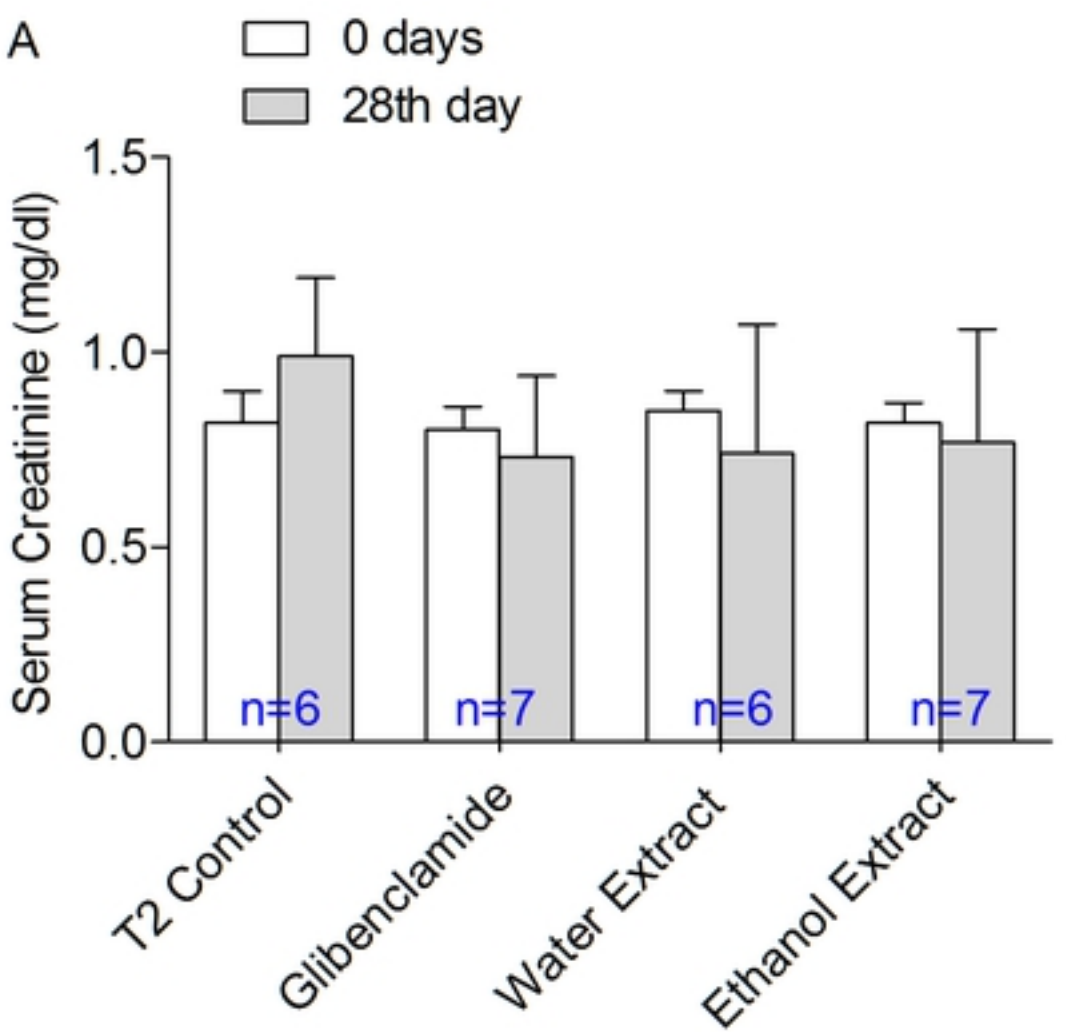


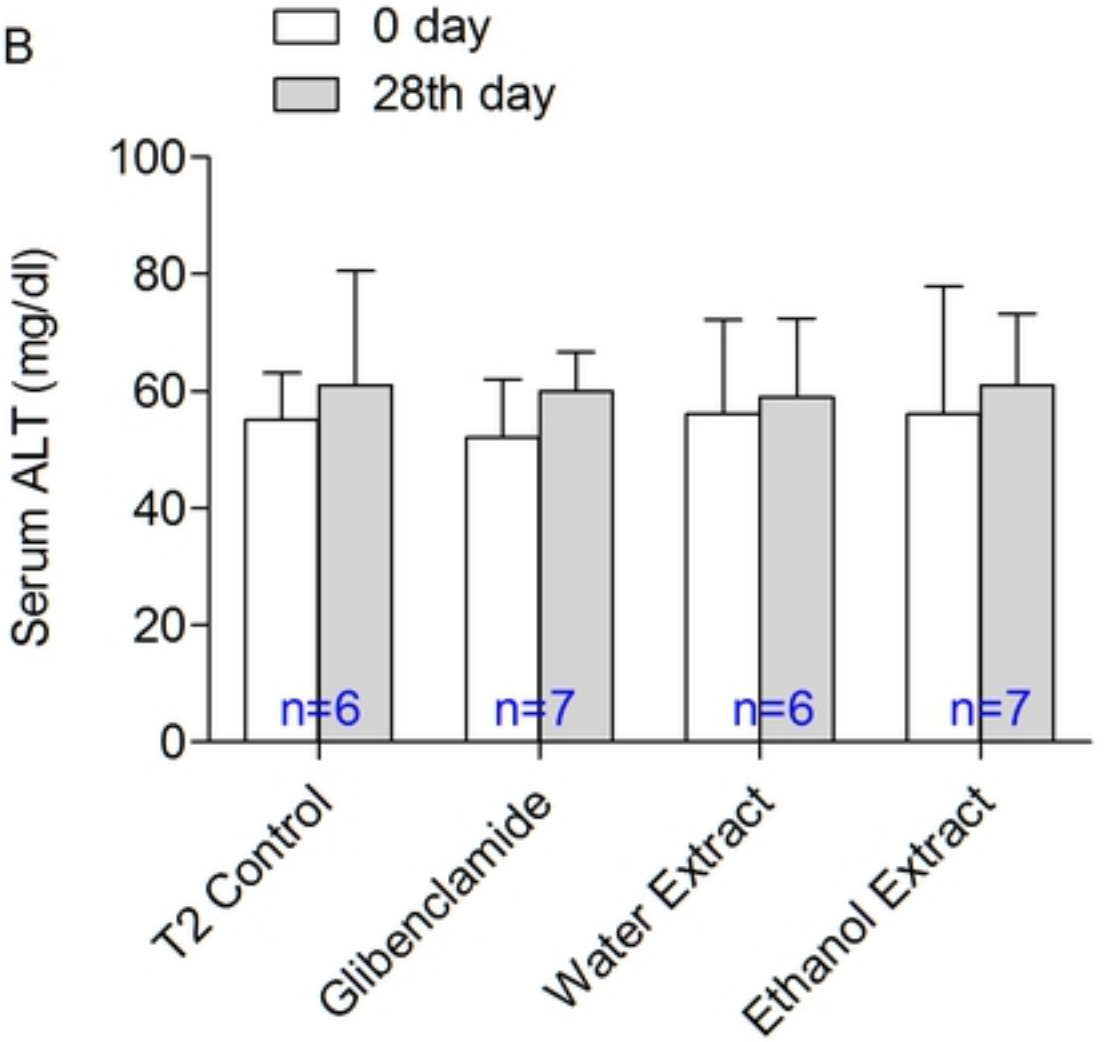


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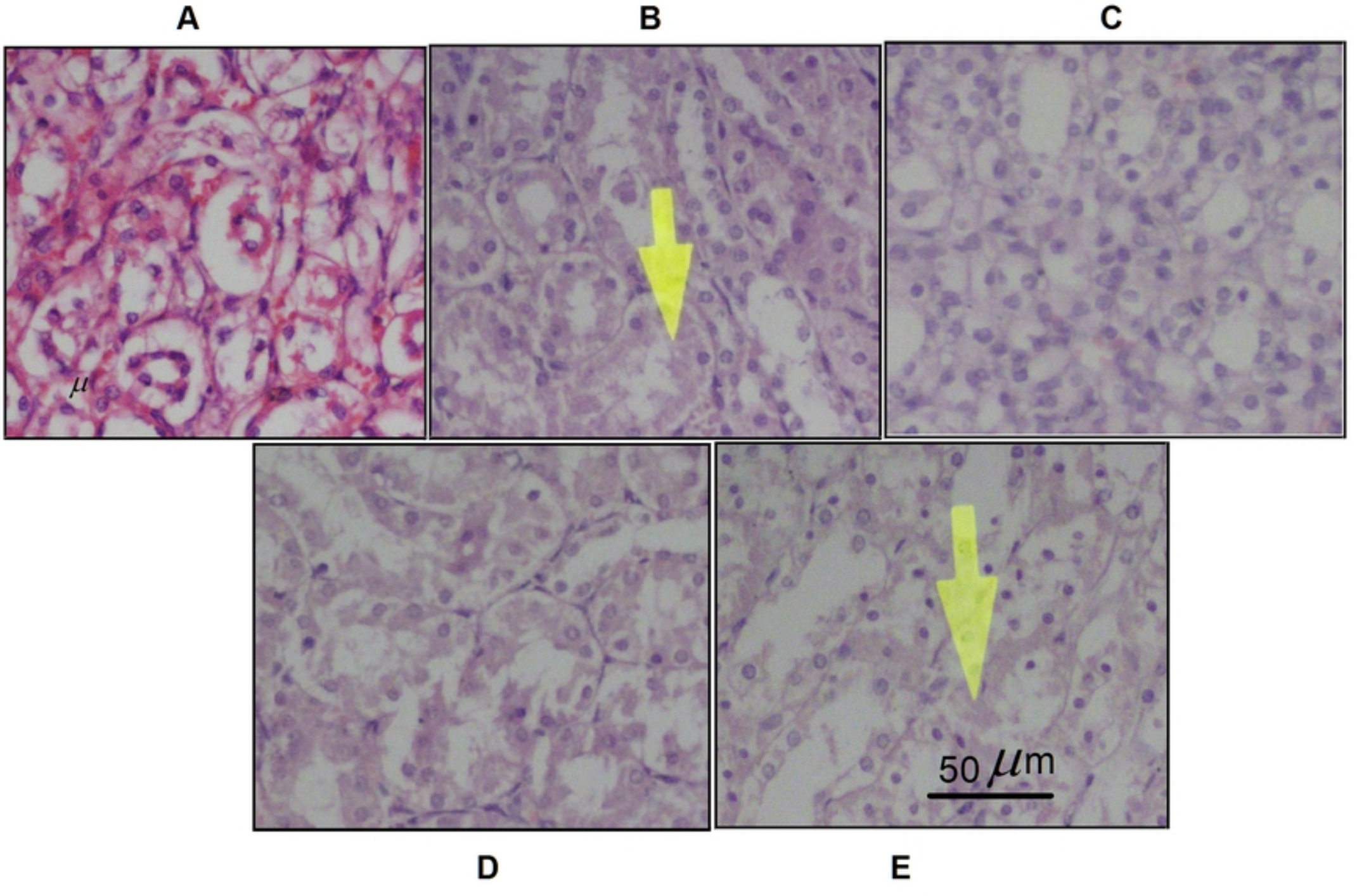


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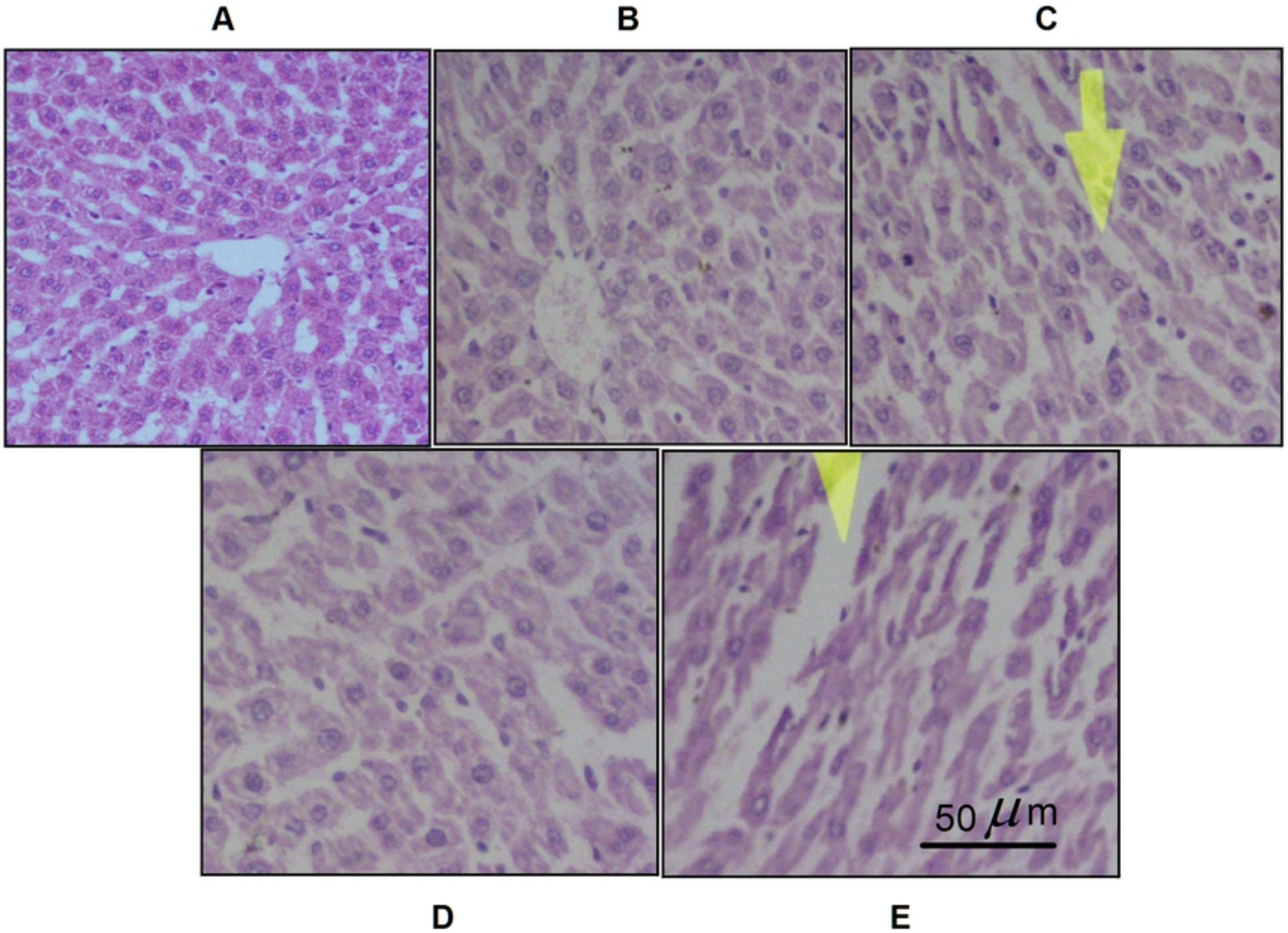


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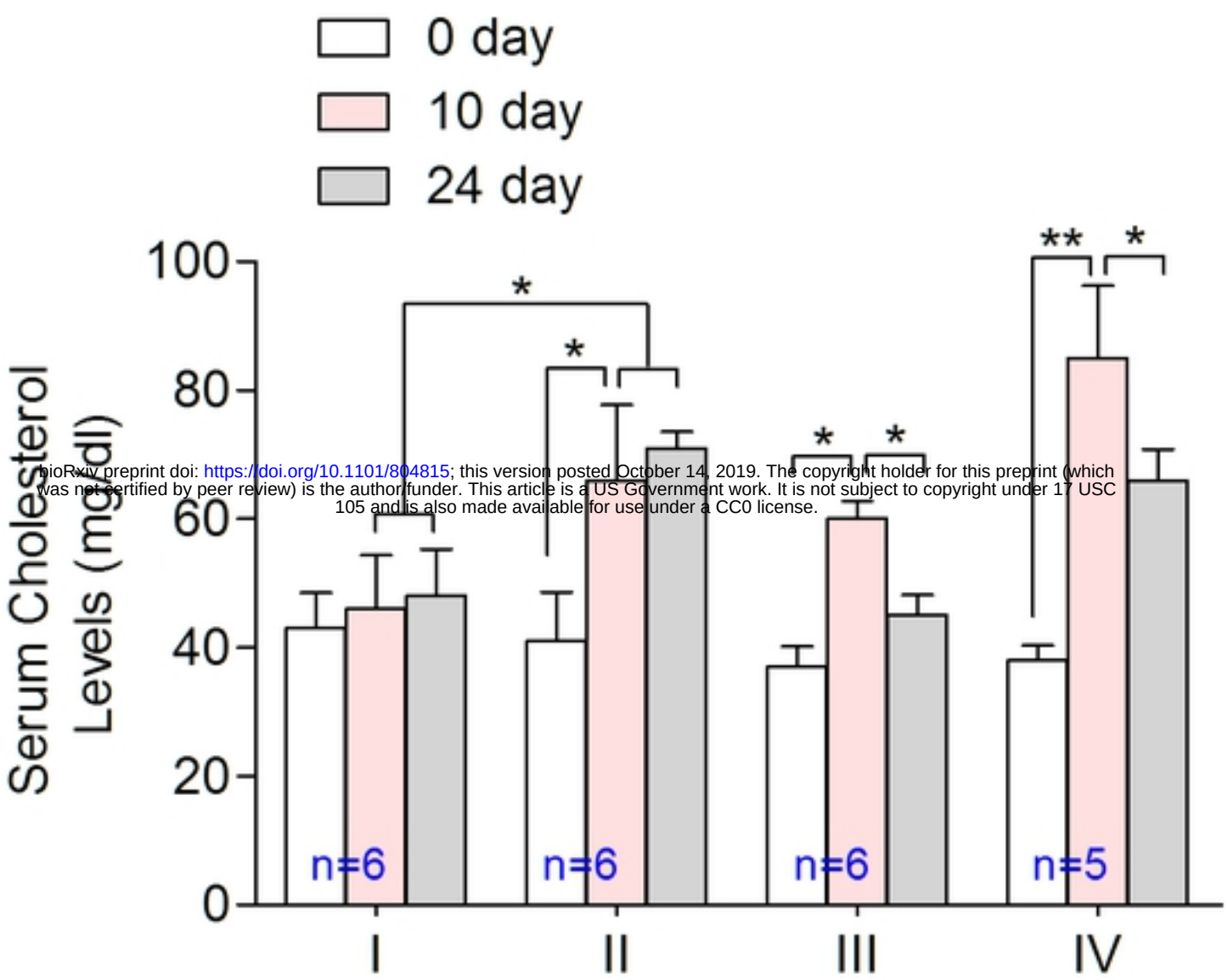




| Groups | Treatments | Serum Cholesterol (mg/dl) | | |
|---|---|---|---|---|
| | | 0 day | 10 days | 24 days |
| I (n=6) | Pellet diet | 43±5.56 (100%) | 46±8.84 (107%) | 48±7.33 (112%) |
| II (n=6) | Pellet diet + 1% cholesterol + 5% coconut oil | 41±7.60 (100%) | 66±11.89 (161%) | 71±2.69 (173%) |
| 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%) |

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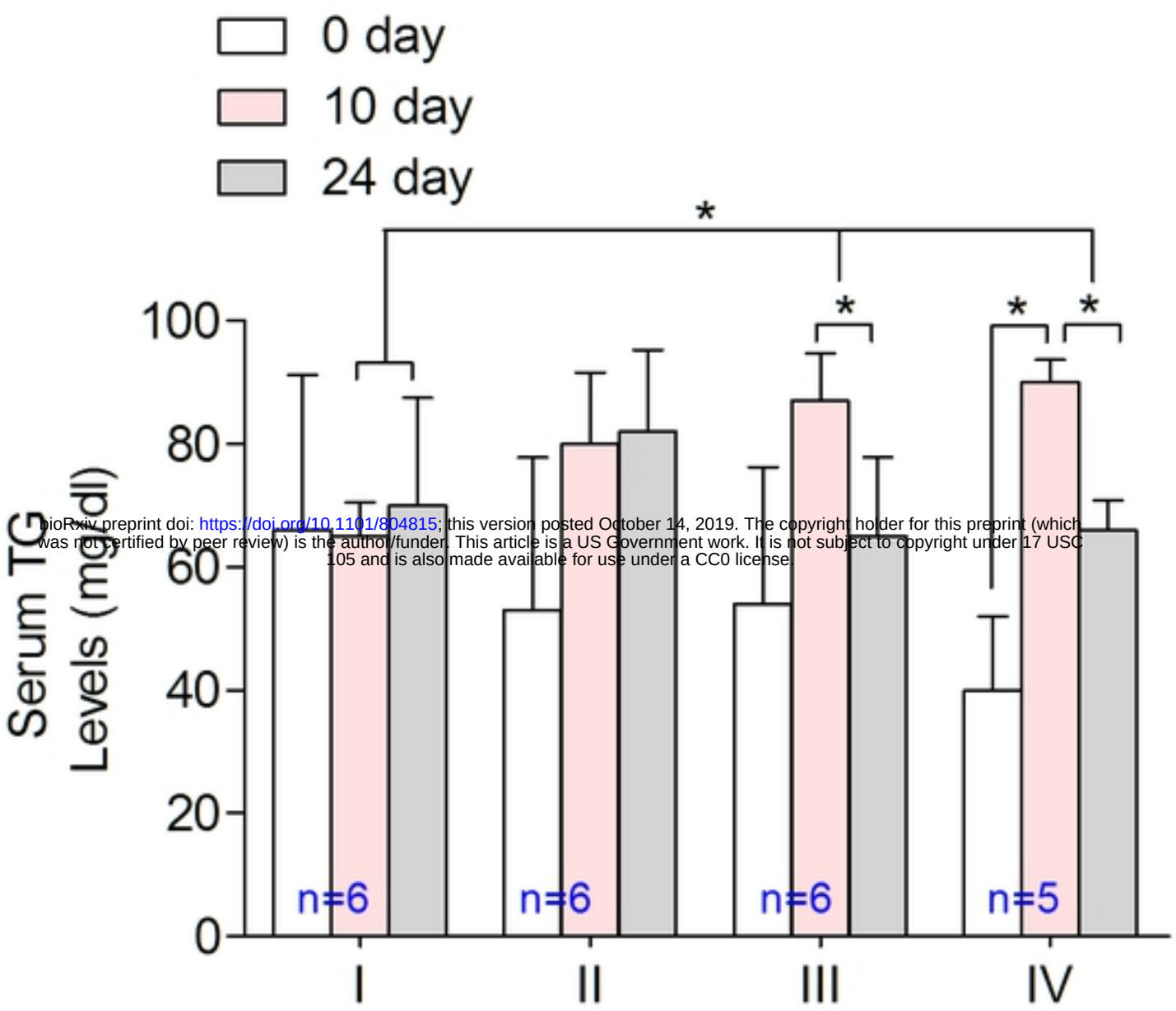




| Groups | Treatments | Serum TG (mg/dl) | | |
|---|---|---|---|---|
| | | 0 day | 10 days | 24 days |
| I (n=6) | Pellet diet | 66±25.3 (100%) | 65±5.56 (98.4%) | 70±17.5 (106%) |
| II (n=6) | Pellet diet + 1% cholesterol + 5% coconut oil | 53±24.9 (100%) | 80±11.26 (151%) | 82±13.3 (155%) |
| III (n=6) | 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%) |

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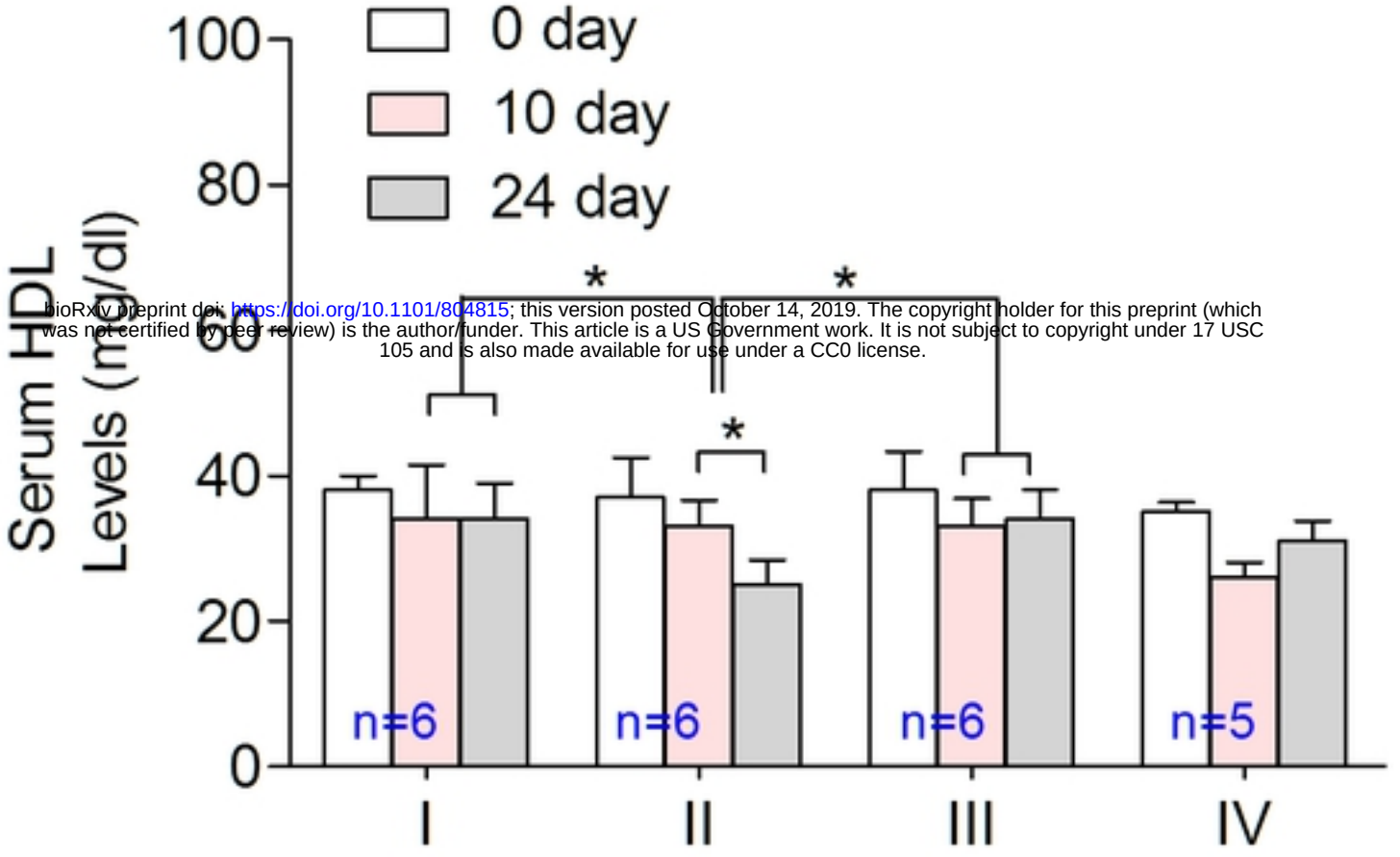


| Groups | Treatments | Serum HDL (mg/dl) | | |
|---|---|---|---|---|
| | | 0 day | 10 days | 24 days |
| I (n=6) | Pellet diet | 38±2.08 (100%) | 34±7.56 (89%) | 34±5.00 (89%) |
| II (n=6) | Pellet diet + 1% cholesterol + 5% coconut oil | 37±5.50 (100%) | 33±3.62 (89%) | 25±3.43 (68%) |
| III (n=6) | 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%) |

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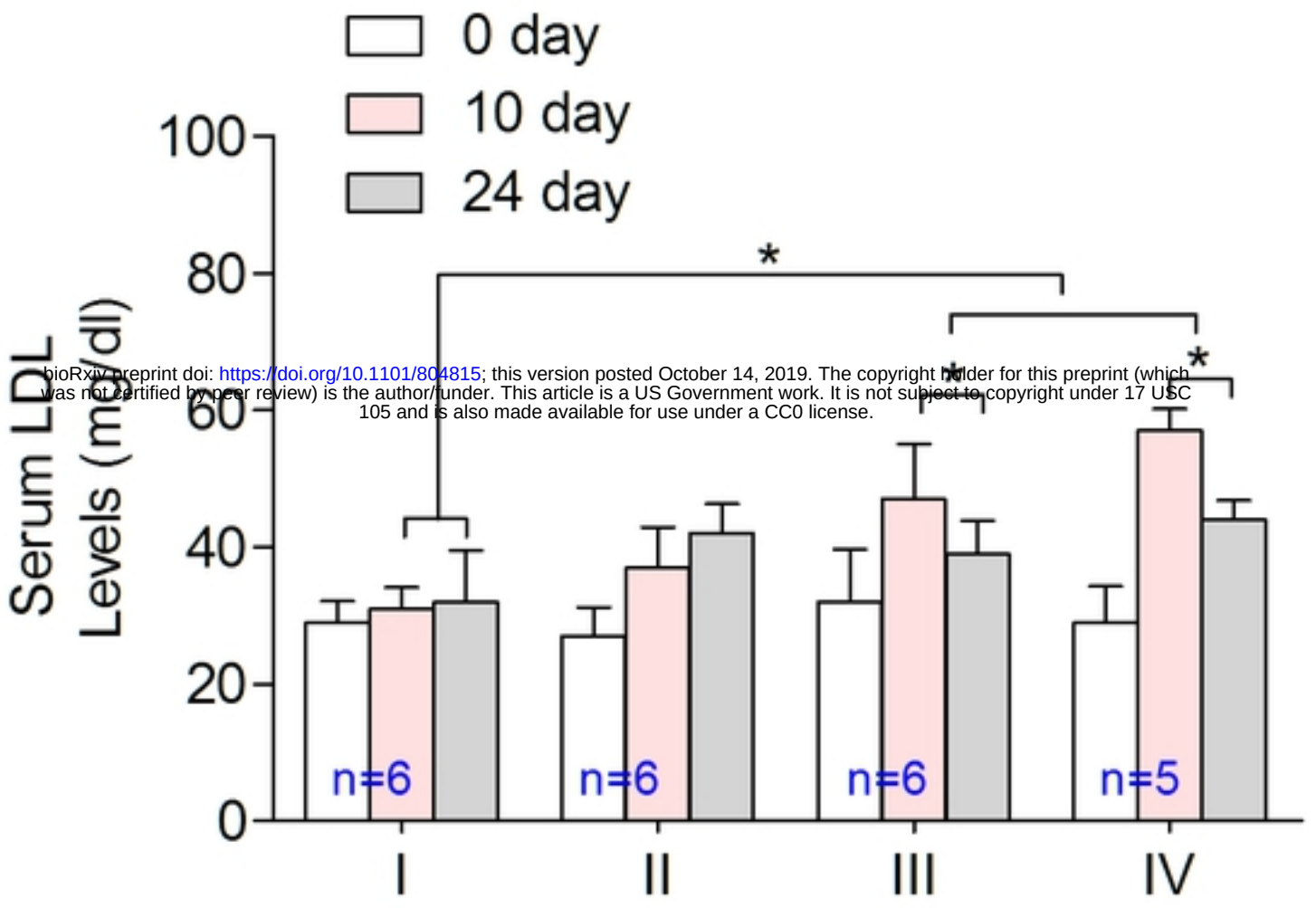


| Groups | Treatments | Serum LDL (mg/dl) | | |
|---|---|---|---|---|
| | | 0 day | 10 days | 24 days |
| I (n=6) | Pellet diet | 29±3.10 (100%) | 31±3.10 (100%) | 32±7.56 (100%) |
| II (n=6) | Pellet diet + 1% cholesterol + 5% coconut oil | 27±4.23 (100%) | 37±5.96 (100%) | 42±3.43 (100%) |
| III (n=6) | 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%) |

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