

Therapeutic effects of *Rhamnus alaternus* on the nephroangiosclerosis in wistar rats

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Abstract: The buckthorn "*Rhamnus alaternus*" is a plant used in traditional medicine especially in the treatment of certain diseases such as diabetes, cardiovascular disease and dyslipidemia. The aim of our study was to analysed and evaluate the effects of buckthorn on nephroangiosclerosis Wistar rats. Thirty male Wistar rats, adult weighing between 120g and 250g were distributed as follows: Five control animals received a standard laboratory diet, thirty four experimental rats received the standard laboratory diet supplemented with palm oil and 4% of NaCl (High Sold Fat Diet). After six month of this diet the HSFd group was subdivided into rats treated for 45 days with aqueous extract of buckthorn or animals HSFd only. Plasma metabolites and endothelin-1 concentration were measured by standard methods, section of kidney were stained by Heindenhain-azan and periodic acid shiff. The examination of renal parenchyma of HSFd rats showed a prominent structural changes such as, obstruction of vascular lumen, ischemia of the glomerular and tubulo-interstitial fibrosis the biochemical and hormonal parameters were significantly improved in the HSFd rats treated with decoction of buckthorn. Moreover, the morphological changes of the renal parenchyma were attenuated in rats HSFd decoction. The buckthorn attenuates renal parenchymal lesions by aggregating at different levels of rats maintained on HSFd.

Keywords: *Rhamnus alaternus*, endothélin-1, renal parenchyma, high-salt-fat-diet.

INTRODUCTION

Nephroangiosclerosis is a nephropathy that is secondary to an arterial hypertension which is frequently inadequately treated, or left untreated (Beaufils, 2005) This nephropathy manifests through vascular remodeling which is essentially characterized by a hypertrophy of the tunica media, an intimal thickening, and therefore a decrease of lumen (Therrien, 2009). These different perturbations lead, downstream, to ischemia of the glomeruli. It shows an impairment of the filtration function, consequently a decrease in glomerular filtration as well as a loss of the functionality of certain nephrons (Therrien, 2009). This loss causes hypertrophy of the remaining glomeruli with increased capillary pressure Glomerular reactions, resulting in macrophage accumulation and release of growth factors (Therrien, 2009) In addition, increased pressure in the glomerular capillaries favors excessive passage of proteins to the proximal tubules, an increased release of endothelin-1 (Flingny, 2010). This neuropeptide via its ET-A receptor induces the release of TGF- β (transforming growth factor) who induces the synthesis of matrix metalloproteinase (MMP), These MMPs foster the differentiation of the tubular epithelial cells into myofibroblasts. These cells synthesize the fibrillar proteins in the extracellular matrix

and cause tubulointerstitial fibrosis (Therrien, 2009), hence the alteration of the kidney tissue and the apparition of renal failure. Although the management of arteriel hypertension and nephropathy by medicine is good , they are still patients who use traditional medicine because of their low cost and ower side effects, the buckthorn includes well-known medicinal plants spicies with diversés biological propreties. indeed, rhamnus alaternus contains active ingredients that have beneficial effects on certain metabolic diseases such as diabetes, cardiovascular diseases, dyslipidemias (Harrar, 2012). It seems that decoction of Rhamnus fruits contains an active ingredient known as "rhamine", which is efficient in the treatment of atherosclerosis (Messaouda, 2004). In vitro studies have shown the antioxidant effect of this Rhamnus alaternus leaves (Harrar, 2012) as well as its effects in the intestinal absorption of lipids and carbohydrates in mice (Khettal, 2014). Its effects antioxidant, diuretic and hypotensive, have left us thought that this plant would have beneficial effects in the treatment of renal parenchymal lesions related to nephroangiosclerosis and the prevention of renal insufficiency in wistar rats subjected to high salt and fat diet (oil palm). A high sodium diet could be behind vascular diseases (Foulquier, 2008) Furthermore, a high lipid diet causes a decrease in the production of nitric oxide and an endothelial dysfunction (Zidane, 2012).

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MATERIALS AND METHODS

Animals

The animals for this study are male adult Wistar rats from the Pasteur Institute in Algiers (Algeria). After an adaptation period spanning 2 weeks in the University's animal facility, the white rats are individually placed in plastic cages equipped with stainless steel lids and free access to water. The ambient temperature is maintained at 25±2°C with a humidity rate between 60 and 80%; lighting is ensured by means of artificial intermittent lights (12h/day).

Standard diet

The Wistar rats received daily in the form of granules of commercial origin provided by national live stock feed office and receive *ad libitum* tap water. This balanced diet consisted of 23.03% proteins, 9% fat, 48% carbohydrates, 3% vitamin, mineral complex and 16.07% water.

High-fat high-sodium diet

The experimental rats are subjected to a standard laboratory diet enriched in palm oil and a saline solution, 4% NaCl. The chosen palm oil for this diet is refined, whitish, solid and enriched in saturated fatty acids: lauric acid (≈0,1%), myristic acid (1%), palmitic acid (43.5%), stearic acid (5%). It is used in liquid form, with a melting point comprised between 38-40°C.

Vegetal materials: Buckthorn

The leaves of *Rhamnus alaternus* were harvested in May in the mountains of Tessala (Western Algeria) and diction was prepared by adding 1500 ml of distilled water in 200 g powder of *R. alaternus*. The decoction obtained after cooling was filtered through an entonnoir containing hydrophilic cotton and then centrifuged at 2500rpm/min for 5 minutes and was preserved in the refrigerator at 4°C.

Experimental protocol

All experimental procedures were authorized by the Committee of animal protection in institutions of the National Administration for Higher Education and Scientific Research in Algeria. Ethical approval number: Law 98-11 of August 22th, 1998.

The experimentation lasted for 7 months and concerned 39 rats divided in 2 groups

The Control Group: C (n=5), received the standard laboratory diet. Daily food intake is 20g corresponding to 62kcal/day of energy consumption and *ad libitum* water. - A second set of 34 experimental animals (E) were subjected to a standard high-salt-fat diet composed of 20g of pellets supplemented with 6 g of palm oil the standard high salt-fat -diet, with an energy consumption of 90kcal/day.

After 6 months on this diet, the high salt- fat groupe was subdivided into two groups: 1- experimental rats:E (n=18)

was sacrificed for the morphological study of the organs and the remaining, 2- Tr rats : (n=16) was treated orally with 4ml/kg of body weight decocted solution of the buckthorn for 45days

Operatory technique

The animals were weighed weekly and samples of blood were collected monthly. The collected blood, in dry heparinized tubes, is centrifuged at 3000 rpm for 15 minutes. The obtained plasma and serum are stored at - 25°C, for further analysis of certain biochemical plasmatic parameters and of endothelin-1.

At the end of the 7 month experimentation, rats were anesthetized via intra-peritoneal injection of urethane 25%, at a rate of 0.4ml/100g of body weight then autopsied. The kidneys were quickly collected and immersed in adequate fixatives during 6 days: aqueous Bouin for the topographical survey and sublimated Hollande Bouin for the histochemical study. Sections, 2 µm thick obtained with a microtome were colored with Heidenhein Azan for topography and with Periodic Acid Schiff (PAS) for histochemistry.

Analytical techniques

Plasma biochemical assays (triglycerides, total cholesterol, HDLc, creatinine, urea, uric acid, Natremia, Kalemia, CRP) were measured using roche hitachi 902 auto analyser (Roche Molecular Systems, Branchburg NJ). The average values of LDL cholesterol were determined by the Friedwald formula:

LDLc concentration (g/l) = CT - TG/5 - HDL, and coronary risk index was calculated as TC/HDLc.

Endothelin-1 was assayed using the rat endothelin-1 ELISA kit (EIA-3111) by the direct sandwich enzyme immunoassay method.

STATISTICAL ANALYSIS

The results are presented under the form of means ± standard error, the values are statistically using student test "t" and Kruskal-wallis test (STATESTICA version 6). The means are considered significantly different at p<0,05. *E Versus C, ● Tr Versus E

RESULTS

Changes of body mass, metabolites and endothelin-1 level of control rats, HSFd and HSFd treated by buckthorn

Compared with controls, rats subjected to the atherogenic diet showed a very significant increase in body weight from the 4th month. This increase becomes highly significant at the end of the experiment; it is of the order of 48.45 % (tab 1). The body mass of HSFd rats decreased significantly after 30days of treatment by decocted of buckthorn by 19.46%

Table 1: Body mass and plasma concentration of lipids of controle and rats kept high salt-fat-diet for six months

Duration of experiment (Month)				
	0	2	4	6
Control group (C)				
Body mass (g)	182.96±13.63	207.68±11.73	196±8	200±9.04
Triacylglycerides (g/l)	0.41±0.01	0.42±0.0096	0.43±0.08	0.40±0.22
Total cholesterol(g/l)	0.43±0.0092	0.42±0.02	0.46±0.096	0.6±0.05
HDL-Cholesterol (g/l)	0.44±0.005	0.43±0.01	0.40±0.067	0.41±0.051
LDL-Cholesterol (g/l)	0.18±0.058	0.194±0.006	0.15±0.0016	0.16±0.038
Atherogenic Index	0.94 ±0.04	0.97±0.03	1.10±0.09	0.4±0.06
Experimental group (E)				
Body mass (g)	202.45±7.72	254.14±7.96	283.1±9.75***	300.5±1112****
Triacylglycerides (g/l)	0.43±0.011	0.47±0.05	0.49±0.08 *	0.89±0.89****
Total cholesterol (g/l)	0.46±0.092	0.49±0.014	0.75±0.026 *	1.02±0.06****
HDL-Cholesterol (g/l)	0.41±0.004	0.42±0.005	0.30±0.0063 ***	0.24±0.06****
LDL-cholesterol(g/l)	0.18±0.0044	0.22±0.060	0.37±0.0077***	0.57±0.05****
Atherogenic Index	1±0.04	1.16±0.05	1.81±0.03**	2.37±0.07****

Data are expressed means ±SEM, Control group (C), Experimental group (E),n=5(C) ,n=34(E) Difference of E Versus C *p<0.05 **p<0.02, ***p<0.01, ****p<0.001 (student test)

Table 2: Body mass and plasma concentration of lipids of Experimental rats treated with buckthorn (Tr) for 45 days

Time of treatment (days)				
	0	15	30	45
Body mass	298±0.05	270 ±0.014	260±0.021●	240±0.03●●●
Triacylglycerides (g/l)	0.89±0.019	0.85±0.023	0.56±0.018●●	0.43±0.017●●●●
Total Cholesterol (g/l)	1±0.011	0.67±0.022	0.65±0.005●●	0.56±0.01●●●●
HDL-cholesterol (g/l)	0.24±0.022	0.29±0.056	0.30±0.006	0.38±0.009●●
LDL-cholesterol (g/l)	0.56±0.015	0.35±0.014	0.26±0.013●●	0.20±0.001●●●●
Atherogenic Index	2.5±0.032	2.4±0,035	2±0.032	1.25±0.04●●●●

Data are expressed means ±SEM Difference of E Versus Tr: ●p<0,05, ●●p<0,02, ●●●p<0,01 ●●●●p<0,001(Kruskal-Wallis test)

Plasma concentration of metabolites

The HSFd increased plasma level of triglycerides and total cholesterol (as compared with the control rats). At the end of experiment plasma concentration of TG and total cholesterol in the HSFd group were 106. 97%, 121. 73% higher than at it is beginning (table 1). When we compared with control group animals the plasma concentration of HDL cholesterol decreased in rats subjected to HSFd starting from 4th month of the experiment (tab 1). By the end of the experiment the value was 41. 46% lower than it start. In rats fed the high salt-fat diet already after 4th month the plasma level of LDL cholesterol were much higher than in control rats (table 1).

The administration of aqueous extract of buckthorn to the HSFd rats decreased plasma concentration of triglyceride and total cholesterol after 30 days of treatment (table 2). Plasma HDL cholesterol levels of the HSFd rats treated with buckthorn showed a significant decrease by 60% after 30days of treatment. The HSFd

rats treated with aqueous extract of buckthorn showed a high decrease of LDL cholesterol concentration after six weeks of treatment (table 2).

After six months of high salt fat diet , the rats showed a higher significant increase in plasma level of creatinin, urea and uric acid respectively by 142.42%, 71.4% and 81%. This metabolites decreased significantly (p<0,01) after 30 and 45 days of treatment by buckthorn (table 4)

The salt fat diet increased plasma concentration of Natremia and kalemia and CRP starting the 4th month. Plasma potassium levels of the HSFd rats treated with the buckthorn showed small variation (table 4). However, plasma levels of sodium and CRP in rats treated with aqueous extract of buckthorn showed a significant decrease by 8% and 22% at 30 days of treatment. In control rats endothelin-1 plasma levels did not undergo any significant variation during experiment, however, rats fed HSFd showed increased concentration of endothelin-1 starting from the 4th month of diet (tab 3). By the end of

Table 3: plasma concentration of creatinin, urea, uric acid, Natremia, kalemia, CRP and endothelin-1 of control and rats kept high salt-fat-diet for six months

Duration of experiment (month)				
	0	2	4	6
Control group (C)				
Creatinin (g/l)	0.36±0.014	0.38±0.03	0.41±0.011	0.47±0.0125
Urea (g/l)	0.33±0.097	0.32±0.01	0.38±0.013	0.36±0.018
Uric acid (g/l)	21.2±0.58	18.8±0.56	24.4±0.4	25±1.32
Natremia (mEq/l)	140.8±0.2	141±0.54	142±0.31	140.5±0.32
Kalemia (mEq/l)	3.77±0.3	3.76±0.22	3.28±0.2	4.35±0.3
CRP	0.38±0.012	0.42±0.013	0.4±0.010	0.42±0.023
Endothelin-1 (pg/l)	4±0.047	4.02±0.09	5.1±0.06	4.78±0.05
Duration of experiment (month)				
	0	2	4	6
Experimental group (E)				
Creatinin (g/l)	0.33±0.011	0.41±0.013	0.47±0.014*	0.8±0.018 ****
Urea (g/l)	0.35±0.058	0.39±0.01	0.54±0.089***	0.6±0.018****
Uric acid (g/l)	19.05±0.58	21.23±0.56	30.34±0.98**	35±0.08****
Natremia (mEq/l)	139.97±0.27	141±0.24	145±0.54*	149.5±0.57***
Kalemia (mEq/l)	4.07±0.073	4.03±0.051	6.27±0.108****	7.04±0.2****
CRP	0.40±0.021	0.45±0.018	0.5±0.015*	0.70±0.022****
Endothelin-1 (pg/l)	4.50±0.047	5±0.09	9±0.06***	10.90±0.07****

Data are expressed means ±SEM ,Control group (C),Experimental group (E),n=5(C) ,n=34(E) Difference of E Versus C *p<0.05 **p<0.02, ***p<0.01, ****p<0.001 (student test)

Table 4: plasma concentration of creatinin, urea, uric acid, Natremia, kalemia, CRP and endothelin-1 of Tr rats treated with buckthorn for 45 days

Time of treatment (days)				
	0	15	30	45
Creatinin (g/l)	0.81±0.016	0.79±0.02	0.56±0.06●●	0.5±0.018 ●●●
Urea (g/l)	0.6±0.058	0.55±0.01	0.54±0.08	0.30±0.020●●●●
Uric acid (g/l)	35±0.58	36±0.56	24±0.98	20±0.09●●●●
Natremia (mEq/l)	149.25±0.27	149±0.26	141±0.52●	136±0.43●●●
Kalemia (mEq/l)	7.01±0.075	7±0.051	6.84±0.108	6.09±0.2●
CRP	0.72±0.031	0.45±0.021	0.5±0.018	0.5±0.017●●●
Endothelin (pg/l)	10.87±0.061	10.43±0.051	9.52±0.08	8±0.09●●

Data are expressed means ±SEM Difference of E Versus Tr: ●p<0,05, ●●p<0,02, ●●●p<0,01 ●●●●p<0,001(Kruskal-Wallis test)

experiment plasma endothelin-1 level was 142.22% higher than the first start.

After 45 days, the HSF D animals treated with buckthorn showed a significant decrease in plasma levels of endothelin-1 by 26. 40%.

The *R. alaternus* treatment, corrects the profound architectonic modification of the arteries of he experimental rats. Thus, we noticed a cellular and reorganization of the various tunicas as well as an improvement of the alterations related to the hyalinosis and to the atherome (fig. f)

II-Condition of the glomeruli and of the tubules in controle, HSF D and HSF D rats

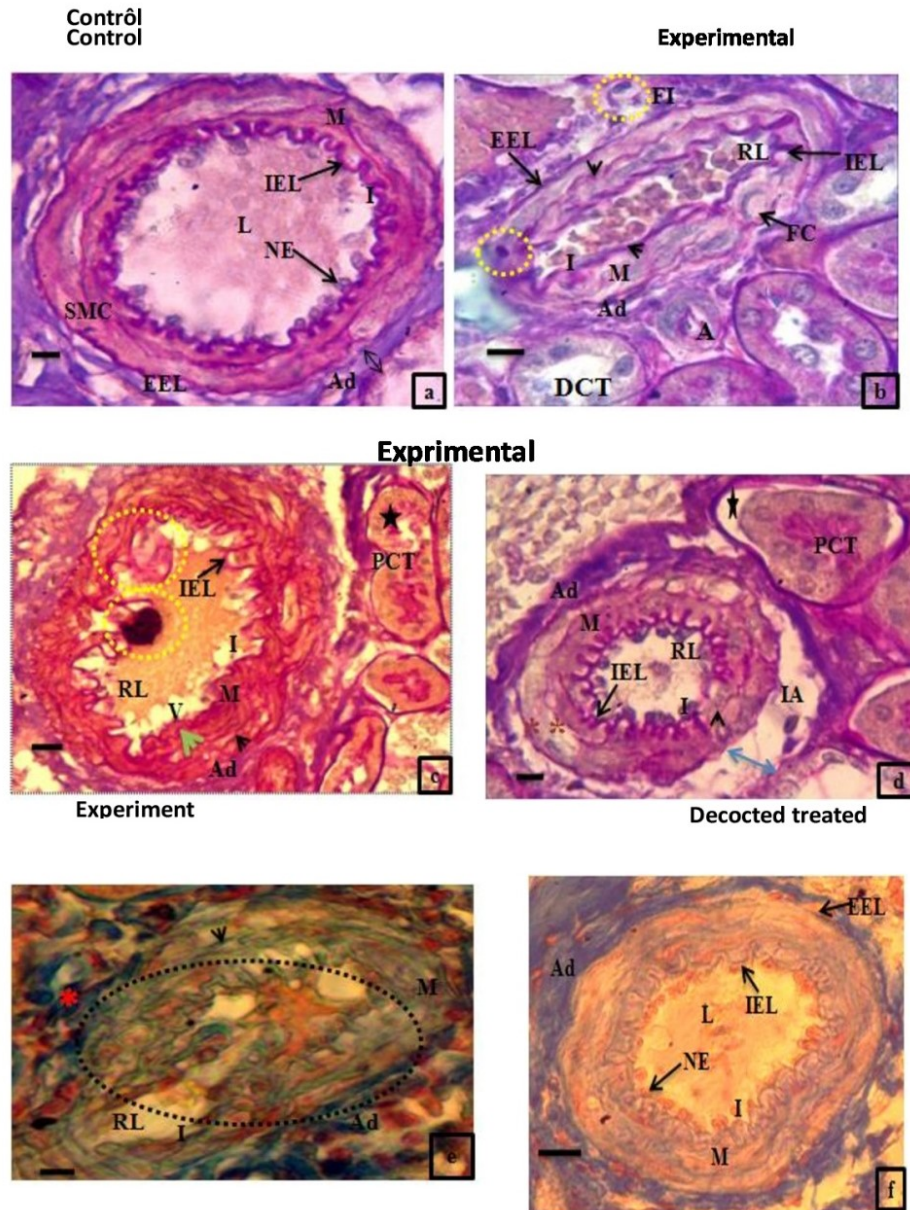
II- a Condition of glomeruli in control, HSF D and HSF D rats treated by decocted buckthorn

All the aforementioned artery lesions cause degeneration and ischemia of the glomeruli, this reflects, on one hand a widening of the urinary room, a loss of cellularity hemosiderin deposits on the other hand causes a hypertrophy and an accumulation of the mesangiale matrix of the remaining glomeruli. Dilatation of the glomerular capillaries, infiltration by the polynuclear celled, necrosis, scarring fibrosis and hyalinoses are the causes of a glomerulosclerosis in the experimental rats.

Histo-Physiopathology of the renal arteries and of nephrons in rats subjected to the high-sodium atherogenic diet and the alaternus treatment

1-Condition of the renal arteries in control rats and experimental

The renal arteries in control rats, compared to those of the control rats, show important structural modification, histological observation of the renal arteries reveals a hypertrophy and an infiltration of the advertitia by the fat cells (fig d[↔]); foamy smooth muscle cells of important size acquiring a phagocytic phenotype (fig b); inflammatory sources (fig b,c [⚡]) and necrotic areas in the media; alterations of the elastic lamina, probably caused by a protcelysis (fig b,c,d,e,[<]);a hyalinosis (fig d[**]) and a duplication of the internal elastic lamina reflecting a thickening of the intima (fig c[↗]). We also noticed the existence of lipids deposits, vacuolitions (figs. c,u) and a luminal stenosis (fig b,c,d,e) that generates an atheromatous plaque in these arteries (fig e [⊙]).



Control (15µm) : Ad (Adventicia); EEL (External Elastic lamina); M(Media); SMC (Smooth Muscle Cells); IEL (Internal Elastic Lamina); NE(Nuclei Endothelial cells)

Fig. 1: aspects structural of arteries in control, HSFD and HSFD rats treated by decocted of buckthorn. Coloration PAS (Periodic Acid shiff) and Azan of Heindenheim

Experimental (b:15µm, c: 15µm;d:6µm,,e:15µm : AI(AdiposeInfiltration); RL (Reduced Lumen) ; Spu(Spumes Cells); V (Faty Vaculisation); Hyalinisation (**), hypertrophy of adventicia (↔); proteolyse des lames elastiques (<); duplication de lame elastique interne (→); recrutment of inflammatory cells (●) desorganization of advticia (*); Lr (Lumiere vasculaire reduite), formation de plaque d' atherome (●●●)

Treated with buckthorn (F: 145µm) : Ad(Adventicia); EFL (Internal Elastic Lamina); M(Media); SMC(Smooth Muscles Cells); I(Intima); IEI(Internal Elastic Lamania); NE (Nucei Endothelial Cells).

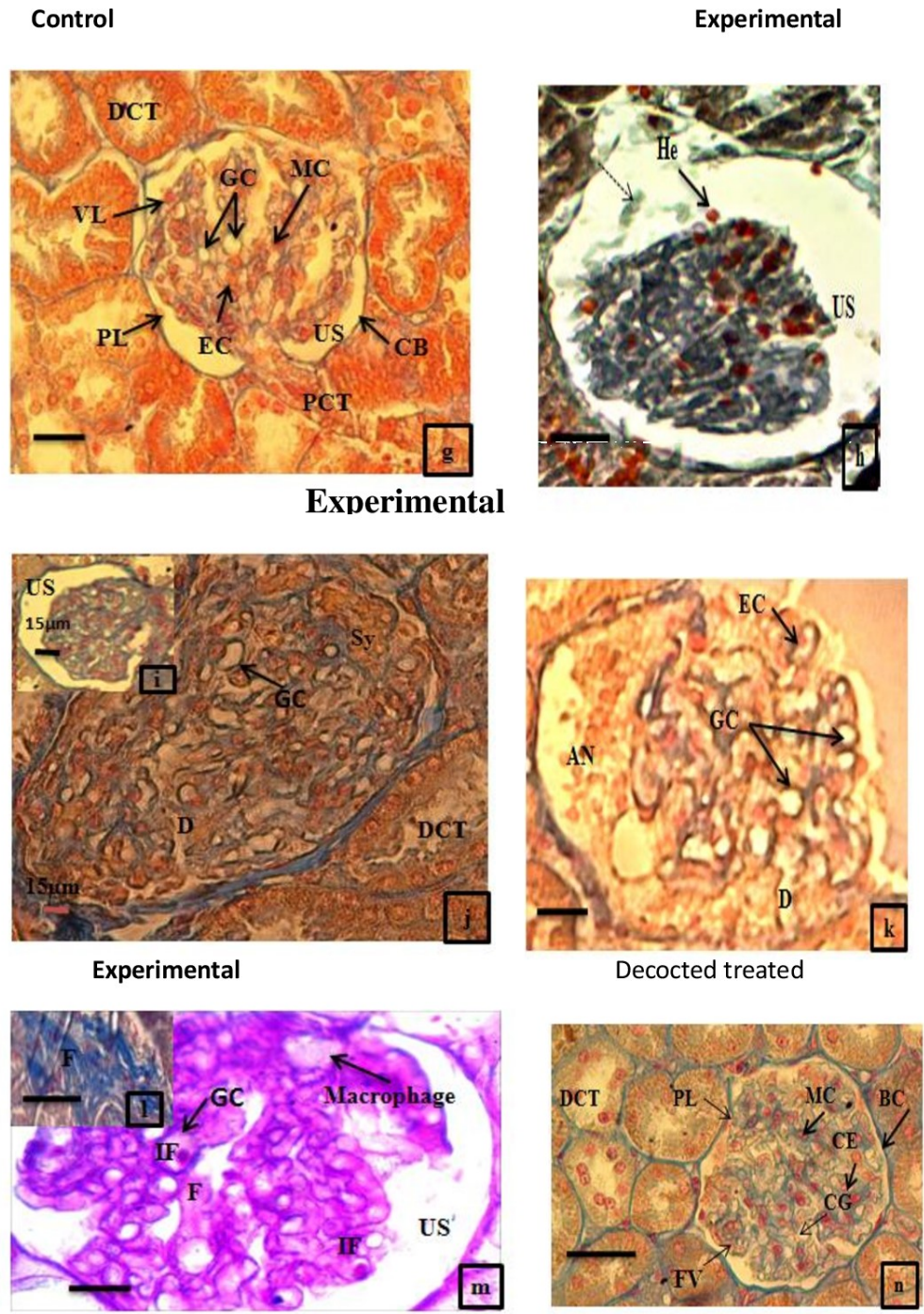


Fig. 2: aspects structural of glomeruli in control, HSFD and HSFD rats treated by decocted of buckthorn. Coloration PAS (Periodic Acid shiff) and Azan of Heindenhein

Control (fig g :15µm) : BC (Bowman’s capsule); PL (Parietal Layer); US(Urinary Space); VL (Visceral Layer) , MC (Mesangial Cells) ; GC (Glomerular Capillaries) , EC (Endothelial Cells)
 Experiment (h:6µm, I :15µm, j:15µm, k:6µm , l: 15µm, m:6µm) :D (Dilatation of capillaries) ; IF (Focus Inflammatory) ; F (Fibrose); FC (Fibrosis Scarring); H (Hyalinosis) ; NA (Necrotic Area), Cellular Debris () ; Sy (Syncytium) ; He (Hemosiderin).
 Decocted treated (fig n :15µm): BC (Bowman’s capsule); PL (Parietal Layer); US(Urinary Space); VL (Visceral Layer) , MC (Mesangial Cells) ; GC (Glomerular Capillaries) , EC (Endothelial Cells) .

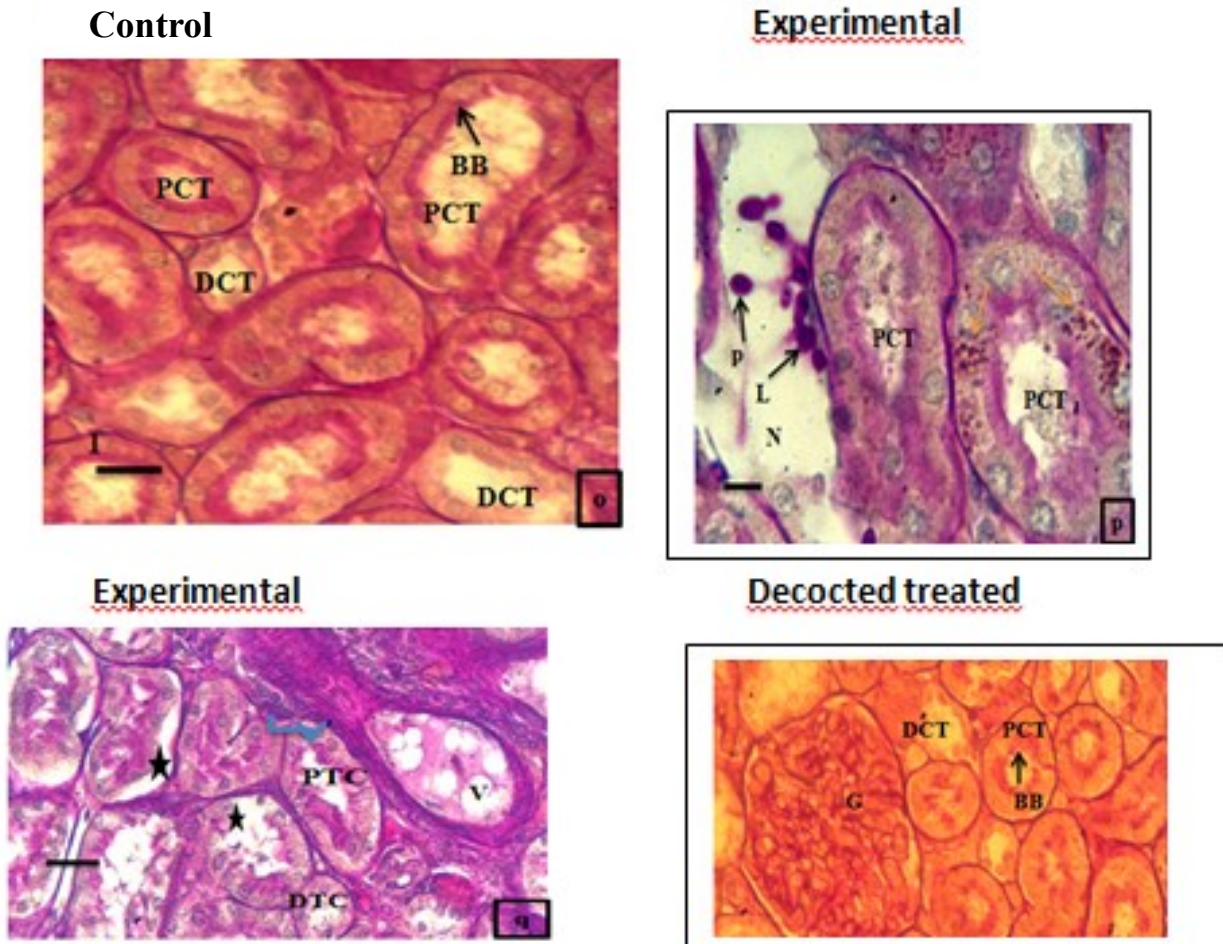


Fig. 3: Structure of tubules in controle, HSF and HSF rats treated by decocted of buckthorn Coloration PAS (Periodic Acide) and Azan Heidenhein.
 Exeperement (p:6µm, q: 15µm) ;G(Glomerule);M(Mastocyte); P(Plasmocyte) N(Necrose) ; DCT (Distal Convulate Tubule); PCT (Proximal Convulated Tubule); V(Faty Vacuolisation); Tubular Atrophy (★); (↙); foyer inflammatoire (↻)
 Decocted treated (r : 15µm): BB(Brush Border); DCT(Distal convulated tubule) PCT(Proximal Convulated tubule);

II-b Condition of the tubules in control, HSF and HSF treated rats

Tubules are affected by the atherogenous high sodium diet in a similar fashion as the glomeruli, atrophied and ischemic . We noticed an infiltration by the inflammatory cells in the in the tubules. In proximal convulated tubules, the epithelia cells lose their brush border, the interstitium studded with plasmocytes and lymphocytes and the PAS positive matrix elements expressscarring fibrosis Treating

these animals restores almost the entirety of the observed perturbations

DISCUSSION

After seven-months investigation, we analysed the impact of buckthorn on renal parenchyma and serum metabolites and endothelin-1 levels in laboratory rats submitted to the standard diet enriched in salt solution and oil palm.

Average change in body weight and in some plasmatic biochemical parameters in experimental Wistar rats and experimental rats treated with buckthorn compared with control rats.

Body weight

The high fat high sodium diet induced a highly significant weight change in our rats ($p < 0.001$), in the order of 48,43%. According to Ammamou (2010), the weight increase in rats who received the high fat diet is due to the activation of the nuclear receptors of the PPAR family (Peroxisome proliferator activated receptors) by the fatty acids which cause the differentiation of pre-adipocytes into adipocytes. This increase of the body mass index can be attributed to a high salt diet in rats according to LASHEEN (2015). and would be expressed by the expansion of the extracellular volume.

Treatment via the aqueous extract of buckthorn, caused in the experimental rats a very significant weight regression ($p < 0,01$). According to khetal *et al* (2015). the anti-adiposity effect of this plant can be linked to its action on lipase, which results in inhibition of intestinal lipids absorption.

Atherogenic lipid-status

The hyperlipemia observed in to the HSFD rats reflects the alteration of the activity of certain lipolytic enzymes, namely LCL (lipoprotein lipase), LCAT (Lecithin Cholesterol Acyl Transferase) and CETP (Cholesterol Ester Transfer Protein). According to Zidane (2012). Hypertriglyceridemia in rats who were given the high fat.diet results from a decrease in the activity of LPL. Wansi *et al* . (2013). showed that inhibition of HMG CoA (3-hydroxy-3-methyl-glutaryl- coA) is the cause of hypercholesterolemia in rats fed with the palm-oil enriched diet. This same author proposes that the alteration of activity of LCAT and of that of CETP brings about not only a considerable decrease in HDLc rates, but also an increase of LDLc.

This dyslipidemia was corrected after administration of buckthorn extract to the experimental rats. This improvement is similar to the one noted by Nwangwa *et al* . (2013) who studied the aqueous extract of *zygophyllum gaetulum*. According to Nwangwa *et al* (2013) the return of triglyceride rates and of total cholesterol to normal levels is explained by an important activity of LPL and by a decrease in concentration of acetyl-CoA that comes from B-oxidation of fatty acids. Zidane (2012) who studied the aqueous extract of *Portulaca orelacae*, noted a decrease in HDLc and LDLc rates, and proposed that this recovery is linked to the increase of the concentration of Apo A-1 on one hand, and to the increase of the synthesis and of the expression of LDLc receptors

Endothelin-1

After 6 months of experimentation, the high salt-fat diet induced a highly significant increase ($p < 0,001$) of endothelin-1 (ET-1) rates in the order of 57.43%. These results concord with those of Moon *et al* (2008) who reported that the atherogenic diet triggers not only an overexpression of the ET-1 gene, but also that of the conversion enzyme. On the other hand, Mir *et al* (2013) showed that this neuropeptide has deleterious effects in that it activates ET_A receptors that foster the increase of the activity of NADPHoxydase during nephroangiosclerose. Furthermore, the stimulation of ET_A via ET-1 leads not only to a vasoconstriction of the vessels but also to a proliferation of smooth muscular cells and a synthesis of extracellular matrix proteins that characterize vascular hypertrophy (Moon *et al.*, 2008).

Luc (2006), showed that CRP leads to the endothelial dysfunction by increasing the secretion of endothelin and decreasing secretion of nitric oxide.

Treatment with aqueous extract of buckthorn corrected the endothelin-1 rates. This is concordant with the results of Moon *et al* (2008) who worked on the aqueous extracts of rhubarb. This leads us to think that our extract inhibit the expression of the conversion enzyme of endothelin-1 and/or the ET_A receptors.

Renal status

Just like the other parameters, renal status (creatinin, urea) was highly increased in the experimental animals. Therrien (2009) suggested that the increase of these parameters is linked to the important number of non-functional ischemic nephrons in one way or another.

Furthermore, Fligny *et al* (2010) reported that the increase of plasmatic rates of endothelin-1 is accompanied by a decrease of the glomerular ultrafiltration coefficient. This could explain the increase of the average rates of creatinin and urea in our experimental rats.

The treatment by decocted extract of *R. alaternus* showed a progressive but significant regression of creatinin and urea. This improvement probably reflects the increase of glomerular filtration. Indeed, histological inspection of kidney sections in the treated animals shows the return of glomeruli and glomerular capillars to their normal size and shrinkage of the urinary space. These data suggest that the buckthorn could have a similar effects of rhubarb, indeed, this plant dilates vascular smooth muscle and activates an endothelium dependant NO /cGMP signaling pathway (Moon *et al.*, 2008)

The highly increased rate of uric acid can be attributed to the close relation between hypertension and hyperuricemia. According to Burnier (2005) hypertension leads to an arteriopathy and thereby a tissular ischemia

which then causes cellular destruction, release of DNA and RNA and degradation of ATP.

The highly significant improvement ($p < 0,001$) of the uric acid rate by decocted extract of *alaternus* in the experimental animals reminds that of Bouhaous *et al* (2011), who observed the same effects in rats that underwent hypercholesterolemic diet and were treated with *Globularia alypom*. This leads us to think that components of our extracts had an action on xanthine oxidase (Bouhaous, 2011)

Macromineral status

Similarly to Lasheen (2015) who observed a hypernatremia in Wistar rats that underwent a high-sodium diet, our experimental rats exhibit sodium rates in the order of 88, 69%. Such hypernatremia results, according to Sedeek *et al* (2013) to an increase in the activity of the epithelial sodium channels located in the collecting ducts (ENaC) via the angiotensin II. This mechanism is mediated by species reactive to oxygen (EROs) produced by NADPH oxydase under the action of the protein kinase C.

The decocted extract of *R. alaternus* leads to a highly significant improvement of Natremia. Since we did not measure aldosterone, we suggest that our extract could have a similar effect of lavender, indeed, the infusion of lavender flowers inhibits carbonic anhydrase in the proximal tubule leading to the decrease in the reabsorption of bicarbonates and consequently that of the sodium associated with these anions (Elhadjili, 2001).

Furthermore, the observed hyperkalemia in the experimental rats is authenticated by the works by Sedeek *et al.* (2013) According to these authors, the observed hyperkalemia can be caused by the overproduction of superoxide in the renal cortex, which decreases the activity of the potassium channels (ROMK), This parameter does not seem to react to treatment by *R. alaternus* extract.

Histophysiology of the kidney tissue in experimental Wistar rats and experimental rats treated with *alaternus* compared to control rats.

Condition of the renal arteries in the experimental Wistar rats and the experimental rats treated compared to control rats

Histological examination of the renal arteries in the experimental rats which were administered the high-salt-fat diet, exhibited important structural modifications when compared to the control rats. Among the observed characteristics are a hypertrophy of the adventitia/media, loosening and severance of the elastic lamina, thickening

of the intima, and multiplication of the internal elastic laminae, subendothelial hyalinization and hence narrowing of the vascular lumen. Boffa (2005) found the same lesions in humans suffering from nephro-angiosclerosis. According to Bouvet *et al* (2007) blood hypertension is the cause of a hypertrophic vascular restructuring. He also reports that arterial pressure stimulates the expression of certain genes and synthesis of certain proteins such as integrins and endothelin-1, which activate MAPKs, hence causing the expression of a transcription factor C-fos. This signaling pathway induces the proliferation of vascular smooth muscle cells that acquire a synthetic phenotype, capable of producing and secreting a large amount of cytokines, growth factors, as well as elements of the extracellular matrix including collagen. According to Baron (2013), hyperplasia and hypertrophy of the vascular smooth muscle cells might be linked on one hand to the synthesis of oxygen-reactive species by the NADPH oxydase and on the other hand to the increase of fibronectin and tenascin C. This supports the hypertrophic remodeling of the media in the rats that were administered the high-fat high-sodium diet.

In advanced stages of the pathology, the larger arteries (relatively to arterioles) undergo atheroma. CRP (C-reactive protein) increases the endothelial permeability to LDLc and fosters their endothelial infiltration and oxidation. The active endothelial cells express ICAM (Intercellular Adhesion Molecule) and VCAM (Vascular Cell adhesion molecule) receptors and E-Selectins (Luc, 2006) which hook on to the monocytes. These then filter through the intima via diapedesis and turn into macrophages capable of phagocytosing the oxidized LDL. Baron (2013) showed that endothelin-1 as well as oxygen reactive species foster the migration of smooth muscle cells towards the intima and acquire the secretory and oxidized LDL phagocytosing phenotype. These foam cells produce proinflammatory and prothrombotic factors that prompt the progression of the atherosclerotic plaque and the secretion of enzymes such as metalloproteases.

This vascular alteration in the experimental rats seems to be particularly remedied by the decocted solution of *R. alaternus*. In response to this phytotherapy, we noticed a cellular and tissular reorganization of all three tunics. This return to near-normalcy is evocative to that observed by Moon *et al* (2008) in rats on an atherogenic diet and treated with rhubarb aqueous extract. The *R. alaternus* decoction might act upon the endothelial dysfunction by decreasing levels of VCAM, ICAM, as well as atherogenic index and endothelin-1 and by improving the vascular eNOS/NO system in HFD rats (Moon *et al.*, 2008). These data give our extract a vasodilator effect.

Condition of glomeruli and tubules in experimental rats and experimental rats treated with alaternus compared to control rats

A profound architectural modification was observed in the nephrotic glomeruli of the rats on the high-fat/high-sodium diet. We reported degeneration as well as an ischemia probably induced by all of the noted alterations in the arteries. This ischemia is characterized by a widening of the urinary chamber and a loss of cellularity. According to Guerrot (2008) the nephron dysfunction causes a hypertrophy of unscathed glomeruli, hinting a proliferation of mesangial cells. These cells, under the influence of angiotensin II, synthesize not only proteins of the extracellular matrix such as fibronectin, laminin and type I and IV collagen⁶, but also endothelin-1, Flingny *et al.* (2010) Furthermore, glomerular hypertrophy is accompanied by a dilatation and increase of the pressure of glomerular capillaries, inducing macrophage recruitment, release of growth factors and a deposit of filtered proteins in the mesangium (Therrien, 2009) In conjunction to these profound tissular and molecular perturbations can be observed a fibrosis, hyalinosis, and focal segmental sclerosis, reflecting an alteration of the glomerular filtration and consequently a loss of functionality of these nephrons.

The tubular ischemia appears later. It manifests through an atrophy of the proximal convoluted tubules. This tubular degeneration shows a loss of the brush border PAS positive and an accumulation of inflammatory cells in the proximal tubular cells. The interstitium is studded with plasmocytes and lymphocytes. These results are close to those of Boffa *et al.* (2012), who proposes that the glomerular hypertension increases the permeability of the glomerular membrane to proteins and consequently fosters their passage to the tubular level. This increases proteinuria and saturates the lysosomal degradation system of the proximal tubular cells. This favors the expression of certain chemoattractant molecules - such as the monocyte chemoattractant protein-1 (MCP-1) - and of osteopontin by these cells, leading to the agglomeration of inflammatory cells in the interstitium. To these perturbations, add a necrosis, most probably caused by the inflammation, an apoptosis, and a detachment of the epithelial cells in the proximal tubules. According to the same author, this cellular detachment in the tubules is due to a failure of the adhesion molecule "E-cadherin" because of the TGF- β 1 inhibitor. The inhibitory expression of this cytokin is stimulated by the increased abnormal synthesis of endothelin-1 (Flingny, 2010). Further more, the TGF- β 1 induces the synthesis of alpha actin and of matrix metalloproteinases (MMP) which are responsible for the alteration of the basal membrane of the tubular cells and their differentiation in myofibroblasts, in turn responsible for tubulointerstitial fibrosis, which aggravates the renal function (Therrien, 2009), These glomerular and tubular alterations are largely improved

following treatment with aqueous extract of buckthorn. We suggest that our buckthorn based treatment has a regulating effect on the endothelin-1 thus inhibiting increase of TGF- β 1 and therefore recovery of the tubular cell adhesion by the E-cadherin

CONCLUSION

From this investigation, it is apparent that the endothelial dysfunction as well as the glomerular and tubular lesions induced by a high-salt-fat diet in laboratory rats is significantly corrected after treatment with aqueous solution. The decocted extract is characterized by his vasodilator, diuretic and hypotensive effects, which improve the glomerular filtration rate. Their antioxidant properties enhance the expression of nitric oxide by influencing the synthesis and action of endotheline-1. All of these data let us suggest that aqueous extract of our plant contains active ingredients having a nephroprotective effect. The determination of the chemical structure of these compounds by chromatography coupled with a mass spectrophotometry will make it possible to better understand the mechanism of molecular action involves in the nephroprotective effect of this medicinal plant.

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