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## ORIGINAL ARTICLE / RESEARCH

# Cholesterol-Lowering Activity of the Aqueous Fruit Extract of *Trichosanthes dioica* Roxb (L.) in Normal and Streptozotocin Diabetic Rats

**SHARMILA BANU G*, KUMAR G**, RAJASEKARA PANDIAN M***

**ABSTRACT**

**Background:** The purpose of this study was to examine the effects of single and repeated oral administration of the aqueous fruit extract of *Trichosanthes dioica* (TD) at a dose of 50 ml/kg b.w in normal and streptozotocin-induced diabetic rats.

**Material-Methods:** The aqueous fruit extracts of TD (50 ml/kg) were administered orally for 15 days, to normal and diabetic rats. The effect of the fruit extracts on cholesterol and triglycerides, were studied. The body weights of the rats were observed. The effect of the fruit extract was compared with vanadate, a reference drug.

**Result:** In normal rats, the aqueous fruit extract of TD induced significant decrease of plasma cholesterol and triglyceride concentrations 6hrs after a single oral administration ($P < 0.05$), and also in 2 weeks after repeated oral administrations ($p < 0.05$). TD treatment caused significant decrease of plasma cholesterol levels after a single administration ($p<0.01$), and after repeated ($p<0.01$) oral administrations. Significant increase of triglyceride levels was observed 6hrs after a single oral administration of the TD aqueous fruit extract ($p < 0.01$). One week after repeated oral administration of aqueous extract of TD, the plasma triglyceride levels were significantly decreased ($p <0.005$). The decreasing trend continued even after 2 weeks ($p <0.01$). On the other hand, repeated oral administration of TD aqueous fruit extract, caused significant decrease of body weight after 2 weeks of treatment in both normal ($p <0.001$) and diabetic ($p <0.01$) rats.

**Conclusion:** The present study indicates that the aqueous fruit extract of TD exhibits cholesterol and body weight-lowering activities in both normal and hyperglycaemic rats.

**Key words:** *Trichosanthes dioica*, cholesterol, triglycerides, body weight, diabetic rats

## Introduction

From the beginning of the last century, evidences on the cholesterol-lowering properties of medicinal plants have been accumulating [1],[2]. The importance of such investigations, are confirmed in the treatment of obesity, diabetes mellitus, heart failure, and atherosclerosis [3]. Many scientists around the world have reported the role of medicinal plants in the control of elevated serum cholesterol, and

the reduction of morbidity and mortality due to vascular diseases associated with it. The influence of diabetes mellitus on lipid metabolism is well established. The association of hyperglycaemia and alteration of lipid parameters present a major risk of cardio-vascular diseases, particularly in diabetic patients [2],[4–6].

Pointed gourd (*Trichosanthes dioica* Roxb.) is one of the most nutritive cucurbit vegetables, and it holds a coveted position in the Indian market during the summer and rainy seasons. It is a perennial crop, highly accepted due to its availability for eight months in a year (February–September). Being very rich in protein and vitamin A, it has certain medicinal properties, and many reports are available regarding its role in lowering of blood sugar and serum triglycerides [7]. The fruits are easily digestible and diuretic in nature. They are also known to have antiulcerous effects [8]. It grows as a vegetable all over India. It is prescribed to improve appetite and digestion [9]. The decoction of TD is useful as a valuable alternative tonic, and as a febrifuge, which is given for boils and other skin diseases [10]. The juice of the leaf is applied to patches of alopecia areata [11]. The root is used as a hydragogue cathartic tonic and febrifuge [12]. The fruits are used as a remedy for spermatorrhoea, and the juice of unripe fruits and also tender shoots, are used for cooling and as a laxative [13]. The fruits and seeds have some prospects in the control of some cancer- like conditions and haemagglutinating activities [14]. The present study was undertaken to evaluate the potential cholesterol and triglyceride lowering activity of a single and repeated oral administrations of the TD aqueous extract in normal and streptozotocin(STZ) rats. The effect of the TD extract on body weight loss was also determined. Vanadate (0.8 mg/kg) was used as a reference drug [2],[15] known for its both hypolipidaemic and hypoglycaemic activities.

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## Materials and Methods

### Plant material

The fruit of TD was collected from Palayapalayam, Namakkal District, Tamilnadu, India, and was authenticated by Fr. K. M. Matthew, Director, Rapinat Herbarium, St.Joseph's College, Tiruchirapalli. Voucher Herbarium specimens have been deposited in the (collection number 23644) Rapinat Herbarium for future references.

### Preparation of the aqueous fruit extract

Fresh raw deseeded fruits of TD (1Kg) were peeled, washed, cut into small pieces, and homogenized in a warring blender, with 2 litres of distilled water. The extraction was carried at a temperature of $20^0 \pm 1^0$C, with constant stirring overnight. The homogenate was then squeezed through a cheese cloth, and was centrifuged at 2000 rpm for 10min at 0-$4^0$C. The supernatant being the TD fruit extract, it was decanted and used for experiments.

### Animals

Experiments were performed in either sex of Wistar rats weighing from 200 to 220 g. The animals were housed under standard environmental conditions (23 ± $1^0$C, with 65 ± 5% humidity and 12:12-h light/dark cycle), and maintained with free access to water and *ad libitum* standard laboratory diet. Six rats were housed per cage, to provide them with sufficient space, and to avoid unnecessary morbidity and mortality. All the studies were conducted in accordance with the National Institute of Health guide [16].

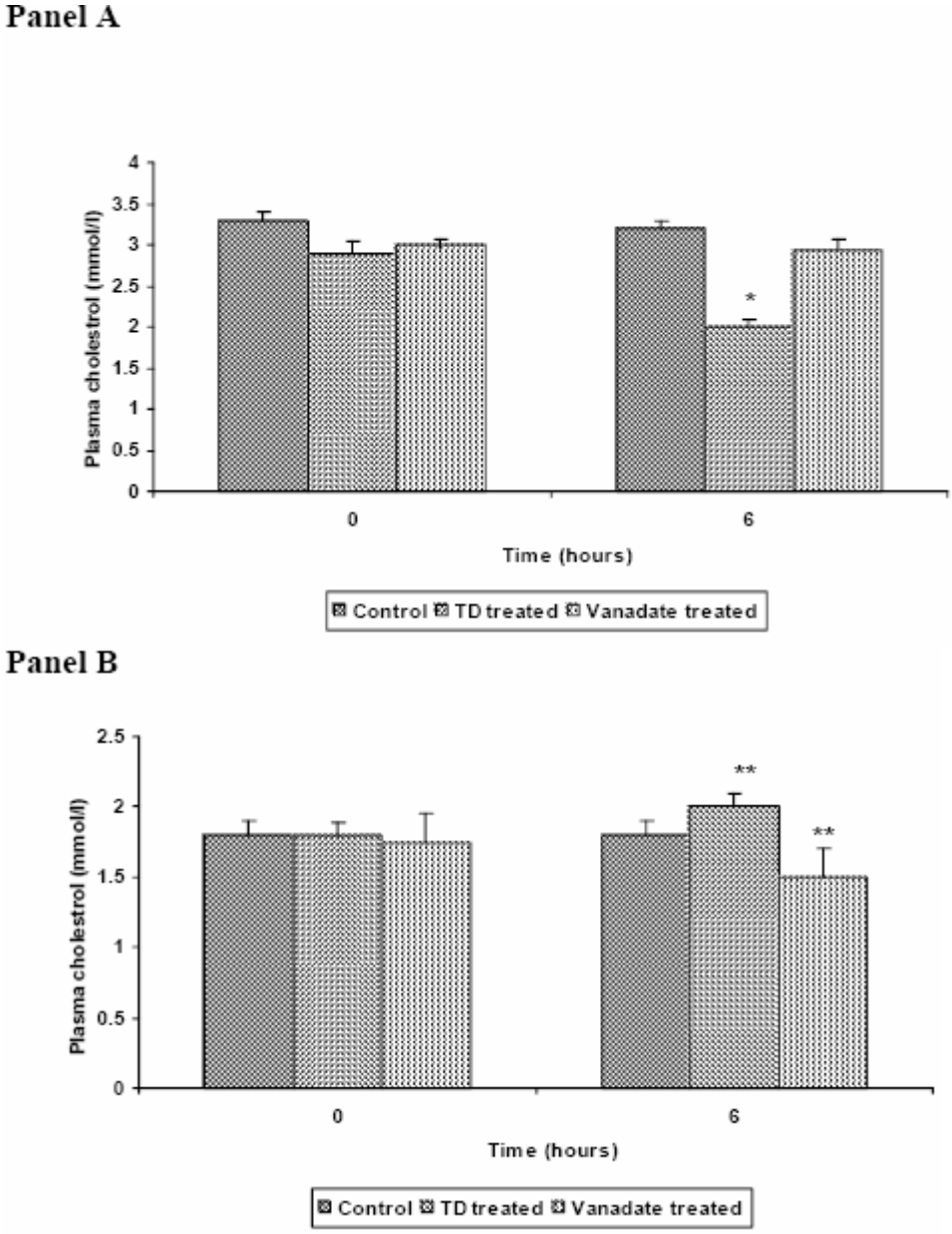
### Induction of diabetes

STZ (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 0.1M of fresh cold citrate buffer at pH 4.5 before use, and was injected intravenously into the tail vein at a dose of 60mg/kg [17]. After 18 hrs, rats with stable fasting blood glucose levels over16 mmol/l were considered as diabetics, and were used in the present study.

### Single oral administration

Normal and diabetic rats were randomly assigned to three different groups, containing six

rats each. One control group received distilled water, a second group received the aqueous extract of TD at a dose of 50 ml/kg, and the third group received a reference drug (vanadate ($Na+VO_3$) at a dose of 0.8 mg/kg). For single oral administration, distilled water (control), Vanadate (0.8mg/kg) [15], or the aqueous fruit extract of TD (50 ml/kg) [19], were administered, and plasma cholesterol and triglyceride levels were measured before and 6hrs after TD treatment.




**[Table/Fig 1]** Plasma cholesterol levels (mmol/l) after a single oral administration of aqueous fruit extract of TD (50 ml/kg) in normal (Panel a) and STZ (Panel b) rats. Data are expressed as means ± S.D., *n* = 6 rats per group. (*) *P* < 0.05, (**) *P* < 0.01, when compared to baseline values (the start of treatment).

## Repeated oral administration

For repeated oral administration, rats were treated once daily at a dose of 50 ml/kg for 2 weeks, and plasma cholesterol and triglyceride levels were measured during this period. Blood samples were collected from the tail vein, and plasma triglyceride and cholesterol levels were determined enzymatically by colorimetric specific kits (Randox, UK), respectively. The kits used in this study for substrate analysis were

specific for both human and rat blood samples, at the same percentage.

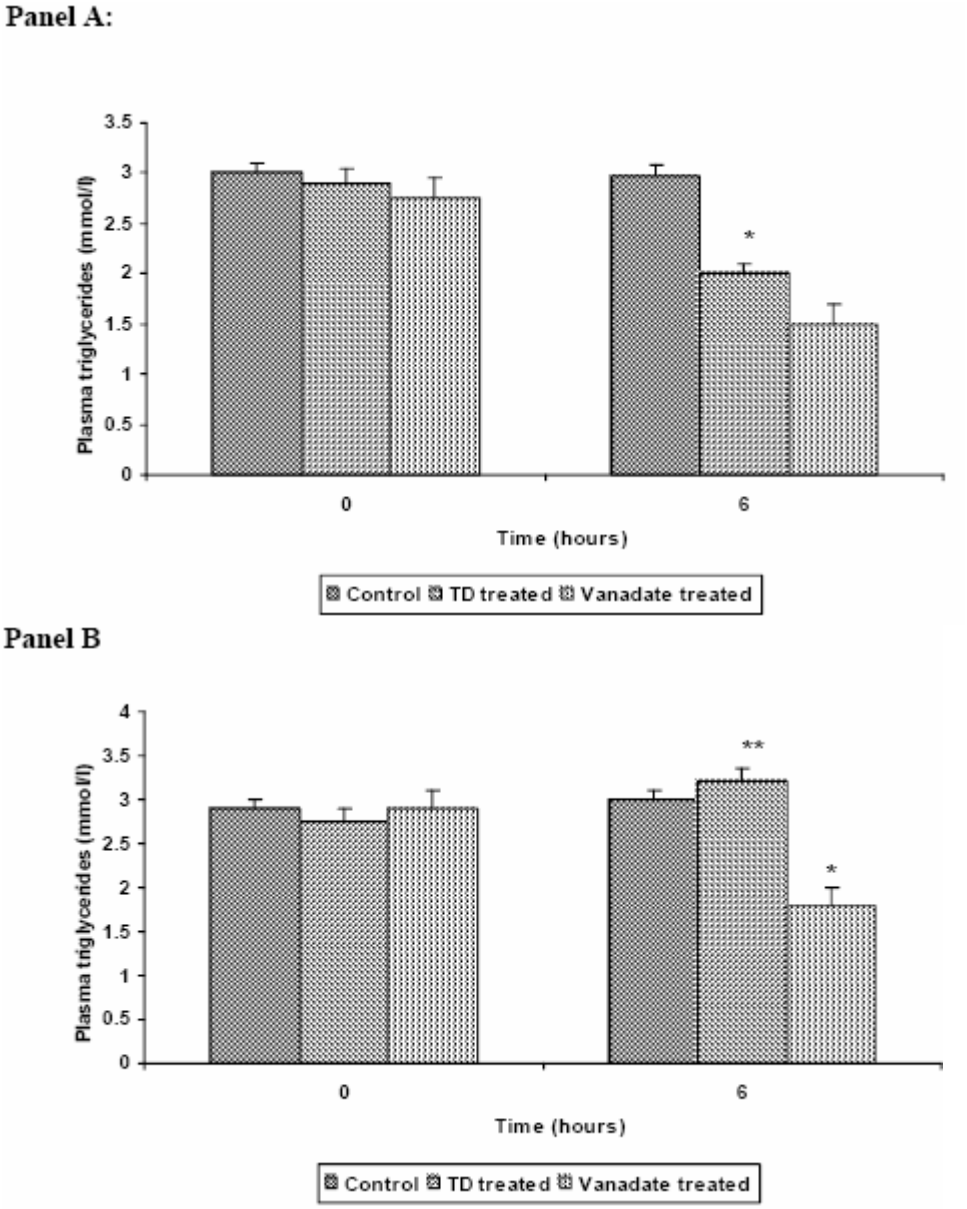
### Statistical analysis

All experimental data were expressed as Mean ± S.D., and statistically assessed by one-way analysis of variance (ANOVA).The difference between test animals and controls were evaluated by Student's *t*-test [18].

## Results

### Single oral administration

In normal rats, a significant decrease of plasma cholesterol levels was observed in the group treated with TD, at a dose of 50 ml/kg, when compared to the control group ($p< 0.05$; [Table/Fig 1a]), 6 hrs after the fruit treatment. The plasma triglyceride levels were also increased, 6 hrs after fruit treatment ($p <0.05$; [Table/Fig 2a]). No statistically significant

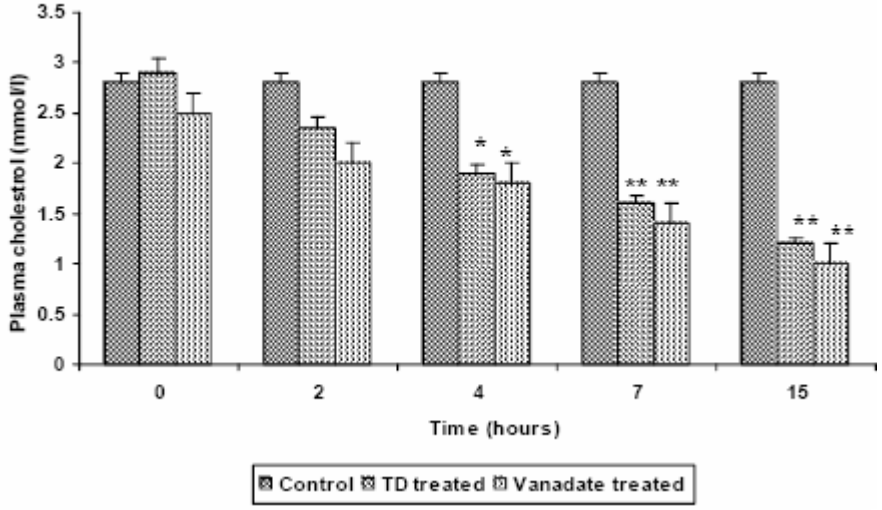



**[Table/Fig 2]** Plasma triglycerides levels (mmol/l) after a single oral administration of aqueous fruit extract of TD (50 ml/kg) in normal (Panel a) and STZ (Panel b) rats. Data are expressed as means ± S.D., $n$ = 6 rats per group. (*) $P < 0.05$, (**) $P < 0.01$, when compared to baseline values (the start of treatment).

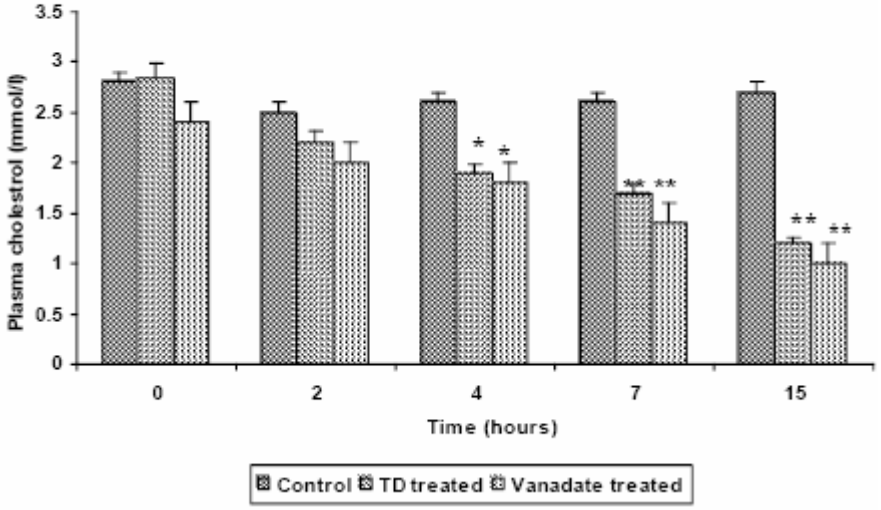
changes were observed for the vanadate-treated group ([Table/Fig 1a] and [Table/Fig 2a]). In STZ rats, the aqueous fruit extract of TD caused significant reduction of plasma cholesterol

levels, 6 hrs after TD treatment ($p$ <0.01; [Table/Fig 1b]), while plasma triglyceride levels were increased ($p$ < 0.01; [Table/Fig 2b]). Vanadate (0.8 mg/kg) reduced both the plasma cholesterol ($p$ <0.01) and triglyceride levels ($p$ < 0.01), after 6 hrs, in diabetic rats ([Table/Fig 1b] and [Table/Fig 2b]).

**Panel A:**




**Panel B:**

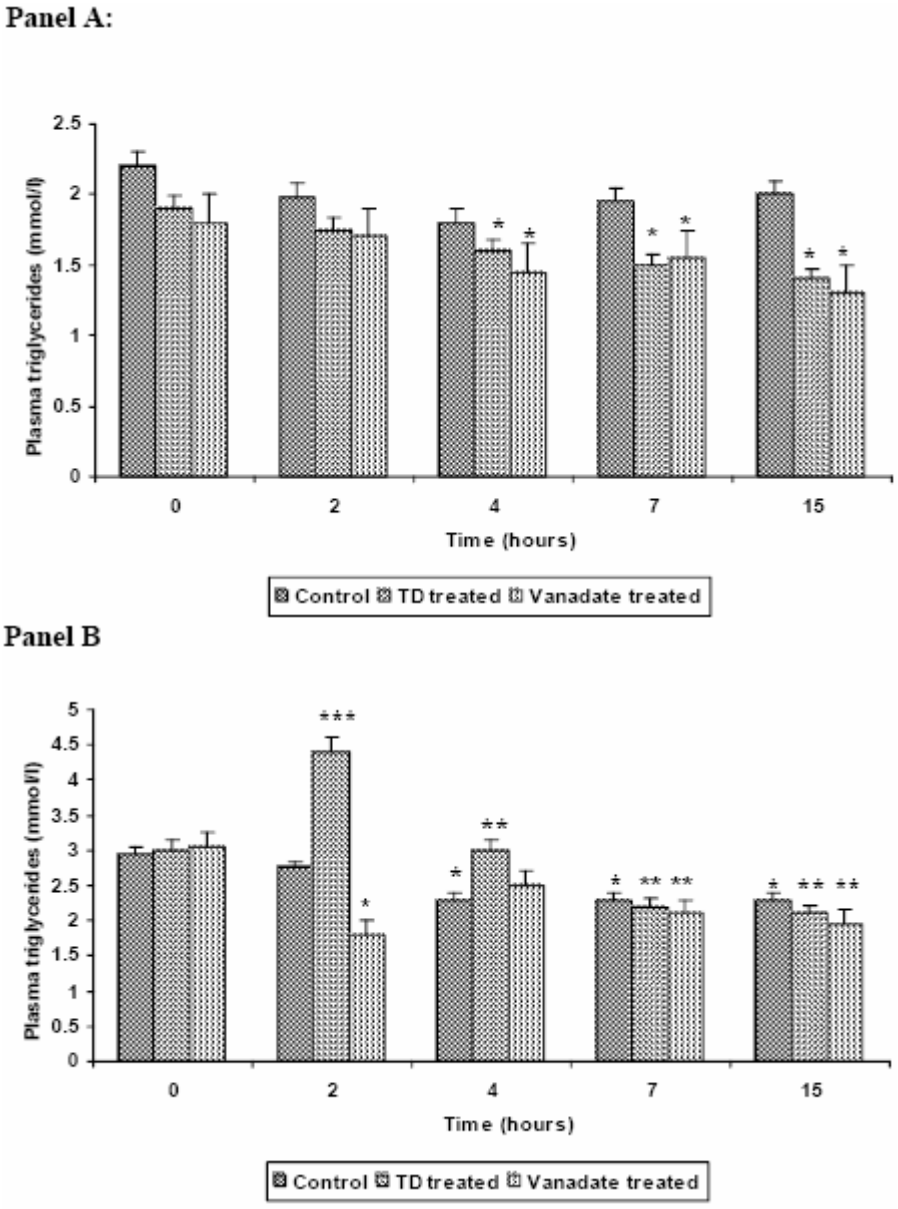



**[Table/Fig 3]** Plasma cholesterol levels (mmol/l) after a repeated oral administration of aqueous fruit extract of TD (50 ml/kg) in normal (Panel a) and STZ (Panel b) rats. Data are expressed as means ± S.D., $n$ = 6 rats per group. (*) $P$ < 0.05, (**) $P$ < 0.01, when compared to baseline values (the start of treatment).

## Repeated oral administration

In normal rats, a significant reduction in plasma cholesterol levels was observed in the TD treated group, from the fourth day to the second week of TD treatment ($p$ <0.05; [Table/Fig 3a]). The plasma triglyceride levels were decreased from the first to the second week ($p$< 0.05) of TD treatment ([Table/Fig 4a]). After 2 weeks of treatment, vanadate caused a significant decrease of plasma cholesterol levels ($p$< 0.01; [Table/Fig 3a]) and triglyceride levels ($p$<0.05; [Table/Fig 4a]). In diabetic rats, TD fruit extract decreased significantly, the plasma cholesterol levels from the fourth day ($p$< 0.05); the most significant effect was reached in the second week of treatment ($p$ < 0.01; [Table/Fig 3b]).

The plasma triglyceride levels were increased 2 days after the fruit treatment ($p < 0.001$), and dropped significantly from the first week ($p<0.01$) to the second week of fruit treatment ($p<0.05$; [Table/Fig 4b]). Daily vanadate administration (0.8 mg/kg) for 2 weeks produced a statistically significant reduction in both plasma cholesterol and triglyceride concentrations ([Table/Fig 3] and [Table/Fig 4]) ($p< 0.01$).




**[Table/Fig 4]** Plasma triglycerides levels (mmol/l) after a repeated oral administration of TD aqueous extract (10 mg/kg/min) in normal (Panel a) and STZ (Panel b) rats. Data are expressed as means ± S.D., $n$ = 6 rats per group. (*) $P < 0.05$, (**) $P < 0.01$, when compared to baseline values (the start of treatment).

## Body weight loss

In normal rats, the TD aqueous fruit extract caused a significant weight loss, 2 weeks after treatment ($p< 0.001$; [Table/Fig 5]). The same effect was noted in STZ rats ($p< 0.01$; [Table/Fig 5]). Vanadate caused also a significant decrease of body weight in both normal and STZ rats ($p< 0.01$) ([Table/Fig 5]).

## Discussion

The aim of this study was to test the effect of the TD aqueous fruit extract on plasma cholesterol and triglyceride concentrations. According to the survey, TD was widely used in diabetes and

cardiac diseases. We have previously reported that TD exhibited a hypoglycaemic and antioxidant activity in STZ rats [19],[20]. The mechanism involved in this pharmacological effect seems to be the inhibition of endogenous glucose production [21]. Vanadate was used as a reference drug, because it has been reported to be a potent insulin mimetic agent in many cells [22],[23]. Administration of this compound to diabetic animals normalizes blood glucose concentration and reduces triglyceride levels [24],[25]. The results demonstrated that the aqueous fruit extract of TD induced a significant decrease of plasma cholesterol levels both in normal and STZ-diabetic rats, for short-term (single) and long-term (repeated) administrations. However, the plasma triglyceride levels were increased when short-term treatment was considered, but decreased significantly after long-term TD treatment in both normal and STZ rats.

**[Table/Fig 5]** Effect of TD aqueous fruit extract treatment (50 ml/kg) on body weight (g) in normal and diabetic rats

| Experimental groups | Body weight (g) | |
|---|---|---|
| | 0 day | 15 days |
| *Normal rats* | | |
| Control | 222.7 ± 2.9 | 230.3 ± 4.7* |
| TD treated | 220.3 ± 4.6 | 199.2 ± 4.5*** |
| Vanadate treated | 217.5 ± 3.7 | 191.7 ± 3.9*** |
| *Diabetic rats* | | |
| Control | 215.5 ± 2.7 | 190.5 ± 3.5*** |
| TD treated | 230.3 ± 4.6 | 214.5 ± 3.1** |
| Vanadate treated | 210.9 ± 3.6 | 181.7 ± 3.4*** |

Data are expressed as means ± S.E.M., $n$ = 6 rats in each group.
*$P$ < 0.05, when compared to baseline values (the start of treatment).
**$P$ < 0.01, when compared to baseline values (the start of treatment).
***$P$ < 0.001, when compared to baseline values (the start of treatment).

Vanadate treatment caused a significant decrease of plasma triglyceride levels in STZ rats. The plasma triglyceride levels were initially increased in STZ rats, because the lipolysis was stimulated by a concomitant insulinopenic state. Recent-onset insulinopenia in STZ-diabetic rats is associated with lipid overproduction in the basal (hyperglycaemic) state [17]. Some studies have reported a similar lipidemic-lowering activity of some medicinal plants [26],[27]. The transient increase of triglyceride levels at short-term experiments, were observed in our study. Such short-term analyses had not been performed in previous studies. This transient increase of triglycerides could be explained by the fact, that glycolysis, glucose oxidation, and lipid synthesis pathways could be activated by the TD aqueous fruit extract [28],[29]. The effect of TD was more prominent, as compared to that of vanadate.

The underlying mechanism of the lipidaemic-lowering activity of TD could be the inhibition of lipid absorption due to the presence of saponins and tanins in the aqueous extract [26],[30–32], and/or inhibition of cholesterol-esterase, activation of fatty acids synthase, acetyl-CoA carboxylase and production of triglyceride precursors such as acetyl-CoA and glycerol phosphate. The main constituents of TD are flavonoids, saponins and tanins [28]. Flavonoids are considered as active constituents of many medicinal plants [32], and natural products with positive effect for human health [33]. We have previously demonstrated that flavonoids are responsible of the antioxidant activity of TD in normal and STZ rats [19],[20]. The effect of some flavonoids on cholesterol metabolism is known [34],[35]. On the other hand, oral administration of saponins from some medicinal plants, significantly reduce triglycerides and cholesterol levels in rat [36]. The usage of diet with high saponins contents is also suggested to reduce heart diseases [35],[36].

It seems then that the TD fruit extract reduced plasma cholesterol and triglyceride levels without stimulating insulin secretion. The TD aqueous fruit extract causes a weight loss in rats. This effect could be explained directly by the lipid-lowering activity of the extract, and/or its influence of rat appetite [37], or indirectly by influencing various lipidaemic regulation systems. The plant extract could be considered relatively free from short-term lethal constituents, since the median lethal dose ($LD_{50}$) by oral administration in mice was 200ml/kg. These values are higher than those reported for many toxic plants [38],[39]. Other advanced toxicological investigations are required to determine the precise eventual toxicity in different organs and tissues.

After such investigations, it can be concluded that the TD aqueous fruit extract could be used in the human healthcare system, especially in the treatment of hypercholesterolemia associated with obesity and cardio-vascular diseases. We concluded that the aqueous fruit extract of TD exhibited potent long-term cholesterol and triglyceride-lowering activities in both normal and STZ-diabetic rats, and confirmed its use in the treatment of cardiac diseases by the Indian population. Additionally, the precise molecular mechanism and active substance(s) need to be determined in further experiments. Such active principle(s) could be precious in atherosclerosis and cardiac diseases therapy and control.

## Conflict of Interest

None declared.

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