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BLOOD BIOCHEMICAL PARAMETERS EFFECT OF SAHARA MYRTLE ON DIABETIC RATS

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ABSTRACT

Sahara myrtle is a Myrtaceae indigenous plant, widely found in central Sahara of Algeria, used for diabetes treatment. In the present study, the chemical content, hypoglycaemic and antioxidant potential of *Myrtus nivellei* decoction were evaluated by using *in vitro*, and *in vivo* methods. After the oral administration of decoction at doses of 100 mg/kg and 300 mg/kg, body weight, blood glucose levels and some biochemical parameters were monitored at specific intervals. Glibenclamide was used as a reference drug at a dose of 5 mg/kg. The experimental data indicated that the decoction of Sahara myrtle demonstrated significant antihyperglycaemic effect in alloxanic rats especially at a dose of 300 mg/kg which confirmed the folkloric utilization. Phenolic, total tannin and flavonoid contents of extract were also determined in this order: 204,67±1.87 µg and 190,62±1,11µg (gallic acid equivalent/mg extract) and total flavanoids 85,32±13,67µg (rutin equivalent/mg extract). It was concluded that this antidiabetic activity of *Myrtus nivellei* was probably, due to its high phenolic, total tannin, flavonoid contents and antioxidant effects.

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Introduction

Medicinal plants and their extracts are natural resources of compounds used in the development of new related drugs in conventional medicine [1]. Diabetes mellitus is a metabolic disorder that leads eventually to the complication of carbohydrates, fats, and proteins metabolism [2]. Divided on two groups, insulin-dependent diabetes mellitus or type I diabetes and non-insulin-dependent diabetes mellitus or type II diabetes, which represents about 90% of cases of diabetes, which may or may not be accompanied by insulin resistance, causes dysregulation in lipids and lipoproteins [3]. Several publications have reported that free radicals are formed in diabetes, mainly by hyperglycaemia, contributing to glucose oxidation, non enzymatic glycation of proteins, which lead to cell damage and diabetes complications [4]. A lot of oral antidiabetic drugs have many serious adverse effects [5]. In order to alleviate this problem, it is necessary to search for new drugs possessing few side effects. Reports on ethnobotanical use of medicinal species in central Sahara of Algeria revealed that a number of plant species were used to cure diabetes disorders [6-9]. While, only a little have been evaluated scientifically to confirm their medicinal uses [10-12]. *Myrtus nivellei* Bath & Trab belongs to Myrtaceae familly which is endemic of central Sahara [11, 13]. It was developed in rocky and sandy wades when the water table is a little deep or on the surface, at an altitude between 500 et 2000 m [6, 14, 15]. The leafed branches, harvested and dried, have been the object of a real trade in all of the sub- Sahara of Africa [7]. Leaves have been used as a condiment [15, 16]. Macerated in the melted butter, the leaves gives a brillantine used in hairdressing [7]. In folklorik medicine, it has been used against dermatosis, fever and diabetes [8]. Leaves added to pancakes of barley have been used against blennorrhea [15]. The present study aimed at investigating the effect of *Myrtus nivellei* Bath & Trab aerial decoction prepared from aerial parts on glucose levels of blood and some biochemical parameters on alloxanic diabetic rats. This study was a continuation of the previous work done by the authors to confirm its traditional use and further pharmacological application in the future.

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Materials and Methods

Plant collection

Sahara myrtle was harvested in 2015 in South Algeria, and identified at the National Institute of Forest Research (in Algeria). After drying, the aerial parts were ground to a fine powder which was traditionally prepared.

Preparation of extract

A decoction (5g) from this plant was prepared with distilled water at boiling temperature during 30min as described in Kaneria *et al* [17]. After that, the extract was concentrated on rotavapour under reduced pressure, and the extract was stored in the dark until analysis.

Estimation of phenolic content

The reaction was prepared by mixing the sample (1 mg/ml) with 1 ml freshly Folin–Ciocalteu reagent (1/10), after that 0,8 ml (75 g/l) of sodium carbonate was added (three replicates). The mixture was shaken, and allowed to stand for 30 min at room temperature. Optical density was measured at 765 nm using the Shimadzu UV–VIS-1240 spectrophotometer. The concentration of the total phenolic was calculated in terms of gallic acid equivalent µg/ mg of plant extract [18].

Dosage of flavonoid

Flavonoid content in the water extract was determined by the spectrophotometric method. In this assay, an aliquot of diluted sample (1mg/ml) was added to 0.5 ml of 2% $AlCl_3$ methanol solution. The absorbance of the mixture was determined at 420 nm after one hour at room. Rutin was used to make the calibration curve [19].

Dosage of total tannin

Gelatine (200 mg) was added into test sample contaning 2.0 ml of water, and 2.0 ml of extract was allowed to stand for 15 min at 4ºC, then the mixture was filtered through Whatman filter paper nº1. After that, water was added to 150 µl of filtrate. Non-tannin phenolics were estimated by the procedure similar to that of total phenolic content estimation, subtracting it from total phenolic content could give total tannin content of extract [20].

In-vitro antioxidant activities

Evaluation of antioxidant capacity by phosphomolybdenum method

Sample solution (0.1 ml) was combined with 0.3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 150 min. After the mixture had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was calculated as µg of ascorbic acid equivalent by using an equation obtained from ascorbic acid calibration curve [21].

FRAP assay

The working FRAP reagent was prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1:1 (v/v); respectively. After that, an aliquot of 2.7ml of prepared reagent was added to 0.3ml of sample (0.1mg/ml). The results were expressed as ascorbic acid equivalents per µg of plant extract [22].

DPPH radical scavenging activity

About 2 ml of (100 mM) solution of DPPH in methanol was thoroughly mixed with 100 µL of test extract at various concentrations, and kept in the dark for 30 min. The absorbance was read at 517 nm using methanol as blank. 2 ml of DPPH solution mixed with 100 µL of methanol was used as control. The inhibition of DPPH radical was calculated using the equation: $I\ (\%) = 100 * (A_0 - A_s)/A_0$, where A_0 is the absorbance of the control (containing all reagents except the test compound), and A_s is the absorbance of the tested sample [23].

Animals

Albino rats (Female) having between 192g and 232 g body weight, which were provided from The Institute of Pasteur in Algeria; were kept in the animal laboratory of El Oued- University, Faculty of Biology and Nature Life for 7 to 15 days of adaptation prior to the experiments, with free access to standard diet and physiologic water at room temperature of 20-22°c. They were fasting overnight, twelve to sixteen hours before the administration of test samples, but free access to physiological water was allowed.

Induction of diabetes

For the induction of diabetes, overnight fasting Rats were injected (150 mg/kg) alloxan monohydrate (Sigma-Aldrich) intraperitoneally (i.p.) [24]. After three days of alloxan injection, the rats were selected, by determination of blood glucose using automated glucometer (CONTOUR PLUS), with a dose more than 200 mg/dl.

So, glibenclamide was used orally as standard per day (with gavage) [4]. To realize this study, the animals were divided to the following groups with five rats in each: Two control groups negative and positive; group 1 (NC): Normal control; group 2 (DC): Diabetic control-(Alloxan injected rats); and three treated groups: one with drug reference; group 3 (Std): Diabetic rats+glibenclamide (5 mg/kg); and two with decoction; group 4 (MN1): Diabetic rats+100 mg/kg aqueous extract of MN; and group 5 (MN2): Diabetic rats+300 mg/kg aqueous extract of MN, the rats were followed for 21 days.

Blood biochemical parameters and body weight

To evaluate the effect of extract on animals, blood parameters were determined. It was collected from the tail vein before the animal sacrifice for determination of blood glucose level using (CONTOUR PLUS) automated Glucometer. Glucose

concentrations and biochemical parameters were then measured by enzymatic method. The influence of the extract on body weights of the rats was determined weekly during the study period.

Statistical analysis

The values have been presented as means ± S.E.M. The statistical differences between the treatments and control were tested by one way analysis of variance (ANOVA) followed by student T test using the Minitab program version 13. A difference in the mean values of $p< 0,05$ or less was considered to be statistically significant.

Results and Discussion

The dry powder of Sahara myrtle aerial parts was submitted to decoction method following traditional preparation, and the yield was about 20,81±0,61%. Total phenol, flavonoid and total tannin of this plant have been shown in table 1. Aqueous extract has presented strong phenolic, total tannin, and flavonoids contents with values of 204,64±1,87 µg AGE/mg extract, 190,62± 1,11µg EAG/mg extract and 85,32±13,67µg RE/mg extract. The results displayed the highest phenolic contents in the aerial parts' extract, generally the maceration with water yielded the higher content of total phenol and total extractable tannin as has been confirmed by Hajji *et al*. [25] who demonstrated that water was a better solvent to dissolve and extract phenolic constituents comparing with other solvents.

The antioxidant activity through PM, FRAP assays and DPPH radical scavenging of the extract was compared with acid ascorbic standard and it has been presented in Table 2. It has been demonstrated that the increased oxidative stress contributed to the development of diabetic complications [4]. Antioxidant activities have been known to have important roles, mainly due to their redox properties which lead to reduced metabolism disorders such as diabetes [26].

Previous works have been published on some biological activities using *in vitro* techniques [11, 15, 26], but there has been no *in vivo* publication that investigated biochemical parameters in normal and diabetic rats, after the administration of decoction extract. This report has been the first which investigated the hypoglycaemic effect of Saharan myrtle.

For 21 days, the body weights of rats were noted. The treated animals showed a little progression of body growth, however the untreated diabetic rats were found to have a significant weight loss of 47,28%. Groups DC, MN administered glibenclamide and *Myrtus* decoction respectively at two different doses, which have restored their body weight slowly (Figure 1). The results illustrated that the samples protected rats from body loss especially at the high dose of 300mg/kg in comparison to the diabetic control, these results were in accordance with those of Al –Shamaony et *al* [24], which might be due to the fact that insulin secretary was restored by the administration of myrtle which protected muscle proteins from degradation [27].

Control diabetic rats showed high levels in the plasma glucose as compared to the normal control group. Using two doses of aqueous extract of *Myrtus nivellei* (100 mg/kg and 300 mg/kg) and standard drug (5 mg/kg) have displayed a significant reduction in the plasma glucose level ($P<0.05$) as compared with the normal control animals; this might be associated to the elevation of insulin which promotes glucose metabolism [4, 28]. However, the diabetic control animal which received no treatment continued to show high plasma glucose in all days of the study (Figure 2). Chemical compositions of the *Myrtus nivellei* species growing in Algeria Sahara have been investigated by Mansour et a*l* [29]; Myricetin 3-*O*-β-D-(6"-galloyl) glucopyranoside, isomyricitrin and myricitrin were identified as the major constituents from Myrtle, therefore, the existence of these constituents might suggest the antidiabetic activity of this species. Furthermore, roseoside, myricetin 3-*O*-β-D(6"galloyl)glucopyranoside, 1,2,3,6-tetra-*O*-galloyl glucose, quercetin 3-*O*-β-D-(6''-galloyl)glucopyranoside, and 3-Oxo-α-ionol-9-*O*-β-D-glucopyranoside were identified for the first time in this plant [29]. At the high dose of 300mg/kg, no toxic effect has been observed and, no mortality has been noted previously at a dose of 1000mg by Touibia *et al* [30]. In addition, Sepici et *al* [31] has confirmed that *Myrtus communis* oil showed hypoglycaemic effect in diabetic rabbits without inducing apparent toxicity. Alloxan in beta cytotoxic pancreatic cells reduced insulin secretion causing a decrease in glucose utilization [27].

The decreased levels of serum urea and creatinine were observed on treating rats with both glibenclamide and samples especially at the dose of 300mg/kg. In the other hand, the diabetic control group demonstrated the high levels of serum urea and creatinine ($P<0.05$; Table 3). The protein plasma level was found to be significantly low ($P<0.05$) in the diabetic control when compared with normal rats, while the supplemented groups with two doses of Sahara myrtle and standard drug showed no significant difference when compared with normal control.

A significant ($p < 0.05$) decline in serum level of proteins in diabetic rats was observed in normal control rats; such disorder might be due to the high protein catabolism [28]. Very low synthesis of protein and more degradation of it influenced on urea and creatinine levels [32] which were increased in diabetic rats. Hence, the utilization of daily treatment of *Myrtus nivellei* decoction and glibeclamide for 21 days leaded to the successful results.

In the present study, the level of lipids like triglyceride, cholesterol was determined. The increase of lipid concentration was observed in diabetic rats ($P<0.05$) in comparison with the normal control. The treatment with decoction and glibenclamide at different doses showed no significant difference with the normal control group (Table 4). Blood lipids' (cholesterol and triglyceride) level was elevated in diabetic rats in high levels; this could be explained by the increase in the mobilization of free fatty acids from peripheral fat depots [33]; treated animals with glibenclamide and two doses of decoction extract

showed decreased serum cholesterol and triglyceride in the study period. However, this was the first report demonstrating the hypolipidemia effect of *Myrtus nivellei* on diabetic rats which could reduce cardiovascular diseases [24].

Conclusion

In conclusion, the results of this study clearly indicated that Sahara myrtle seemed to be a promising plant with high phenolic content and antioxidant activity with its hypoglycaemic effect of ameliorating the body weight. Further comprehensive pharmacological investigation is in progress to elucidate the exact mechanism of action of this extract.

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Table 1: Yield and total phenols flavonoidsand tannins of aqueous extract of *M.nivellei*

yield %	Phenolic content	Flavonoid content	Tannin content
20,81±0,61	204,64±1,87	85,32±13,67	190,62± 1,11

Phenolic and total tannins contents values were determined as µg equivalent to gallic acid /mg of extract
Flavonois contents were determined as µg equivalent rutin/ mg of extract

Table 2: Antioxidant activities of aqueous extract of *M.nivellei* and standard

	PM	FRAP	DPPH assay IC_{50} (µg/ml)
Aqueous extract	190,97±15,64	180,74±5,98	17,74 ±0,08
Ascorbic acid	/	/	5,86 ±0.02

PM and FRAP values were determined as equivalent to ascorbic acidµg/mg of extract

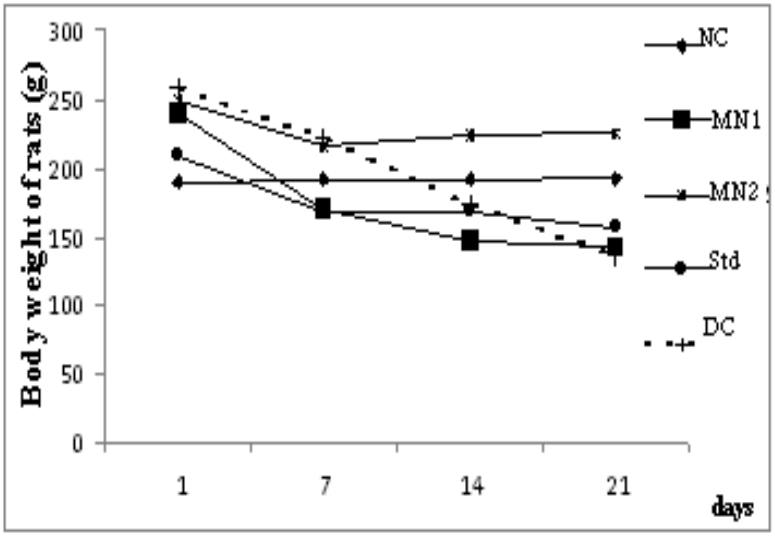

Figure 1. Effect of *Myrtus nivellei* decoction on body weight rats.
Values are given as mean ± SD for groups of five rats ineach.

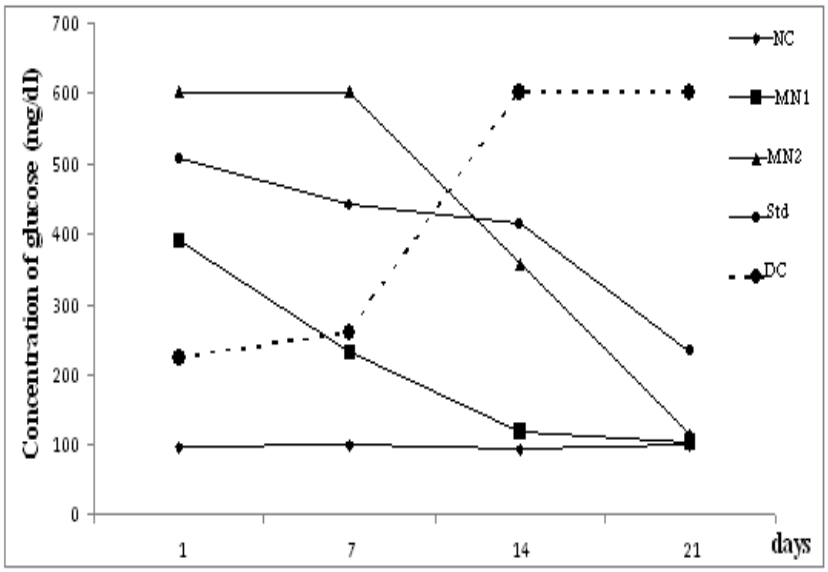

Figure 2. Effect of *Myrtus nivellei* decoction on glucose level in alloxanic diabetic rats.
Values are given as mean ± SD for groups of five rats ineach.

Table 3: Urea, creatinine and total proteins blood concentration in all groups

Groups	parameters		
	Urea (g/l)	**Creatinine (mg/dl)**	**Protéins (g/l)**
CD	0,57 ± 0,028 ***	11,85 ± 2,76*	65,67 ± 1,31*
NC	0,53 ± 0,028	9,25 ± 2,62	70,03 ± 1,56
MN1	0,41 ± 0,042	6,10±1,131	68,53 ± 2,97
MN2	0,47 ± 0,021	10,05 ± 2,90	70,53 ± 4,17
Std	0,43 ± 0,028	7,90 ±0,14	65,23 ± 0,438

Significantly different from normal control *p< 0,05, ***p< 0,001, syudent's *t*-test
CD: Control diabetic; NC: Normal contol; MN1: Diabetic rats+100 mg/kg aqueous extract of MN; MN2: Diabetic rats+300 mg/kg aqueous extract of MN; Std: Diabetic rats+glibenclamide (5 mg/kg). Values are given as mean ± SD for groups of five rats ineach.

Table 4: Blood lipid level il all groups

Groups	Blood lipid level (g/dl)	
	triglyceride	**Cholesterol**
CD	0,57 ± 0,021***	1,08 ± 0,049**
NC	0,45 ± 0,021	0,45 ± 0,063
MN1	0,48 0,205	0,53 ± 0,170
MN2	0,38 ± 0,028	0,49 ± 0,113
Std	0,48 ± 0,09	0,48 ± 0,035

Significantly different from normal control *p< 0,05, ***p< 0,001, syudent's *t*-test
CD: Control diabetic; NC: Normal contol; MN1: Diabetic rats+100 mg/kg aqueous extract of MN; MN2: Diabetic rats+300 mg/kg aqueous extract of MN; Std: Diabetic rats+glibenclamide (5 mg/kg). Values are given as mean ± SD for groups of five rats ineach.