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Effects of an aqueous extract of *Triticum repens* on lipid metabolism in normal and recent-onset diabetic rats

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Abstract

The aim of this study was to demonstrate the effects of single and repeated oral administration of the aqueous rhizomes extract of *Triticum repens* (TR) (20 mg/kg) on lipid metabolism in normal and streptozotocin-induced diabetic rats. In normal rats, the aqueous extract of TR induced a significant decrease in the plasma triglycerides concentrations 4 days ($P < 0.05$) and 1 week after repeated oral administration ($P < 0.05$). This reduction was abolished 2 weeks after once daily repeated oral administration. A significant decrease of plasma cholesterol levels was observed only 1 week ($P < 0.05$) after repeated oral administration.

In diabetic rats, TR treatment caused a significant decrease in plasma triglycerides levels after a single ($P < 0.01$) and repeated ($P < 0.001$) oral administration. A strong decrease in cholesterol level was observed 6 h after a single oral administration of the aqueous extract TR ($P < 0.001$). Four days after repeated oral administration of TR aqueous extract, the plasma cholesterol level was significantly decreased ($P < 0.05$) and still dropped after 2 weeks ($P < 0.001$).

On other hand, the repeated oral administration of aqueous TR extract caused a significant decrease in body weight 2 weeks after repeated oral treatment in diabetic rats ($P < 0.05$).

We conclude that the aqueous extract of TR exhibits lipid and body weight lowering activities in severe hyperglycaemic rats after repeated oral administration of aqueous TR extract at a dose of 20 mg/kg.

Keywords: *Triticum repens*; Cholesterol; Triglycerides; Body weight; Aqueous extract and diabetic rats

1. Introduction

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 cardiovascular diseases in diabetic patients (Jensen et al., 1988; Motta et al., 2001). Diabetes mellitus is a disease with profound effects on lipid metabolism. Insulin affects lipid metabolism, e.g. by inhibition of the activity of lipoprotein lipase, therefore it decreases the mobilization of free acids from peripheral fat depots. On other hand, it stimulates the synthesis of fatty acids in the liver, adipose tissue and intestine. The diabetogenic effect of streptozotocin (STZ) is the result of irreversible damage to pancreatic β cells. The STZ-induced diabetic animal is thus considered as an animal model of hyperlipidemia (Burcelin et al., 1995).

Triticum repens P. Beauv. (TR), locally named as "N'jm L'bouri or Outara" is a spontaneous plant belonging to the Gramin family. By reviewing the current literature, we know of no previous research on the pharmacological properties of this plant. However, according to a recent ethnobotanical survey in south-eastern region of Morocco (Tafilalet), TR is prescribed by traditional healers for diabetes control (Eddouks et al., 2003).

The present study was undertaken to evaluate the potential cholesterol and triglycerides lowering activity of a single and repeated oral administration of the aqueous TR extract in normal and STZ-induced diabetic rats. The effect of TR extract on body weight loss was also monitored. Sodium-metavanadate (0.8 mg/kg) was used as a reference drug known by its both hypolipidemic and hypoglycaemic activities.

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2. Material and methods

2.1. Plant material

The plant used in this study was collected from its natural habitat, from Tafilalt region (Morocco) in May 2002, and dried under hot air (40–60 °C). The plant was identified and authenticated as *Triticum repens* with assistance of Prof. M. Rejdali (Veterinary and Agronomy Institute, Rabat, Morocco). Voucher specimen (EM 3a) was deposited at the herbarium of the Faculty of Sciences and Techniques Errachidia.

2.2. Preparation of the aqueous extract

An aqueous extract was prepared according to the traditional method used in Morocco: 1 g of the dried powdered rhizomes of TR were boiled in 100 ml of distilled water for 10 min and cooled to room temperature for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm, St. Quentin en Yvelines, France) to remove particulate matter. The filtrate was lyophilized and the desired dose (milligrams of lyophilized aqueous extract of TR per kilogram body weight) was then prepared and reconstituted in 1.5 ml of distilled water. The aqueous extracts were prepared daily, just before administration. The extract yield was 14%. Animals were treated orally, i.e. intubation using a syringe at an overnight fasted state.

2.3. Animals used

Experiments were performed in adult male Wistar rats weighing from 200 to 230 g. The animals were housed under standard environmental conditions (23 ± 1 °C, with 55 ± 5% humidity and a 12-h light/dark cycle) and maintained with free access to water and ad libitum standard laboratory diet (70% carbohydrates, 25% proteins, 5% lipids).

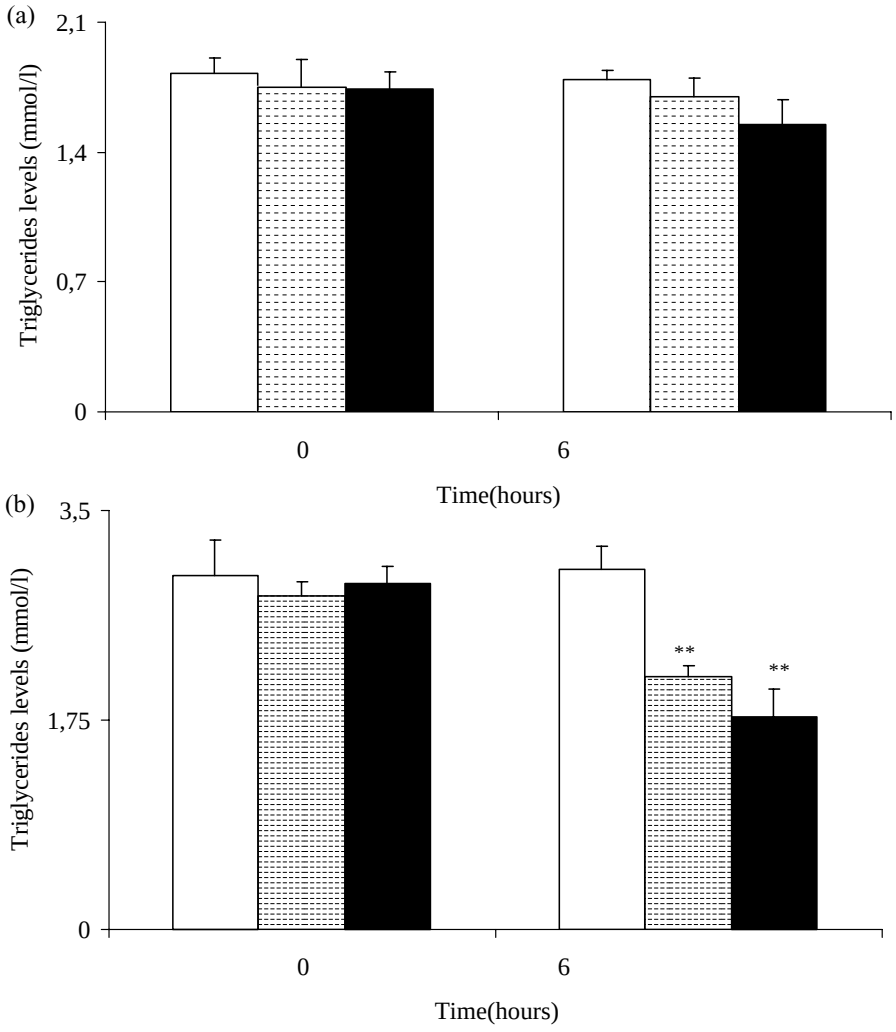

Fig. 1. Plasma triglycerides levels (mmol/l) after a single oral administration of TR aqueous extract (20 mg/kg) in normal (Panel a) and STZ (Panel b) rats. Data are expressed as means ± S.E.M., $n = 6$ rats per group. $^{**}P < 0.01$ when compared to baseline values (the start of treatment). (□) control group; (▤) TR-treated group; (■) vanadate-treated group.

2.4. Induction of diabetes

Streptozotocin (Sigma, St. Louis, MO, USA) was dissolved in 0.1 M fresh cold citrate buffer at pH 4.5 before use, and injected intravenously into the tail vein at a dose of 65 mg/kg (Burcelin et al., 1995). After 18 h, the rats with stable fasting blood glucose levels greater than 16 mmol/l were considered as diabetic and used in the present study. The percentage of response to streptozotocin injection was 90%.

2.5. 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 treated group received the aqueous extract of TR at a dose of 20 mg/kg (20 mg of lyophilized aqueous extract of TR per kilogram body weight) 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 (0.8 mg/kg) or the aqueous extract (20 mg/kg) was administered and plasma cholesterol and triglycerides levels were measured before and 6 h after TR treatment.

2.6. Repeated oral administration

For repeated oral administration, rats were treated once daily at a dose of 20 mg/kg for 2 weeks and plasma cholesterol and triglycerides levels were followed during this period. The number of rats was six in each group ($n = 6$). Blood samples were collected from the tail vein and plasma triglycerides and cholesterol levels are determined enzymatically by colorimetric technique using specific kits (Randox, UK), respectively. The kits used in this study for substrates analysis were specific for both human and rat blood samples at the same percentage.

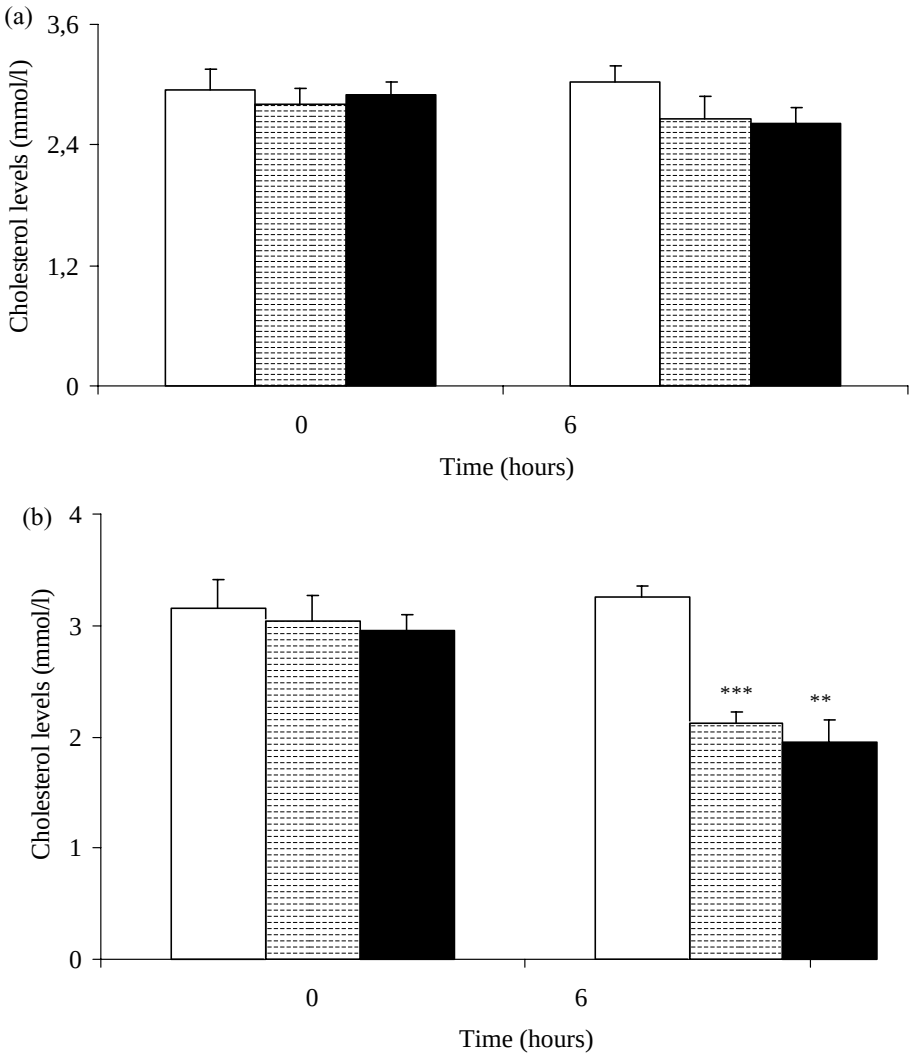

Fig. 2. Plasma cholesterol levels (mmol/l) after a single oral administration of TR aqueous extract (20 mg/kg) in normal (Panel a) and STZ (Panel b) rats. Data are expressed as means ± S.E.M., $n = 6$ rats per group. $^{**}P < 0.01$; $^{***}P < 0.001$ when compared to baseline values (the start of treatment). (□) control group; (▨) TR-treated group; (■) vanadate-treated group.

2.7. Statistical analysis

Data were expressed as mean ± S.E.M. The statistical analysis was performed by the Student's *t* test. The values were considered significantly different when the *P* value was less than 0.05 in comparison to baseline values (starting values).

3. Results

3.1. Single oral administration

In normal rats, no significant changes of both plasma cholesterol and triglycerides concentrations after a single administration of TR (20 mg/kg) were noted (Figs. 1a and 2a). In vanadate-treated group, the plasma cholesterol and triglycerides concentrations were significantly decreased over 6 h of a single oral dose of vanadate ($P < 0.01$) (Figs. 1a and 2a).

In STZ rats, the aqueous extract of TR caused a significant reduction of plasma triglycerides ($P < 0.01$) and cholesterol ($P < 0.001$) levels 6 h after TR treatment (Figs. 1b and 2b). Vanadate (0.8 mg/kg) reduced both the plasma cholesterol ($P < 0.01$) and triglycerides levels ($P < 0.01$) 6 h after TR administration in diabetic rats (Figs. 1b and 2b).

3.2. Repeated oral administration

In normal rats, the aqueous extract of TR induced a significant decrease of the plasma triglycerides concentrations 4 days ($P < 0.05$) and 1 week after repeated oral adminis-

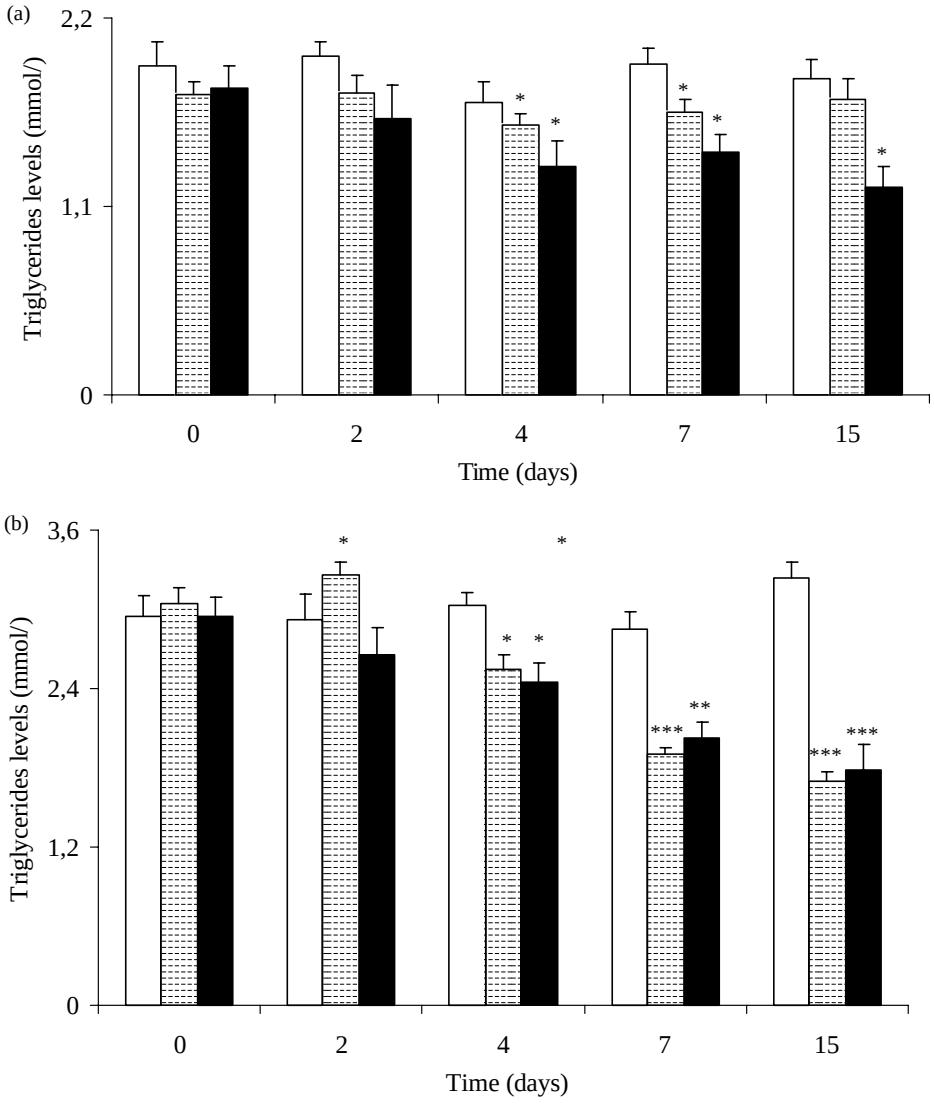

Fig. 3. Plasma triglycerides levels (mmol/l) after a repeated oral administration of TR aqueous extract (20 mg/kg) in normal (Panel a) and STZ (Panel b) rats. Data are expressed as means ± S.E.M., $n = 6$ rats per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ when compared to baseline values (the start of treatment). (□) control group; (▤) TR-treated group; (■) vanadate-treated group.

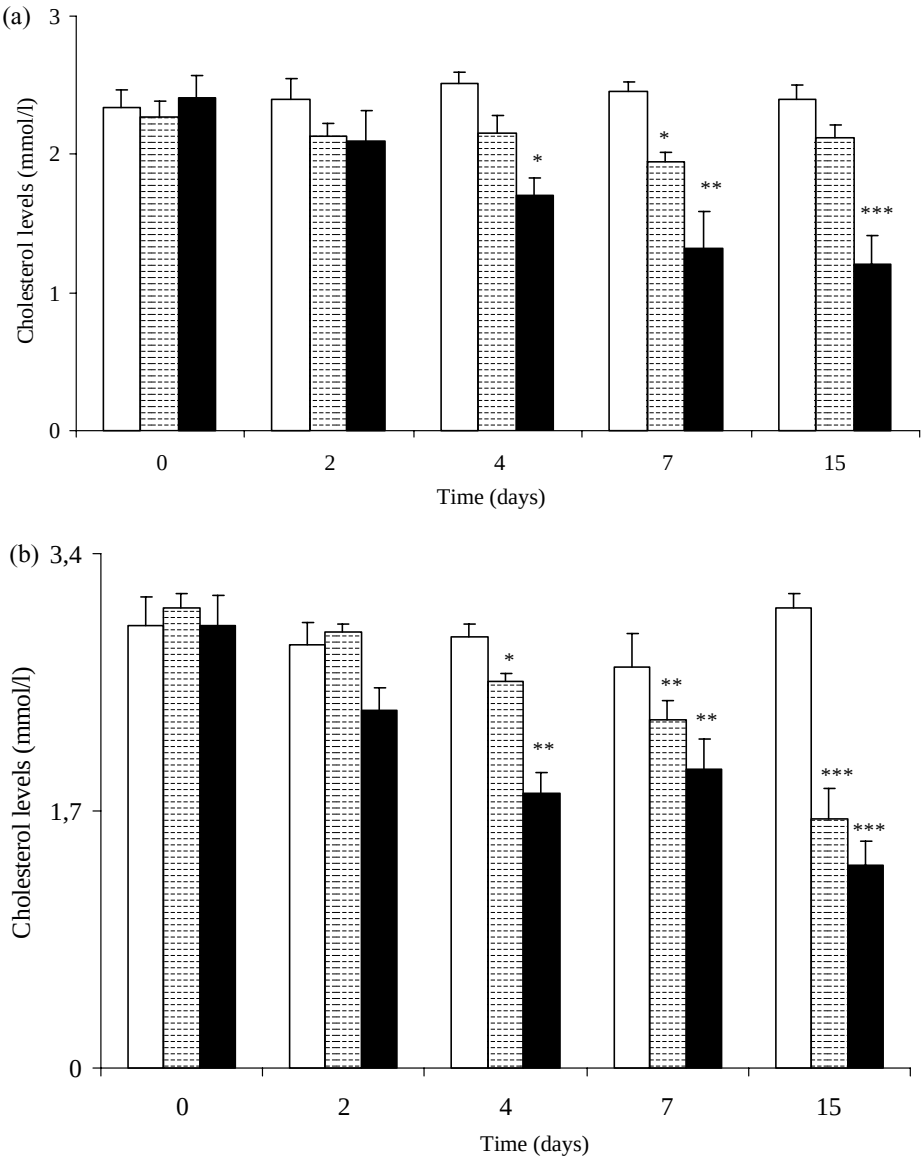

Fig. 4. Plasma cholesterol levels (mmol/l) after a repeated oral administration of TR aqueous extract (20 mg/kg) in normal (Panel a) and STZ (Panel b) rats. Data are expressed as means ± S.E.M., $n = 6$ rats per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ when compared to baseline values (the start of treatment). (□) control group; (▤) TR-treated group; (■) vanadate-treated group.

tration ($P < 0.05$) (Fig. 3a). This reduction was abolished 2 weeks after once daily repeated oral administration. A significant decrease of plasma cholesterol levels was observed only 1 week ($P < 0.05$) after repeated oral administration (Fig. 4a). After 2 weeks of treatment, vanadate caused a significant decrease of both plasma triglycerides ($P < 0.05$) (Fig. 3a) and cholesterol levels ($P < 0.001$) (Fig. 4a).

In diabetic rats, TR extract decreased significantly the plasma cholesterol levels from the fourth day ($P < 0.05$); the most significant effect was reached from the first to the second week of treatment ($P < 0.001$) (Fig. 3b). The plasma cholesterol levels were decreased from the fourth day ($P < 0.05$) to the first ($P < 0.01$) and second week ($P < 0.001$) after TR treatment (Fig. 4b). Daily vanadate administration (0.8 mg/kg) for 2 weeks produced a statistically significant decrease in both plasma cholesterol and triglycerides concentrations ($P < 0.001$) (Figs. 3b and 4b).

3.3. Body weight loss

In normal rats, the TR extract caused a slight increase of body weight 2 weeks after daily oral administration of TR (20 mg/kg) ($P < 0.01$) (Fig. 5a). A significant decrease in body weight was observed in distilled water treated group from the second day of STZ injection. TR aqueous extract (20 mg/kg) caused a significant weight loss 2 weeks after once daily repeated oral treatment ($P < 0.01$) in STZ rats (Fig. 5b). Vanadate caused also a significant decrease in body weight in both normal and STZ rats after repeated oral administration ($P < 0.001$) (Fig. 5).

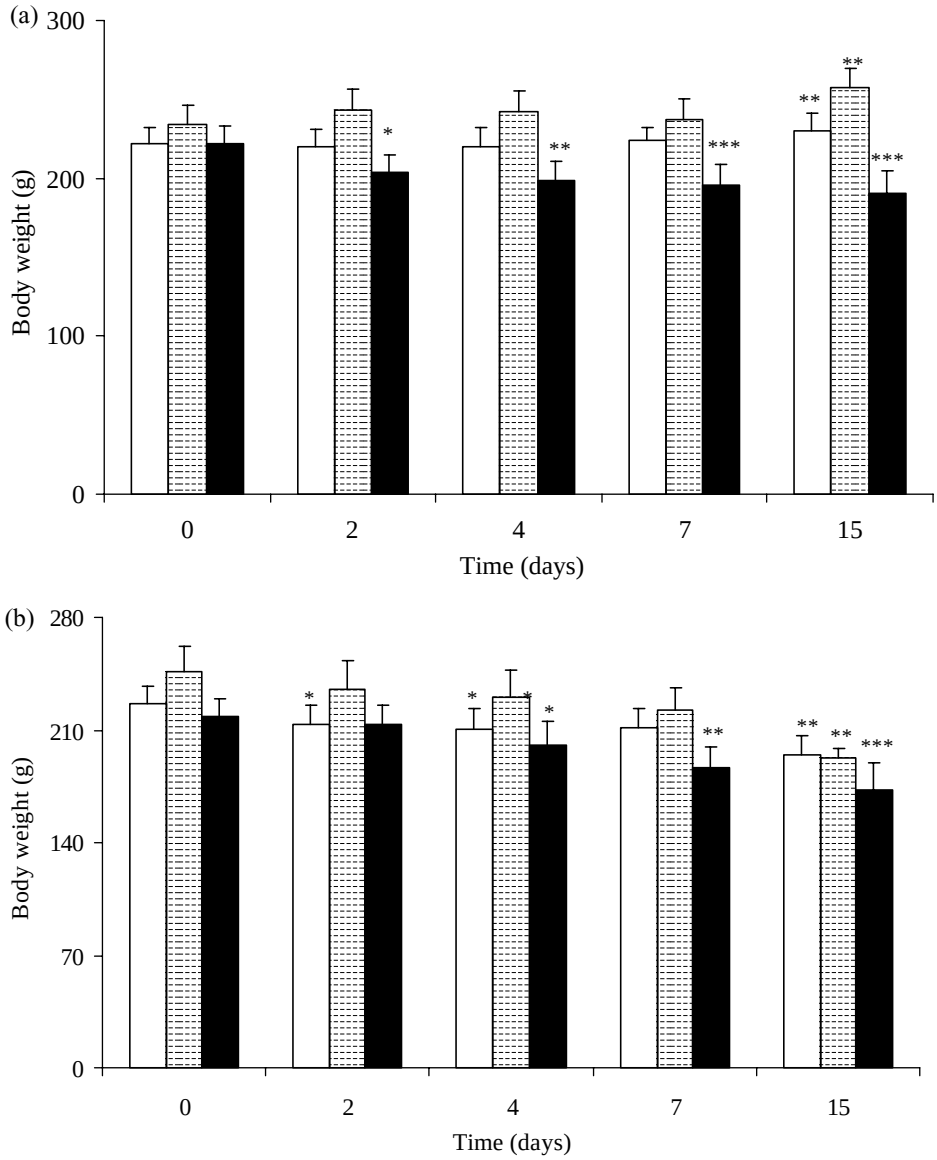

Fig. 5. Effect of TR aqueous extract treatment (20 mg/kg) on body weight (g) in normal and diabetic rats. Data are expressed as means ± S.E.M., $n = 6$ rats per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ when compared to baseline values (the start of treatment). (□) control group; (▨) TR-treated group; (■) vanadate-treated group.

4. Discussion

In the present study, the effect of aqueous TR extract on plasma cholesterol and triglycerides concentrations was evaluated in the normal and STZ-induced diabetic rats, a model of type 1 diabetes mellitus. Until now, TR remains unknown as a medicinal plant. We have used the rhizomes of this plant based on an ethnobotanical information in Tafilalet region (Eddouks et al., 2003). According to this survey, TR was largely used in diabetes phytotherapy and control. Tafilalet region is considered as a great reserve of medicinal plants in which the phytotherapy knowledge is very developed (Eddouks et al., 2002). Diabetes is associated with hypertriglyceridaemia (Rodrigues et al., 1986) which is due to increase in adipose tissue lipolysis in absence of insulin, a decrease in lipoprotein lipase activity and reduced carnitine levels. In diabetic state due to absence of insulin sensitivity (Burcelin et al., 1995), there is mobilization of fatty acids from adipose tissues and proteins leading to increase in free fatty acids. In addition, it has been well documented that rats treated with STZ had increased plasma cholesterol and triglycerides levels (Shah et al., 1995).

Vanadate was used as a reference drug because it has been reported to be a potent insulin mimetic agent in many cells (DeFronzo, 1988; Reaven, 1988). Administration of this compound to diabetic animals normalized blood glucose concentration and reduced the triglycerides levels (Kashiwagi et al., 1983; Brichard and Henquin, 1995).

The results demonstrated that aqueous extract of TR induced a significant decrease of plasma cholesterol and

triglycerides levels in STZ-diabetic rats for both short term (single) and long term (repeated) administrations. However, in normal rats, only the long term treatment had caused a significant drop of lipidic parameters. Vanadate treatment caused a significant decrease of both plasma cholesterol and triglycerides levels in normal and STZ rats after short term as well as long term treatment. The plasma triglycerides levels were initially increased in STZ rats because the lipolysis was stimulated by concomitant insulinopenic state. Recent-onset insulinopenia in STZ-diabetic rats is associated with lipid overproduction in the basal (hyperglycaemic) state (Burcelin et al., 1995).

Some studies have reported a similar lipid lowering activity of some medicinal plants (Ram et al., 1997; Sharma et al., 1997). Parallely we have previously reported a hypocholesterolaemic activity of *Spergularia purpurea* in normal and STZ rats (Jouad et al., 2003).

The underlying mechanism of the lipid lowering activity of TR could be the inhibition of lipid absorption due to the presence of saponins and tannins in the aqueous extract (Dwivedi and Agarwal, 1994; Vaidya, 1994; Ram et al., 1997) and/or inhibition of cholesterol-esterase, activation of fatty acids synthase, acetyl-CoA carboxylase and production of triglycerides precursors such as acetyl-CoA and glycerol phosphate.

The TR aqueous extract caused a weight loss in STZ rats. This effect could be explained directly by the lipid lowering activity of the extract and/or its influence of rat appetite (Trejo-Gonzàlez et al., 1996) or indirectly by influencing various lipidic regulation systems.

Other advanced toxicological investigations are required to precise eventual TR toxicity in different organs and tissues. After such investigations, aqueous TR extract could be used in human healthcare system, especially in the treatment of hypercholesterolaemia associated with diabetes, obesity and cardiovascular diseases.

We conclude that aqueous extract of TR exhibited long term cholesterol and triglycerides lowering activities in both normal and STZ-diabetic rats and confirms its use in Moroccan phytomedicine. This activity is concomitant with body weight loss in type 1 diabetes mellitus. In this study, the period of plant collection, the dose and duration of TR treatment were respected according to Moroccan traditional usage. Further experiments dealing with the effect of seasonal as well as geographical variability of the plant material are still to be performed. Additionally, precise molecular mechanism and active substance(s) need to be determined in further experiments. Such active principle(s) could be precious in obesity, atherosclerosis and cardiac diseases therapy and control.

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