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Full Length Research Paper

Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats

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The present study was carried out to investigate the antidiabetic properties of aqueous leaves extract of *Leonotis leonurus* in streptozotocin (45 mg/kg intraperitoneal) induced diabetic rats for 15 days. The induced diabetic rats exhibited high blood glucose level, cholesterol, high density lipoprotein (HDL) and triglycerides accompanied with weight loss while the level of low density lipoprotein (LDL) was very low. In addition, the water intake was remarkably high while feed intake was decreased as compared with normal control group. The continuous oral administration of the extract at the dose of 125, 250 and 500 mg/kg for 15 days was able to lower the blood glucose level, HDL, feed and water intake while that of LDL was increased. Also, the weight loss of diabetic rats (31 g) after extract treatment was near that of glibenclamide treated groups. The extract yielded high phenolics content (48 mg/g tannic acid equivalent) and flavonoids (4.8 mg/g quercetin equivalent). These compounds have been reported to potentiate insulin secretion. The present study revealed that aqueous extract of *L. leonurus* possesses antihyperglycemia and antilipidemic potential and thus could support ethnotherapeutic usage of this plant.

Key words: *Leonotis leonurus*, lipids, blood glucose, phenolics, flavonoids.

INTRODUCTION

Diabetes mellitus is a complex chronic disorder that affects the metabolism of carbohydrates, fats, proteins and electrolytes due to deficiency of insulin or insensitivity of target organs to insulin. This disorder is characterized by chronic hyperglycemia and abnormality of lipid profile such as cholesterol, low and high density lipoprotein and triglyceride leading to series of secondary complications (Rang et al., 1991; Ravi et al., 2005). These complications include polyuria, polyphagia, polydypsia, ketosis, retinopathy as well as cardiovascular disorders (Kumar and Clark, 2002). Presently, the frequency of diabetes is increasing with a major impact on the population of developing countries due to the absence of effective and affordable interventions of diabetes (Marx, 2002). According to Wild et al. (2004), about 366 million peoples were projected to be diabetic by the year 2030. Several hypoglycemic agents have been used for the treatment of diabetes mellitus but are reported to produce serious adverse side effect such as liver problems, lactic acidosis and diarrhea (Rajalakshmi et al., 2009). In addition, they are not suitable for use during pregnancy. Therefore, the search for more effective agents with low cost and low side effect from plant source has continued to be an important area of research because of their ready availability, affordability and low adverse side effect.

In South Africa, *L. leonurus* (Lamiaceae) is a shrub indigenous plant found along forest margins, on rocky hillsides and riverbanks as well as grasslands of the Eastern and Western Cape, Kwazulu-Natal and Mpumalanga Provinces of South Africa (Van Wyk et al., 2000).

The leaves of the plant have a characteristic aromatic-pungent odour, bright yellow- green colour and rough texture. It has long been used traditionally for the

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treatment of cough, cold, influenza, chest infections, diabetes mellitus, eczema, epilepsy, delayed menstruation; intestinal worms, constipation, scorpion stings, spider and snake bite (Van Wyk et al. 2000; Ososki et al., 2002; Jager et al., 1996). Our previous work reported the toxicological effect of sub-chronic administration of *L. leonurus* in male Wistar rats (Oyedemi et al., 2010b). Similarly, we also assessed *in vitro* and *in vivo* antioxidant activities of this plant (unpublished). Evidences from traditional healers have shown that *L. leonurus* may have antidiabetic activity but have not been proved scientifically (Oyedemi et al., 2009).

Before the commencement of this study, there was scanty information on the antidiabetic effect of this plant in male Wistar rats induced with streptozotocin. Therefore, the objective of this study was aimed to investigate the antidiabetic and antilipidemic potential of aqueous leaves extract of *L. leonurus* in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant material

The leaves of *L. leonurus* were collected from rockhill field near Ntselamanzi location in Nkokonbe Municipality (Eastern Cape, South Africa) between May and June, 2008. It was authenticated by Prof. DS. Grierson of the Department of Botany, University of Fort Hare. Voucher specimen (Sun MED 2) was deposited at the Giffen herbarium of the University.

Animals used

Male Wistar rats (*Rattus novergicus*) with average weight of 250.00 ± 7.22 g were obtained from the animal house of the Agricultural and Rural Development Research Institute, University of Fort Hare. The animals were maintained at a controlled temperature of 28°C with a 12 h light-dark cycle at room temperature and humidity of 45 to 50%. The animals were allowed free access to food (Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and water for 15 days. The experiment was approved by the Animal Ethics Committee of the University of Fort Hare.

Assay kits and reagents

The assay kits for triglycerides, cholesterol and high and low density lipoproteins were obtained from Roche Diagnostic GmbH, Mannheim, Germany. All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Induction of experimental diabetes in animals

Streptozotocin was freshly prepared in 10 mmol/citrate buffers, pH 4.5, and injected to experimental animals (25 rats) intraperitoneally at the dosages of 125, 250 and 500 mg/kg body weight (Siddique et al., 1987). After 48 h of STZ administration, rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose > 8.1 mmol/L) were taken for the experiment.

Animal grouping and extract administration

Thirty-six male rats were randomized into six groups of six animals each (30 diabetic surviving rats, 6 normal rats) were used in this study. The extract was administered orally into the rats using gavages throughout the experimental period. Group 1: Diabetic rats received distilled water only (0.5 ml) on daily basis repeatedly for 15 days. Groups 2: Diabetic animals treated daily with 0.5 ml of glibenclamide (0.6 g/kg body weight). Group 3 to 5 animals were treated daily with 0.5 ml doses of 125, 250 and 500 mg/kg body weight of aqueous leaves extract of *L. leonurus* respectively. The blood samples were collected every fifth day from the tail vein of the animals to determine the blood glucose level using glucometer (Bayer Health Care, Japan). On day 16, the rats were sacrificed by ether anesthesia.

Preparation of extract

The leaves of the plant were air dried at room temperature for 7 days. The dried leaves were thereafter pulverized using an electric blender (Waring Products Division, Torrington, USA). The powdered plant material (200 g) was extracted in distilled water on a mechanical shaker (Stuart Scientific Orbital Shaker, UK) for 48 h. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was freeze-dried using Savant Refrigerated Vapor Trap (RV T41404, USA) to give a yield of 30 g. The resulting extract was reconstituted with distilled water to give the required doses (125, 250 and 500 mg/kg body weight) used in this study.

Preliminary phytochemical screening

The aqueous extract of *Strychnos henningsii* was tested by subjecting the extract to phytochemical analysis to determine the presence of phenols, flavonoids, alkaloids, saponin, glycoside and tannins using the general chemical test of Zafar and Mujeeb (2002).

Total phenolics

The total phenolics content in the aqueous leaf extract of *L. leonurus* was determined spectrophotometrically with Folin Ciocalteau reagent using the modified method of Wolfe et al. (2003). An aliquot of the extract (0.5 ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (75% w/v). The resulting mixture was vortexed for 15 s and incubated at 40°C for 30 min for colour development. The absorbance of the samples was measured at 765 nm using Hewlett Packard, UV spectrophotometer. Total phenolics content was expressed as mg/g tannic acid equivalent from the calibration curve using the equation: $Y = 0.1216x$, $R^2 = 0.936512$, where x was the absorbance and Y was the tannic acid equivalent (mg/g). The experiment was conducted in triplicate and the results are reported as mean ± SD values.

Total flavonoids

The method of Ordonez et al. (2006) was used to estimate total flavonoids contents of the extract solution based on the formation of a complex flavonoids - aluminums. A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of extract solution. After one hour of incubation at the room temperature, the absorbance was measured at 420 nm using UV-VIS spectrophotometer. A yellow colour indicated the presence of flavonoids at the final concentration of 0.5 mg/ml. All determinations were done in triplicate and values were calculated from calibration curve obtained

from quercetin using the equations: Y= 0.0255x, R^2 = 0.9812, where x was the absorbance and Y the quercetin equivalent (mg/g). The experiment was conducted in triplicate and the results are reported as mean ± SD values.

Total proanthocyanidins

Total proanthocyanidins was determined based on the procedure of Sun et al. (1998). To 0.5 ml of 1 mg/ml extract solution was added 3 ml of vanillin-methanol (4% v/v), and 1.5 ml of hydrochloric acid and then vortexed. The absorbance of resulting mixture was measured at 500 nm after 15 min at room temperature. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the following equation from the calibration curve: Y = 0.5825x, R^2 = 0.9277, where x was the absorbance and Y is the catechin equivalent (mg/g).

Preparation of serum

The preparation of serum was carried out using the method described by Yakubu et al. (2005). The blood samples were collected into clean dry centrifuge tubes. An aliquot (5 ml) of the blood was collected into sample bottles containing EDTA (BD Diagnostics, Pre-analytical Systems, Midrand, USA) and was allowed to clot at room temperature for 10 min. This was centrifuged at 1282 g × 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay of lipid profiles.

Serum lipids analysis

The levels of low density lipoprotein, high density lipoproteins, triacylglycerol and cholesterol in the serum of the animals were determined using the method of Tietz et al. (1994). They were determined spectrophotometrically using assay kits from Randox Laboratories Limited, Ardmore, Co Antrim, UK.

Effect of extract on the weight, feed and water intake of the animals

Feed and water intake were measured everyday at the same hour during the experimental periods while the body weight of the animals were measured before the start and every fifth day throughout the experimental period (15 days).

Statistical analysis

Data were expressed as mean ± SD (standard deviation) of six replicates and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by the Duncan multiple test using SAS (SAS, 2002). Values were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The phytochemical screening showed the presence of flavonoids, tannins, phenolics and saponins (Table 1). These compounds especially flavonoids and phenolics have been reported to enhance insulin secretion and scavenge free radicals that are generated during diabetic state (Marles and Farnsworth, 1995). The results of quantitative analysis of polyphenolics compounds investigated in this study revealed the high phenolics and flavonoids in the aqueous leaves extract of *L. leonurus* phenolics contents (48 mg/g tannic acid equivalent) and flavonoids (4.8 mg/g quercetin equivalent) as shown in Table 1. Flavonoids are well known to regenerate the damaged beta cells in the diabetic rats while phenolics are found to be effective antihyperglycemic agents (Chakravarthy et al., 1980; Manickam et al., 1997).

The intraperitoneal injection of streptozotocin at the dose of 45 mg/kg into rats was characterized by polydipsia, polyuria, weight loss and hyperglycemia. These symptoms agree with the previous findings of Shenoy and Ramesh (2002). The elevated level of blood glucose observed after 48 h of streptozotocin induction confirmed the diabetic state in rats which may be attributed to the selective cytotoxicity effect of streptozotocin on the beta cells (Bedoya et al., 1996). The continued treatment of diabetic rats for 15 days with the plant extract caused a significant reduction of blood glucose level (10.5 to 14 mmol/L) comparable to glibenclamide which is used for the treatment of type II diabetes (Table 2). Glibenclamide is a standard hypoglycemic drug that stimulates insulin secretion from beta cells of islet of Langerhans. The result obtained from this study was in accordance with that of Ojewole et al. (2005) who observed antihyperglycemic effect of this plant in mice. The glucose lowering activity of plant extract was compared with that of glibenclamide. The possible mechanism though not investigated in this study may be attributed to the ability of the extract to potentiate insulin secretion from pancreatic beta cells or sensitizing insulin receptors (Ratnasooriya et al., 2004). The presence of flavonoids and phenolics compounds in the extract may be responsible for this observation.

The serum cholesterol level in diabetic untreated rats increased significantly ($p< 0.05$) above the normal rats throughout the experimental period (Table 3). The abnormal high concentration of serum lipids in the diabetic rats induced by STZ was in agreement with the findings of Nikkila and Kekki (1973). Similar observation was made by Bopanna et al. (1997) who linked the rise in serum lipid to increase mobilization of free fatty acids from the peripheral fat depots, where free fatty acid esterification is balanced in lipolysis cycle. In addition, deficiency of insulin had been reported to be associated with hypercholesterolemia and hypertriglyceridemia due to inactivation of lipases to hydrolyze these lipids. In the present study, the continuous administration of aqueous leaves extract of *L. leonurus* and glibenclamide for 15 days reduced the level of cholesterol, triglyceride, HDL and rise in LDL at certain doses. This observation corroborates with several studies reported on antilipidemic effect of plant extract used traditionally in experimental diabetic animals (Daisy et al., 2009; Marles

Table 1. Phytochemical analysis of aqueous extract of *L. leonurus.*

Phytochemical compounds	Plant extract	Total content
Alkaloids	+	ND
Tannins	+	ND
Saponin	+	ND
Flavonoids	+	34.16 mg/g[A]
Cardiac glycosides	+	ND
Proanthocyanidins	+	25 mg/g[B]
Phenolics	+	220 mg/g[C]

+ = Presence. [A] Tannic acid equivalent. [B] Quercetin equivalent. [C] Catechin equivalent

Table 2. Effect of oral administration of *L. leonurus* extract on plasma blood glucose levels of STZ induced diabetic rats.

Treatment	Plasma blood glucose			
	0 (day)	5 (day)	10 (day)	15 (day)
Normal	5.60 ± 0.01	5.60 ± 0.01	5.42 ± 0.02	5.40 ± 0.00
Diabetic control	25.60 ± 0.24	26.30 ± 0.21	31.30 ± 0.16	33.30 ± 0.16
Diabetic + LL (125 mg/kg)	27.47 ± 0.02	20.43 ± 0.05	17.40 ± 0.06	16.97 ± 0. 06
Diabetic + LL (250 mg/kg)	28.50 ± 0.06	18.30 ± 0.04	15.30 ± 0.12	15.23 ± 0.00
Diabetic + LL (500 mg/kg)	27.83 ± 0.05	20.50 ± 0.07	16.30 ± 0.16	13.60 ± 0.04
Diabetic + glibenclamide	27.40 ± 0.02	25.95 ± 0.04	22.30 ± 0.11	18.03 ± 0.02

Values are expressed as means ± SD (n = 6 rats).

Table 3. Effect of aqueous extract of *L. leonurus* extract at doses investigated on serum lipid profiles in streptozotocin induced diabetic rats.

Serum lipids parameter	Normal control	Diabetes control	Do	D1	D2	D3
Cholesterol (mmol/L)	1.57 ± 0.12	2.33 ± 0.05	1.50 ± 0.16	1.55 ± 0.05	1.60 ± 0.0	1.67 ± 0.12
Triacylglycerol (mmol/L)	2.17 ± 0.34	3.53 ± 0.33	1.10 ± 0.57	1.60 ± 0.30	1.00 ± 0.00	1.60 ±0.22
HDL-C (mmol/L)	1.10 ± 0.08	0.47 ± 0.05	1.30 ± 0.22	1.35 ±0.05	1.30 ±0.00	1.37 ±0.05
LDL-C (mmol/L)	0.92 ± 0.05	3.24 ± 0.02	1.27 ± 0.20	1.30 ± 0.03	1.28 ± 0.01	1.32 ± 0.03
Atherogenic index (LDL-C/HDL-C)	0.84	6.90	0.97	0.96	0.98	0.96

Values are expressed as means ± SD (n= 6 rats). Do = diabetes+125 mg/kg extract; D1 = diabetes + 250 mg/kg extract; D2 = diabetes + 500 mg/kg extract; D3 = diabetes + glibenclamide (0.6 mg/kg). HDL-C High density lipoprotein-cholesterol. LDL-C =Low density lipoprotein-cholesterol.

and Farnsworth, 1995; Grover et al., 2002) (Figure 1).

The induction of diabetes into the rats resulted into loss of body weight between 20 to 31 g in comparison with the control rats. The feed and water intake of the diseased animals was significantly increased throughout the study period. These symptoms are well known marker of diabetes mellitus in both human and animal models which is a direct consequence of insulin deficiency (Shenoy and Ramesh, 2002). Oral administration of plant extract at the three doses investigated was able to improve the body weight of the animals. The extract at the dose of 500 mg/kg significantly decreased the level of feed and water intake comparable to glibenclamide treated group while the dose at 250 mg/kg did not have any significant effect. The result obtained in this study support the report of Kim et al. (2006) who reported the effect of Morus *alba* in controlling the desire for food and water intake under diabetic condition. The significant body weight gain observed in diabetic rats was nearly similar to the control group after oral administration of plant extract in a dose dependent manner. This result agrees with other investigators who noticed increase in body weight gain upon improvement of diabetes status (Schwechter et al., 2003; Craft and Failla, 1983). The

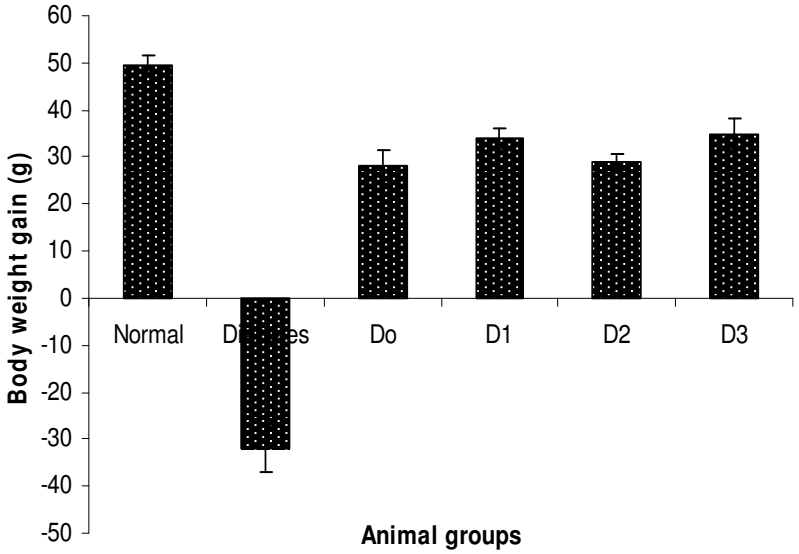

Figure 1. The effect of plant extract on body weight gain after 15 days of experimental periods. Values are expressed as means ± SD (n= 6 rats). Do = diabetes + 125 mg/kg extract; D1 = diabetes + 250 mg/kg extract; D2 = diabetes + 500 mg/kg extract; D3 = diabetes + glibenclamide (0.6 mg/kg).

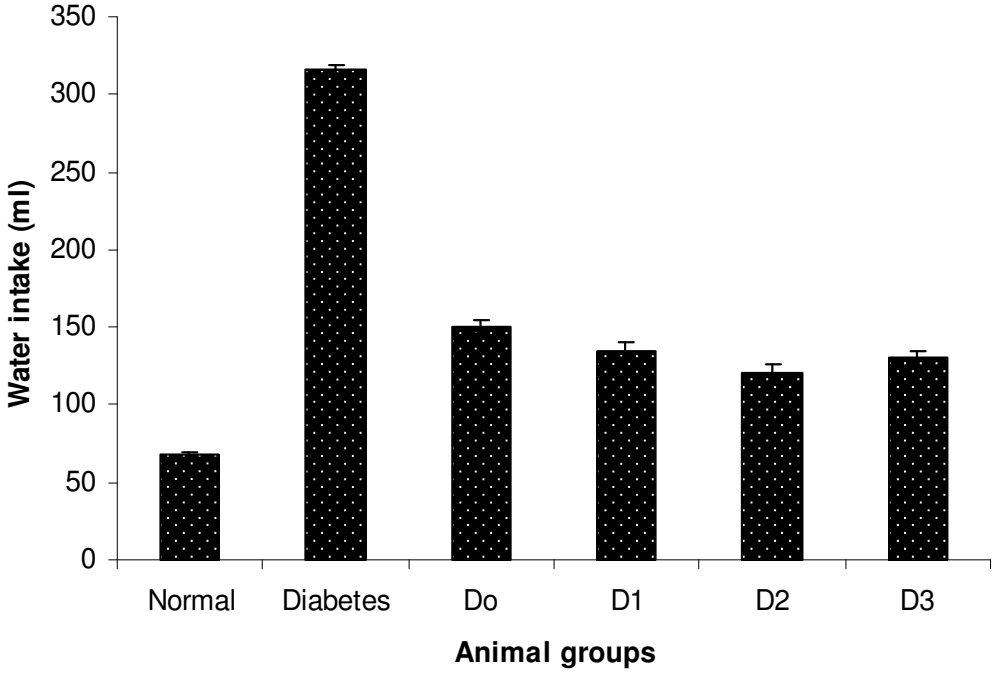

Figure 2. Effect of plant extract on water intake. Values are expressed as means ± SD (n = 6 rats). Do = diabetes+125 mg/kg extract; D1 = diabetes + 250 mg/kg extract; D2 = diabetes + 500 mg/kg extract; D3 = diabetes + glibenclamide (0.6 mg/kg).

mechanisms of action is unknown but suggest a protective effect of *L. leonurus* in controlling muscle wasting (Swanston-Flatt et al., 1990).

In conclusion, the present findings reveal that oral administration of aqueous extract of *L. leonurus* leaf has a beneficial effect in reducing the blood glucose levels as well as lipids. This study also showed that the plant extract improves the polydipsia, polyuria and body weight loss of diabetic rats. Further studies on the pharmacological and biochemical investigation to elucidate the mechanism of antidiabetic effect of this plant will be needed to justify its usage (Figures 2 and 3).

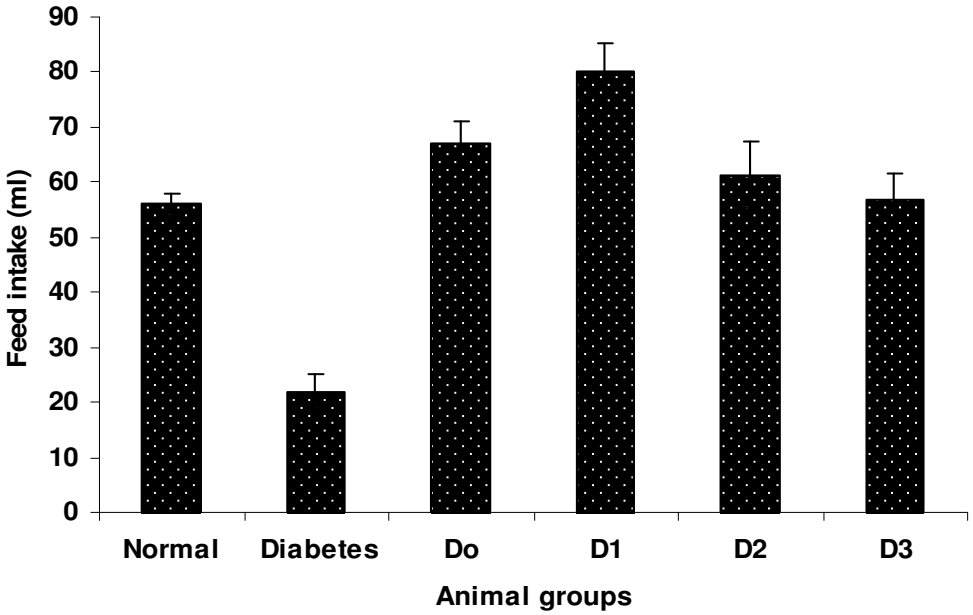

Figure 3. Effect of aqueous extract of *L. leonurus* on feed intake in STZ induced diabetic rats. Values are expressed as means ± SD (n = 6 rats). Do = diabetes + 125 mg/kg extract; D1 = diabetes + 250 mg/kg extract; D2 = diabetes+500 mg/kg extract; D3 = diabetes + glibenclamide (0.6 mg/kg)

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