Research Article

Hypolipidemic effect of *Tamarindus indica* L fruit on Triton X-100-induced hyperlipidemia in Wistar rats

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Abstract

Background: *Tamarindus indica* L is widely used as a traditional medicine. In Indonesia *T. indica* L is commonly used to treat gastritis, rheumatism, constipation, and fever. **Aims and Objective:** The purpose of this study was to examine the hypolipidemic effect of 70% ethanolic extract of *T. indica* L fruit flesh and rind and their chemical compounds. **Materials and Methods:** A total of 40 Wistar male rats were divided into 8 groups. group I was negative control (*aquabidest*); group II was positive control (Simvastatin 0.72 mg/gbw); groups III, IV, and V were treated by 70% ethanolic extract of the flesh fruit with doses of 200, 100, and 50 mg/kgbw, respectively; groups VI, VII, and VIII were treated by 70% ethanolic extract of the rind fruit with doses of 200, 100, and 50 mg/kgbw, respectively. The extract was given for 7 days after the third day. Measurement of plasma total cholesterol and triglyceride were carried out on days 0, 3, and 10. **Result:** The results show that the 70% ethanolic extract of *T. indica* L fruit rind and flesh with doses of 200, 100, and 50 mg/kgbw can reduce plasma total cholesterol and triglyceride significantly ($P < 0.01$). Chemical content of 70% ethanolic extract of *T. indica* L fruit rind and flesh by thin-layer chromatography examination are alkaloids, flavonoids, terpenoids, and phenolic. **Conclusion:** The 70% ethanolic extract of *T. indica* L fruit flesh and rind exert effect for lowering total cholesterol and triglyceride on hyperlipidemia induced by Triton X-100 in Wistar rats.

KEY WORDS: *Tamarindus indica* L; total cholesterol; triglycerides; Triton X-100

Introduction

The greatest risk factor for coronary heart disease (CHD) is hyperlipidemia.[1] Of the approximately 9.4 million deaths each year, 51% are caused by stroke and 45% are by CHD.[2] *Tamarindus indica* L is widely used as a traditional medicine in Indonesia and is commonly used to treat gastritis, rheumatism, constipation, and fever.[3,4] In India too, this plant is traditionally used to treat fever, stomach disease, diarrhea, and infection.[5] Several studies have examined the pharmacological effect of *T. indica* L. The results showed that pectin of *T. indica* L believed to have antioxidants that can reduce serum blood total cholesterol and triglycerides and increase high-density lipoprotein (HDL).[6] The extract of *T. indica* L has strong antioxidant effect.[7–9] The methanolic extract of *T. indica* L seed has antioxidant properties. This extract contains procyanidin.[7] In vitro, *T. indica* L fruit pulp has radical scavenging ability that is measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radicals and causes a decrease in serum lipid peroxidation, which is assessed by thiobarbituric acid.[9] *T. indica* L has also antibacterial properties.[10,11] *T. indica* L stem bark and leave were subjected to extraction using aqueous, ethanol, and acetone-inhibited growth of both gram-positive and gram-negative bacteria. These extracts contain tannin, saponin, sesquiterpenes, alkaloids, and phlobatannins.[11] The extract of *T. indica* L has hypoglycemic effects.[12–16] Aqueous extract of *T. indica* L seeds with dose 80 mg/0.5 mL distilled water/100 g/day for 14 days reduces blood glucose level after 7 days in

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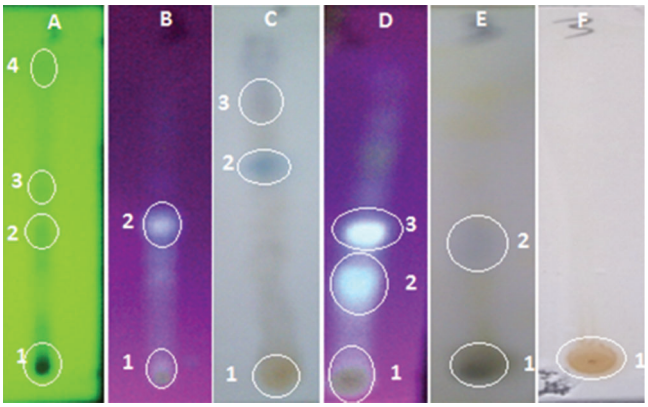

Figure 1: TLC Profile of 70% ethanolic extract of *T. indica* L fruit rind with silica gel GF254 plates and mobile phase with toluene/ethyl acetate (3:9) v/v (A) The appearance of under UV254, (B) appearance under UV366, (C) derivatization with sulfate vanillin, (D) derivatization with sitroborat, (E) derivatization with $FeCl_3$, and (F) derivatization with dragendorf.

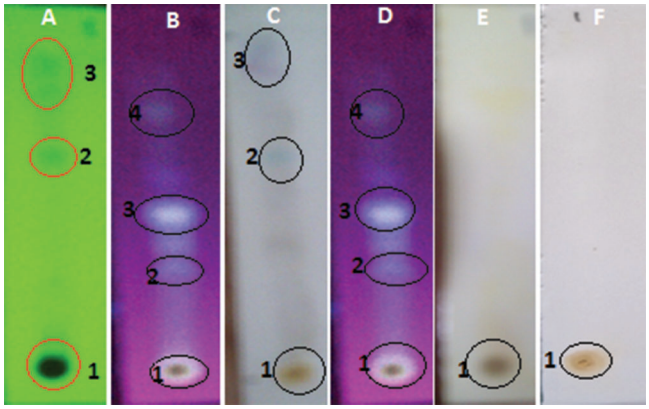

Figure 2: TLC Profile of 70% ethanolic extract of T. indica L fruit rind with silica gel GF254 plates and mobile phase with toluene/ethyl acetate (3:9) v/v (A) The appearance of under UV254, (B) appearance under UV366, (C) derivatization with sulfate vanillin, (D) derivatization with sitroborat, (E) derivatization with FeCl3, and (F) derivatization with dragendorff.

streptozotocin-induced diabetic rat.[12] The methanolic extract of *T. indica* seeds with doses 200 and 400 mg/kgbw orally on alloxan-induced diabetic mice reduced blood glucose level in a 5-day study.[13] The aqueous extract of *T. indica* L seed (500 mg/kgbw, p.o.) decreases fasting blood glucose and increases plasma insulin.[14] Aqueous extract of *T. indica* L. seeds was predicted to restore pancreatic beta cells in streptozotocin-induced diabetic rats.[17] The methanolic extract of *T. indica* L seed has cytotoxic activity against sea urchin embryo cells.[18] The ethanol, chloroform, and aqueous extracts of *T. indica* L have anti-inflammatory activity on mice and rats after intraperitoneal and topical administration.[19] Dried and pulverized pulp of *T. indica* L fruits (15 mg/kgbw, p.o.) can reduce total cholesterol level and low-density lipoprotein (LDL)-cholesterol in human subjects.[20] The methanol extract of *T. indica* L has spasmolytic effect on yeyunum rabbits.[21] The aqueous extracts of tamarind leaves, fruits, and unroasted seeds administered for 9 days have hepatoregenerative effect on paracetamol-induced hepatotoxicity in rats.[22] The aqueous and alcoholic extracts of *T. indica* L seed coat have anti-inflamatory, anti-arthritic, and anti-nociceptive effects. These extracts inhibit expression of interleukin (IL) and decrease the production of prostaglandin E2.[23] The methanolic extract of *T. indica* L seeds with doses of 100 and 200 mg/kgbw administered orally can reduce the total volume of gastric juice and free and total acidity of gastric secretion in rats' pylorus-ligation-induced ulcer model.[24] The methanolic extract of *T. indica* L leaves has antiasthmatic activity. Doses of 175, 350, and 700 mg/kgbw (p.o.) of these extracts exhibit mast-cell-stabilizing activity.[25] *T. indica* L also has laxative effect.[26–27] The juice of *T. indica* L leaves with a dose of 40% has laxative properties.[27] The methanol and butanol extracts of *T. indica* L leaves have antiemetic properties.[28] Besides, *T. indica* L has also anthelmintic activity. The juice of *T. indica* L (20%, 50%, and 100%, respectively) causes paralysis on pheretima posthuma.[29]

Objectives

The objectives of this study were to determine the hypolipidemic effects of ethanolic extract of *T. indica* L fruit flesh and rind on hyperlipidemia in Wistar rats induced by Triton X-100.

Materials and Methods

The materials used in this study include fruit tamarind (*T. indica* L) obtained from Boyolali area in Indonasia, harvested in August 2013, Triton X-100 (Sigma), Simvastatin® StarDust FC15 analyzer, silica plate GF254 7 × 6 cm (Merck).

Animals tested were male Wistar rats obtained from Laboratory of Pharmacology of Universitas Muhammadiyah, Surakarta. The age of Wistar rats was approximately 2–3 months and weight was 175–225 g. This research was approved by health research ethics committee of Dr. Moewardi Hospital of Surakarta.

Preparation of Extract

Extracts used were fruit rind and flesh of *T. indica* L. They were separated, then dried under the sun, and blended into a powder. Each powder was extracted by maceration method by soaking in 70% ethanol for 5 days and then was filtered. Filtrates were evaporated on a water bath temperature of 60–70°C as they were stirred and aerated to obtain thick extract.

Methods of Evaluating of Hypolipidemic Effect

Subjects used in the study were male Wistar rats. They were divided into eight groups randomly. Each group consisted of five rats. Group I was the negative control (*aquadest*); group II was the positive control (Simvastatin 0.72 mg/200 gbw); groups III, IV, and V were treated by 70% ethanolic extract of the fruit flesh with doses of 200, 100, and 50 mg/kgbw;

Table 1: The mean of plasma level of cholesterol ± SD on days 0, 3, and 10

Groups	Cholesterol level (mg/dL)			*P*-value (compared by negative control)
	Day 0	Day 3	Day 10	
Negative control	68.4 ± 15.4	128.2 ± 43.4	128.7 ± 42	
Positive control (Simvastatin 0.72 mg/200 gbw)	84.8 ± 4.8	150 ± 33.1	79.8 ± 7.8	0.00
The 70% ethanolic extract of fruit flesh with dose 200 mg/kgbw	77.8 ± 20.7	184.8 ± 56.7	86.02 ± 19.1	0.00
The 70% ethanolic extract of fruit flesh with dose 100 mg/kgbw	87 ± 28.20	185.2 ± 38.6	103.4 ± 37.7	0.01
The 70% ethanolic extract of fruit flesh with dose 50 mg/kgbw	49.2 ± 13.4	160 ± 14.9	88.8 ± 14	0.01
The 70% ethanolic extract of fruit rind with dose 200 mg/kgbw	55.00 ± 22.30	161.16 ± 28.28	98.50 ± 30.62	0.00
The 70% ethanolic extract of fruit rind with dose 100 mg/kgbw	55.40 ± 18.86	152.58 ± 63.39	94.50 ± 18.37	0.01
The 70% ethanolic extract of fruit rind with dose 50 mg/kgbw	59.60 ± 12.81	153.40 ± 30.26	76.53 ± 4.97	0.01

Table 2: The mean of plasma level of triglycerides ± SD on day 0, 3, and 10

Groups	Triglycerides (mg/dL)			*P*-value (compared by negative control)
	Day 0	Day 3	Day 10	
Negative control	64.4 ± 17.6	158 ± 20.6	208.3 ± 20.7	
Positive control (Simvastatin 0.72 mg/200 gbw)	76.8 ± 12.4	139 ± 18.9	102.5 ± 18.9	0.00
The 70% ethanolic extract of fruit flesh with dose 200 mg/kgbw	88.4 ± 16.7	160.8 ± 11.8	112.5 ± 11.9	0.00
The 70% ethanolic extract of fruit flesh with dose 100 mg/kgbw	101.6 ± 26.9	160.4 ± 16.9	115.2 ± 16.9	0.01
The 70% ethanolic extract of fruit flesh with dose 50 mg/kgbw	92.2 ± 13.9	138 ± 12	83.6 ± 12	0.01
The 70% ethanolic extract of fruit rind with dose 200 mg/kgbw	86.00 ± 12.35	145.60 ± 45.02	105.50 ± 17.25	0.00
The 70% ethanolic extract of fruit rind with dose 100 mg/kgbw	73.80 ± 16.63	189.40 ± 60.92	88.12 ± 23.19	0.00
The 70% ethanolic extract of fruit rind with dose 50 mg/kgbw	89.60 ± 19.32	148.00 ± 52.24	101.33 ± 43.14	0.01

groups VI,VII, and VIII were treated by 70% ethanolic extract of the fruit rind with doses of 250, 200, and 100 mg/kgbw, respectively. The extract was given for 7 days. Plasma total cholesterol and triglyceride levels were measured on days 0, 3, and 10. Cholesterol and triglyceride measurements were carried out by a StarDust FC15 spectrometer with reagent cholesterol oxidase-peroxidase for the measurement of total cholesterol and glycerol phosphate oxidase-peroxidase for the measurement of triglyceride (Diasis).

Thin-Layer Chromatography

A total 100 mg of 70% ethanolic extract of the fruit rind and flesh of *T. indica* L were dissolved in 1 mL methanol p.a. From this solution, 0.5 mL was spotted on thin-layer chromatography (TLC) plates (silica plate GF254). The TLC plate was eluted with mobile phase ethyl acetate/toluene (1:3). The spotting was observed in UV 254 nm and UV 366 nm. The coloring agents were sulfate vanillin for terpenoid, $FeCl_3$ for polyphenols, dragendorff for alkaloids, and sitoborat for flavonoid. The movement of active compound was expressed by its retention factor (R_f). This R_f was calculated by the following formula:

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

Results

1. The lowering of total cholesterol and triglycerides

Treatment by the extract was done for 7 days. The plasma level of cholesterol and triglycerides was measured on days 0, 3, and 10. The plasma levels of cholesterol on days 0, 3, and 10 are shown in Table 1.

Table 1 and 2 show consequence on the calculation of the percentage reduction of total cholesterol and triglycerides. The result can be seen in Table 3.

2. Thin-layer chromatography

a. The 70% ethanolic extract of *T. indica* L fruit flesh

The result of TLC of *T. indica* L fruit flesh can be observed in Figure 1.

The TLC results show the 70% ethanolic extract of *T. indica* L fruit flesh contains flavonoids, phenolics, terpenoids, and alkaloids

b. The 70% ethanolic extract of *T. indica* L fruit rind.

The result of TLC of *T. indica* L fruit rind can be observed in Figure 2.

On the basis of Figure 2, several compounds may be identified and are listed in Table 5.

The TLC results show the 70% ethanolic extract of *T. indica* L fruit rind contains flavonoids, phenolic, terpenoids, and alkaloid.

Table 3: The percent reduction of total cholesterol and triglycerides ± SD

Groups	The mean of the percent reduction (% ± SD)	
	Cholesterol	Triglycerides
Positive control (Simvastatin 0.72 mg/200 gbw)	37.95 ± 6.1	50.82 ± 14.9
The 70% ethanolic extract of fruit flesh with dose 200 mg/kgbw	33.19 ± 14.8	41.18 ± 19.9
The 70% ethanolic extract of fruit flesh with dose 100 mg/kg bw	19.64 ± 7.2	44.7 ± 13.9
The 70% ethanolic extract of fruit flesh with dose 50 mg/kgbw	30.99 ± 10.9	59.89 ± 10.9
The 70% ethanolic extract of fruit rind with dose 200 mg/kgbw	23.45 ± 23.79	49.36 ± 8.28
The 70% ethanolic extract of fruit rind with dose 100 mg/kgbw	26.56 ± 14.28	57.70 ± 11.13
The 70% ethanolic extract of fruit rind with dose 50 mg/kgbw	40.52 ± 3.85	51.36 ± 20.71

Table 4: The separation of 70% ethanolic extract of *T. indica* L fruit flesh

Detection	No	hRf	Description of color	Chemical compound
UV_{254}	1	0	Strong quenching	
	2	55	Weak quenching	
	3	57.5	Weak quenching	
	4	95	Weak quenching	
UV_{366}	1	0	Weak yellow fluorescence	Flavonoid
	2	55	Yellow fluorescence	Flavonoid
Vanilin H_2SO_4	1	0	Brown	Terpenoid
	2	60	Purple-green	Terpenoid
	3	92.5	Blackish	Terpenoid
Sitroborat	1	0	Weak yellow fluorescence	Flavonoid
	2	45	Yellow fluorescence	Flavonoid
	3	55	Yellow fluorescence	Flavonoid
$FeCl_3$	1	0	Blackish	Phenolic
	2	55	Blackish	Phenolic
Dragendorff	1	0	Brown	Alkaloid

Table 5: The separation of 70% ethanolic extract of *T. indica* L fruit rind

Detection	No	hRf	Description of color	Chemical compound
UV_{254}	1	0	Strong quenching	
	2	75	Weak quenching	
	3	95	Weak quenching	
UV_{366}	1	0	Weak yellow fluorescence	Flavonoid
	2	37.5	Blue yellowish fluorescence	Flavonoid
	3	62.5	Yellow fluorescence	Flavonoid
	4	90	Blue yellowish fluorescence	Flavonoid
Vanilin H_2SO_4	1	0	Blackish brown	Terpenoid
	2	75	Purple-green	Terpenoid
	3	95	Blackish	Terpenoid
Sitroborat	1	0	Weak yellow fluorescence	Flavonoid
	2	37.5	Blue yellowish fluorescence	Flavonoid
	3	62.5	Yellow fluorescence	Flavonoid
	4	90	Blue yellowish fluorescence	Flavonoid
$FeCl_3$	1	0	Blackish	Phenolic (Tanin)
Dragendorff	1	0	Brown	Alkaloid

Discussion

Hyperlipidemia is one of the major contributors to coronary heart disease (CHD) and other cardiovascular diseases.[30] The extract, which has a lipid-lowering effect, is expected to reduce the risk of CHD and other cardiovascular diseases. This study used Triton X-100 to induce hyperlipidemia. The possible mechanism is Triton X-100 blocks TGs-rich lipoproteins from causing acute hyperlipidemia.[31] Data in this study show that the 70% ethanolic extract of *T. indica* L fruit rind and flesh was able to lower plasma cholesterol and triglyceride in rats induced by Triton X-100. This study is in line with the previous research. Martinello et al.[9] found that the pulp of fruit of *T. indica* L is able to reduce the levels of total cholesterol (50%), non-HDL cholesterol (73%), and triglycerides (60%) but increases HDL 60% on hamster. Research by Jindal et al.[32] stated the extract of tamarind pulp can decrease total cholesterol and triglycerides significantly in Wistar rats.[32]

Aqueous extract of *T. indica* L was able to decrease levels of total cholesterol, LDL, and triglycerides. It also reduced body weight of obesity-induced Sprague–Dawley rats.[33] Research by Koyagura et al.[34] showed that extracts of *T. indica* L decrease levels of total cholesterol, LDL, very low-density lipoprotein, triglycerides, glucose, and increase HDL. Studies in humans indicate that administration of *T. indica* L fruit (dried and pulverized pulp) with a dose of 15 mg/kgbw lowered the total cholesterol and LDL cholesterol significantly.[35] Research by Yerima et al.,[36] showed that extract of *T. indica* L has effect on hypolipidemia and hypoglycemia.

This result is slightly different from that reported in the study by Ukwuani et al.,[37] which found that the aqueous extract of pulp of *T. indica* L decreases levels of total cholesterol and LDL but increases those of HDL and triglycerides.[37]

Chemical constituents in 70% ethanolic extract of *T. indica* L fruit rind and flesh in this study were flavonoids, alkaloids, terpenoids, and phenolic. Several other studies mentioned *T. indica* L contains chemical constituents such as malic acid,[18] tartaric acid, mucilage and pectin, arabinose, xylose, galactose, glucose, and uronic acid,[38–39] and phenolic compound and cardiac glycosides.[40] Research by Razali et al.,[41] showed that *T. indica* L contains many flavonoids. The leaves of *T. indica* L contain many polyphenols.[42] Research by Yerima et al.[36] stated that the chemical constituents of *T. indica* L among others are carbohydrates, glycosides, saponins, flavonoids, tannins, alkaloids, and triterpenes.

Hypolipidemic mechanism of this extract is suspected by its polyphenols (flavonoids, limonene, etc.)[34] and the presence of the antioxidant effect of the extract.[9,15] The content of phenolic in *T indica* L is a contributor to the antioxidant effects of this plant.[7] Polyphenols in *tamarindus* are dominated by proanthocyanidins groups such as procyanidin B2, apigenin, catechin, epicatechin, procyanidin dimers, procyanidin trimers, eriodictyol, taxifolin, and naringenin.[43] Polyphenols are a group of antioxidants that are most abundant in plant metabolites and are an integral part of both human and animal diets including the simple phenolic molecules.[44] Hypocholesterolemic and antioxidant effects, presumably through an increase in Apo A1, ABCG5, and LDL receptor gene expression in liver, decrease in HMG CoA reductase and inhibit *MTP* gene expression. Flavonoids also trigger an increase in the excretion of cholesterol but decrease in the biosynthesis of cholesterol, increase in intake LDL cholesterol from peripheral tissues. They prevent the accumulation of TG in the liver. Treatment by *T. indica* L fruit pulp in hypercholesterolemic hamsters can protect oxidative damage by increasing hepatic antioxidant enzymes, preventing hepatic lipid peroxidation, and increasing antioxidant activity.[45]

Conclusion

The 70% ethanolic extract of *Tamarindus indica* L. fruit flesh and rind can reduce total cholesterol and triglycerides in hyperlipidemia induced by Triton X-100 in rats. The chemical compounds of these extracts are flavonoids, terpenoid, phenolic (tannins), and alkaloids.

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