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Impact of the methanolic extract of *Marrubium vulgare* on biochemical parameters of wistar rats

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RESUME:
Evaluation de l'effet de l'extrait méthanolique de Marrubium vulgare (Utilisé traditionnellement comme antidiabetique, antihypertension et pour soigner plusieurs maladies), sur les Rats wistar males. Ces animaux ont été subdivisés en 4 groupes (n=6) et l'extrait méthanolique de Marrubium vulgare a été administré à de doses de 50, 100 et 200 mg/Kg de poids corporel d'animal en expérimentation, pendant 21 jours. Puis, le sang des animaux a été prélevé à partir de la ponction cardiaque, afin d'obtenir le sérum, qui a permis de mesurer les paramètres biochimiques. Les résultats obtenus n'ont pas montré un changement significatif (p < 0.05) pour les paramètres dosés : cholestérol, bilirubine, glucose, protéine, urée, phosphatase alcaline et de transaminase (ASAT). Alors que le taux d'Alanine Transaminase (ALAT) pour les groupes des rats ayant reçu 100 et 200 mg/Kg de poids corporel (16.44 et 14.94U/L respectivement) a montré une différence significative (p < 0.05) quand on le compare avec le taux d'animaux de control 25.36 U/L et ceux traité à 50mg/Kg de poids corporel (17.2 U/L)..

Mots clés : Marrubium vulgare, extrait méthanolique, paramètres biochimiques

ABSTRACT :
Problem statement: To evaluate the effects of the methanolic extract of the M. vulgares (traditionally used antidiabetic, antihypertensive and some diseases) in male Wistar rats. Approach: The animals were divided into 4 groups (n = 6). Methanolic extract of M. vulgare was administered in graded doses of 50, 100 and 200 mg/kg body weight of experimental animals for 21days. Blood samples were collected by cardiac puncture. The serum harvested was analyzed for some biochemical parameters, using assay kits.
Results: There were no significant changes (p>0.05) in the levels of cholesterol, bilirubin, glucose, protein, urea, alkaline phosphatase and aspartate transaminase (AST), alanine transaminase (ALT). Activities of the groups of rats given 100 and 200 mg/kg body weight extracts (16.44 and 14.94 U/ L respectively) showed significant decreases (p<0.05) when compared with the control (25.36 U/L) and the group fed 50 mg/kg body weight of extract (17.2U/L). Computed ALT/AST showed decreases in the test groups (0.84-0.87) when compared with the control (1.06).

Keywords : lived experience, people, old age, environment, family, community.

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I. INTRODUCTION

Marrubium vulgare L. (Lamiaceae) commonly known as "Horehound" in Europe, or "Marute" in Mediterraneean district, is naturalized in Mediterranean district and Western Asia and America. In Mediterranean, *M. vulgare* is frequently used in folk medicine to cure a variety of diseases. The plant is reported to possess hypoglycemic (Romanand al., 1992; Novaes and al, 2001), relaxant (El-Bardai and al, 2003), antihypertensive (El-Bardai and al, 2004), analgesic [Brand and al, 1999; De Souza and al, 1998; Sahpaz and 2002; De Jess and al, 1999), anti-inflammatory (Schlemper and al, 1996), antioxidant activity (Weel and al, 1999), antioedematogenic activity (Stulzer and al, 2006), and many other biological activities.
However, information is scanty on the effects of the plant on biochemical parameters in experimental animals and on the possible risks associated with consumption of *Marrubium vulgare* extracts. The present study was designed to determine the biochemical changes associated with oral consumption of methanolic extracts of the leaves of the *Marrubium vulgare.*

II. MATERIAL AND METHODS

II.1. Collection of plant materials and preparation of extract:

M.vulgare plant samples were collected from the Tamara district of Morocco in May 2007. The plant was authenticated at the Department of Biology, Faculty of Science-Rabat. A specimen of the original collection was placed in the herbarium of the Faculty of Medicine and Pharmacy of Rabat.
The Whole plant of *M. vulgare* was dried in shade and crushed into fine powder. The dried powder from the plant (200 g) was extracted in Soxhlet apparatus with methanol. The extract was evaporated to dryness in vacuo using a rotary evaporator at 70 ºC to give a yield of 39.2 %.

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II.2. Experimental animals and treatment

Twenty four male Wistar rats, weighing between 150.4 and 180.6 g were obtained from the animal house of the Laboratory of Pharmacology, Faculty of Medicine and Pharmacy of Rabat. The animals were divided into 4 groups of 6 rats each. All rats housed in well-ventilated cages (room temperature 24±1°C), without any restriction to food and drinking water during the experimental period. Rats control (R-c) that received distilled water. Animals in the test groups were orally fed once daily with 1ml/body weight, of the extract respectively (prepared on each day of the experiment). The doses used were 50, 100 and 200 mg/kg body weights respectively. Administration of the extract was carried out daily for 21 days.

II.3. Preparation of serum

24 h after the last administration of extract, the animals were anaesthetized under chloroform vapor and dissected. Blood for serum preparation was collected by cardiac puncture, using sterile syringes into sterile plain tubes without anticoagulant. The serum was stored in the refrigerator for the analysis of biochemical parameters. The analyses on serum were completed within 24 h of sample collection.

II.4. Biochemical assays:

Serum Alkaline Phosphate (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT), Total cholesterol, Bilirubin and total protein were estimated using assay kits. Urea was estimated by the diacetyl method. Glucose was assayed by the glucose oxidase-peroxidase method using a kit (Sigma Diagnostics, India). All the tests were determined using a spectrophotometer.

II.5. Statistical analysis

Results were expressed as means ±SD. The data were analyzed by exploring the Student's t-test, $p<0.05$ was regarded as significant.

III. RESULTS

The activities of the enzymes assayed as well as the concentrations of other biochemical parameters are shown in **Table 1**. The results indicated dose-related decreases in serum activities of ALT and AST in the test groups (14.24 -17.2 and 17.16 -20.35 U/L respectively) when compared with those of the control (25.36 and 23.89 U/L respectively). However, the groups fed 100mg/kg body weight and 200mg/kg body weight extracts had significantly lower ALT activities (16.44 and 14.94 U/L respectively) relative to those of the control ($p<0.05$). There were mild variations in the ALP activities (30.87-33.4 U/L) of the experimental rats as were the variations in the ALT/AST ratios of the experimental animals (0.85-1.06). **Table 2** shows the serum concentrations of bilirubin, total protein, total cholesterol, glucose and urea in the experimental animals. There were mild alterations in the serum concentrations of bilirubin (12.3-16.4µmol/L), total protein (88.2-90.5 g/L) and total cholesterol (170.6-182.1 mg/dL). There were very slight dose dependent decreases ($p>0.05$) in the serum glucose concentrations of the test rats (3.98-4.4 mmol/L) when compared with those of control rats (4.9 mmol/L). The urea concentrations of test rats (6.6-7.4mmol/L) were slightly higher ($p>0.05$) than those of the control rats (6.5 mmol/L).

Table 1: Effects of methanolic extract of *M. vulgare* on enzyme activities of rats

		R-Tests		
	R-c	**50 mg/Kg**	**100 mg/Kg**	**200 mg/Kg**
ALT (U/l)	25,96 ±5,2	17,2±6,32	16,44±3,1*	14,94±0,4*
AST (U/l)	23,89±3,2	20,35±3,6	19,2±2,2*	17,16±2,3*
ALP (U/l)	30,87±2,7	33,4±4,3	31,75±2,1	31,29±4,5
ALT/AST	1,06±0,2	0,84±0,14	0,85±0,11	0,87±0,13

*: Values are means ± SD (n = 6 rats per group). Significantly different ($p<0.05$); ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; R-c:Rat control

Table 2: Effects of methanolic extract of *M. vulgare* on serum chemistry of rats

		R-Tests		
	R-c	**50 mg/Kg**	**100 mg/Kg**	**200 mg/Kg**
Cholesterol tot (mg/dl)	182,1±3,4	177,6±3,2	173,9±3,1	170,2±3,6*
Bilirubin tot (µmol/l)	13,83±1,8	12,3±1,5	14,33±2,2	16,49±2,4
Glucose (mmol/l)	4,9±0,50	4,4±0,2	4,2±0,17	3,9±0,3*
Protein (g/l)	88,2±2,1	90,5±4	88,4±5,1	86,2±4,3
Urea (mmol/l)	6,5±0,5	6,6±0,4	7,1±0,6	7,4±0,86

*: Values are means ±SD (n = 6 rats per group) Significantly different ($p<0.05$)

IV. DISCUSSION

The decreases in the serum AST activities and ALT/AST of the test groups when compared with the control are not significant ($p>0.05$). Serum ALT/AST has been used as an index to monitor liver pathology (Eteng and al. 1998). Ratios higher than unity are indicative of adverse pathological effects on the liver. From the studies, it has been shown that the M. vulgare maintain the ALT/AST ratios at favorable levels. This might suggest a non-toxic effect or absence of hepatocellular damage at the investigated concentrations. Serum ALP is a sensitive detector for intrahepatic and extrahepatic bile obstruction, the presence of diseases in the liver and diseases associated with osteoblastic activity (Vasudevan, 2005). From the results obtained, it is likely that the concentrations of methanolic extract of *M. vulgare* used in this study did not adversely interfere with the calcification and/or metabolic activities involving the liver.

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There were decreases in the glucose concentrations of the test rats (dose-dependent), when compared with those of control rats. Reports by Roman and al. (1992) indicated a significant reduction in blood glucose when crude extract of *M. vulgare* were administered to rats.

Normal bilirubin concentration is 4-17 µmol/L. The sight variations (which are within normal limits) in bilirubin concentrations are indicative of non-adverse effects on haemoglobin metabolism. There were decreases in the glucose concentrations of the test rats (dose-dependent), when compared with those of control rats.

The total cholesterol levels of the Rats test were lower than those of the control in a dose-dependent fashion.

V. CONCLUSION

From the foregoing, methanolic extract of *M. vulgare* did not induce adverse alterations in biochemical parameters such as total cholesterol, protein, urea, total bilirubin, glucose, alkaline phosphatase and aspartate transaminase. Thus the use of infusions of *M. vulgare* in the management of hypertension, diabetes and coronary artery and heart diseases should be encouraged.

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