Therapeutic effect of alkaloids and glycosides of colocynth seeds on liver injury, associated with metabolic syndrome in wistar rats, subject to nutritional stress

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Abstract: The *Citrullus colocynthis*, commonly called colocynth, is known because of its purgative effects and whose seeds are commonly used as certain diseases treatment, namely liver diseases, in the Mediterranean countries traditional medicine. This study aims to analyze the effect of two colocynth extracts « glycosides » and « alkaloids » on metabolic and histological disorders associated with liver function in Wistar rats (*Rattus norvegicus*). This pathology is due to an enriched oil palm diet. For this purpose, Wistar male rats n = 18, weighing between 130g and 150g, are divided into two lots. A control group (C) n = 6, receives a standard laboratory diet ; an experimental group (E) n = 12, receives a standard laboratory diet supplemented with palm oil. After seven months of experimentation, 8 experimental rats were sacrificed for the morphological study and the remaining 12 rats undergo a colocynth treatment (Tr) for eight weeks. They are subdivided into: The first six experimental rats receive a 70mg/kg single intraperitoneal injection of ethanol extract of cucurbitacin glycosides (Glc). The second lot receives a 70mg/kg single intraperitoneal injection of total alkaloids extract (Alc). The animals of (E) group showed hyperglycemia, hyperinsulinemia, hyperlipemia, dyslipoproteinemia, a significant increase of the enzymatic activity of transaminase (AST and ALT) and alkaline phosphatase (ALP). Histological examination of the liver gland shows major damages Non-alcoholic steatohepatitis [NASH]. Treatment with colocynth glycosides and alkaloids reveals a significant improvement at different levels in plasma as well as in tissue. Treatment with colocynth glycosides and alkaloids shows a hypoglycemic effect, lipid-lowering a well as a hepato-protective effect.

Keywords: High-fat diet, metabolic syndrome, *Citrullus colocynthis*, glycosides, alkaloids.

INTRODUCTION

The metabolic syndrome components (MS) are the most important risk factors for our today's health (Poudyal *et al*, 2011). Indeed, food quality and the fatty acids kind of the diet are essential elements in the setting of this pathology (Dalle Grave *et al*, 2010).

The MS is a complex disorder associated with abdominal obesity as well as an excess of free fatty acids (FFA); following an intense lipolysis of the adipose tissue, an insulin resistance, hyperglycemia, hypertriglyceridemia, a rate of low HDLc and a high blood pressure (Vaidya *et al*, 2009).

As a result of this pathogenesis, an increase in hepatic glucose production and a reduction of its peripheral uptake (muscular) - due to an alteration of the GLUT4 carrier translocation - is noted. The plethora of FFA causes, directly and indirectly, a Langerhansien exhaustion, resulting in hyperinsulinemia and insulin resistance.

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The FFA delivery to the liver promotes an excessive synthesis of VLDL rich in triglycerides and apo B. The evolution of these particles reports hypertriglyceridemia, a decrease in HDLc, as well as the formation of small dense LDLc particles, particularly atherogenic (Buysschaert, 2006).

For the analysis of metabolic disorders, it is accepted that fat-rich diets can be used to choose a good rodent model for metabolic syndrome and insulin resistance (Lingohr *et al*, 2002). It has been reported by several authors that a fat-rich diet is the cause of cardiovascular diseases and of type 2 diabetes (Riserus, 2008). However, for our study, this syndrome was induced, in laboratory rats, by a palm oil-rich diet. This oil is rich in saturated fatty acids (50% of palmitic acid). It has a hypercholesterolemic effect (Cater *et al*, 1997) by increasing more the LDLc (Vega-Lopez *et al*, 2006) as well as hypertriglyceridemic (Guelzim and Hermier, 2010) and hyperinsulinemic (Shinji *et al*, 1996).

The main interest of our investigation is to appreciate the evolution of metabolic and histopathological complications of two « hepato-pancreatic » endocrine

glands for their importance in the homeostasis maintenance, faced to nutritional stress caused by a high-calorie diet rich in palm oil (HFDP) in Wistar rats and evaluate the effects of total alkaloids and glycosides ethanolic extracts from a hypolipidemizing natural substance, namely by its inhibiting action of HMG-CoA reductase hepatic the "*Citrullus colocynthis* "(Jeyanthi *et al*, 2009). The same parameters are followed and studied in experimental rats.

The *Citrullus colocynthis*, commonly known as colocynth, is a native arid soils cucurbit. It is used as treatment of certain diseases in the traditional medicine of Mediterranean countries. This plant phyto-chemical tests show from the isolation various active substances that have therapeutic properties such as anti-inflammatory (Sayed Darwish, *et al*, 1973). Anti-hepatotoxic (Agil *et al*, 1999), insulin-stimulating (Nmila *et al*, 2002), lipid lowering (Daradka *et al*, 2007), antioxidant (Kumar *et al*, 2008) and hypoglycemic (Benariba *et al*, 2013).

MATERIALS AND METHODS

Animals and diets

In this study 26 adult male Wistar rats are divided into two lots:

- A Control group of six animals (C), receives the standard laboratory diet (30g of pellets ≈ 93 calories per day)
- A Second lot of twenty experimental animals (E) receives the lipid diet (30g of pellets + 9g fat ≈ 174 calories per day) that is 46% supplementation of palm oil (HFDP).

After seven months of experimentation, 8 experimental rats were sacrificed for the morphological study and the remaining 12 rats undergo a colocynth treatment (Tr). To assess the *Citrullus colocynthis* therapeutic effect, two types of extracts were tested for eight weeks.

- Six experimental rats receive a single intraperitoneal injection of 70mg/kg of cucurbitacins glycosides ethanol extract (Glc).
- Other Six experimental animals receive a single intraperitoneal injection of 70mg/kg of total alkaloids extract (Alc).

All experimental procedures were authorized by the Institutional Animal Care Committee of the National Administration of Algerian Higher Education and Scientific Research. Ethical Approval Number: Law 98-11 of 22 August 1998.

The standard diet

Wistar rats are fed daily with food, in the form of granules of commercial origin, provided by the National Agency for Livestock Food (O.N.A.B.); it is a balanced diet to meet rats' needs. They also receive water "*ad libitum*".

Lipid diet

Palm oil is obtained by hot pressing of oil palm fruit: The drupes. These oil palms are mainly grown in Africa, Asia and Latin America. Palm oil has a composition of 100% fat in the form of glycerides. It contains about 50% saturated fatty acids and 50% unsaturated fatty acids.

In addition to these triglyceride major components, palm oil, like any vegetable oil, contains « minor » compounds, consisting of vitamin E, carotenoids, phytosterols, phenolic compounds, known for its antioxidant properties. However, some of its nutritional properties decrease or disappear once it is heated and refined.

Indeed, it loses its vitamin properties and therefore it lightens and becomes solid at room temperature. Thus palm oil is found in the form of lumps (like butter).

Extremely rich in saturated fatty acids, palm oil is harmful for health if consumed in large quantities. In fact, saturated fatty acids promote an increased of cholesterol - particularly the bad one (LDL). This latter, itself acts as a harmful agent on the cardiovascular system. One gram of oil has an energy density of nine calories, so it is a concentrated food source.

Refined palm oil, solid, whitish with a melting temperature of 38-40 ° was the subject of our study. We impregnate pellets (standard diet) in liquid palm oil and let soak, thus we obtain the high fat diet (HFDP).

Preparation of plant material

The colocynth fruits (*Citrullus colocynthis* L. Schard); of a cucurbitaceae family were harvested at maturity during the month of September in the region of Ain Sefra, Wilaya of Naama (south-west of Algeria).

Seeds are recovered from fruit, dried away from light and crushed. A plant material defatting is performed, with Bruneton technique (1999), to eliminate substances that disrupt the extractive process.

Total alkaloids extraction, in acidic medium, is carried out Harborne method (1998) while glycosides extraction is conducted using the method of Natiq *et al.* (1989).

Blood samples

The animals are monitored metabolically through blood tests performed by puncturing the retro-orbital sinus on the fasting animal, once a month, throughout the experiment. Blood is collected on heparinized tubes and centrifuged at 3000rev/min. for 10 minutes. The plasma obtained is stored at -25°C until the analyses.

Biochemical analyzes

Biochemical assays (blood glucose, total cholesterol, triglycerides, HDL-C and transaminases) at plasma levels

are determined by enzymatic colorimetric methods (Spin react kits, Spain), The insulin asset is determined by the imunoenzymatique ELISA method (Enzyme Linked Immuno Sorbent Assay).

Organ removal

After nine months of experimentation, the organs are removed after in situ fixation by perfusion (Baleydier, 1973). After anesthesia by intraperitoneal injection of urethane at 25% - at 0.4ml/100g of body weight - the perfusion is carried out by the intracardiac way with suitable fixatives such as the aqueous bouin for morphological study and the sublimated Holland bouin for histochemical study. The perfusion time is 15 to 20 min.

The liver, object of our study, is removed and stored in the appropriate fixative for 5 to 6 days. It is then washed, dehydrated with ethanol and included in paraffin for the morpho-histochemical study. The cuts of 2 µm thickness are stained with the heidenhain Azan of and the PAS (GABE 1968).

STATISTICAL ANALYSIS

Results are presented under the form of means ± standard error. The values are statistically compared by the Mann-Whitney "U" test (Statsoft Statistica software). The means are considered significantly different at P <0.05. *E versus C, ●Tr versus E

RESULTS

Compared with controls, average body weight of animals, subject to high-fat diet, increases significantly (P <0.02) from the first month of the diet and becomes highly significant (P <0.001) at the sixth month. This increase continues until the end of the experiment, it reaches 117.18%. In animals subject to fat diet, glycemia gradually increases compared to those subject to the standard laboratory diet.

Indeed, this increase is not significant (p <0.05) in the first month but becomes optimal from the third month (p <0.001). It reaches 233.77% and is maintained until the end of the experiment. Similarly to glycemia, triglycerides of the experimental group animals increases compared to that of the control group. The increase is observed within the first month of the diet (p<0.02) and becomes maximal from the third month (P<0.01). The values increase from 0.69±0.03 to 2.55±0.32g/l. The increase is of 269.56%.

As for of cholesterol, our experimental rats showed an optimum (P <0.01) in the third month of the experiment compared to that of the control rats. The increase is approximately of 101.32%. The increase is maintained until the end of the experiment (P<0.001).

As for HDL, a highly significant decrease (p<0.001) is observed in our experimental rats during the fourth month of the experiment, compared to the control rats. This drop reaches 37.5%. However, LDLc knows a highly significant rate (P<0.001) for the third month experimental group animals, compared to control group animals. The increase is around 120%. It persists until the end of experimentation.

Concerning the liver function markers, we also observed a highly significant increase of aspartate aminotransferase levels in our experimental animals (P <0 .001), comapred to those of control group, starting from the third month and remains until the end of the experiment. This elevation is of 120.74%.

In addition, ALT levels also increased very significantly (P<0.01) in the experimental group animals compared to those of the control group, from the fourth month. The increase continues until the end of the experiment. It is of 116.04%.

Similarly, alkaline phosphatase marks a very significant increase (P<0.01) in animal subject to a fat diet, compared to the control animals in the third month. The increase is maintained until the end of the experiment. It is of 101.02%.

The observed weight regression in experimental group, treated with alkaloids, compared to those treated with glycosides is significant (P<0.02) starting from the sixth week. It becomes very significant (P<0.01) at the end of treatment.

Note that for the animals treated with alkaloids compared to experimental animals, the weight regression is highly significant (P<0.01) from the sixth week of treatment. Whereas for those treated with glycosides, the answer is little insignificant (P<0.02).

The recorded hyperglycemia, in our experimental animals, improves when administering colocynth glycosides treatment. This improvement is very significant (p <0.01) in the fourth week and becomes highly significant (p <0.001) from the sixth week of treatment, whereas it is of low amplitude with alkaloids treatment (P <0.05), therefore treatment with glycosides compared to that of alkaloids remains the best solution, regarding glucose regulation. As for alkaloids treatment, this improvement is of low amplitude.

Regarding triglycerides, experimental rats treated with colocynth alkaloids show a highly significant regression (P<0.001) in the sixth week of treatment and remains highly significant (P<0.01) until the end of treatment. With the colocynth glycosides treatment, hypertriglyceridemia improves in the sixth week of

The percentage composition of this diet is as follows

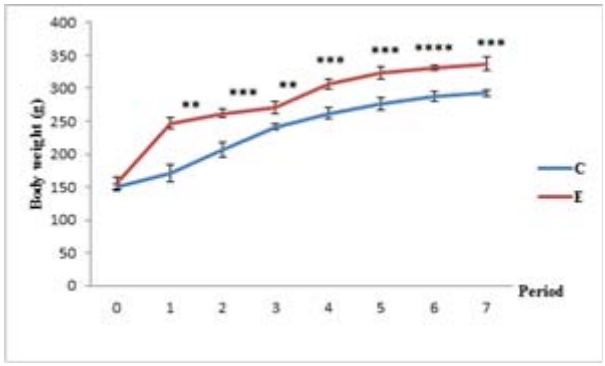
Food	Carbohydrate	Protein	Fat	Vitamin-Mineral Complex
Quantity in %	49,80	23,50	5,00	5,70

Fatty acid composition of palm oil.

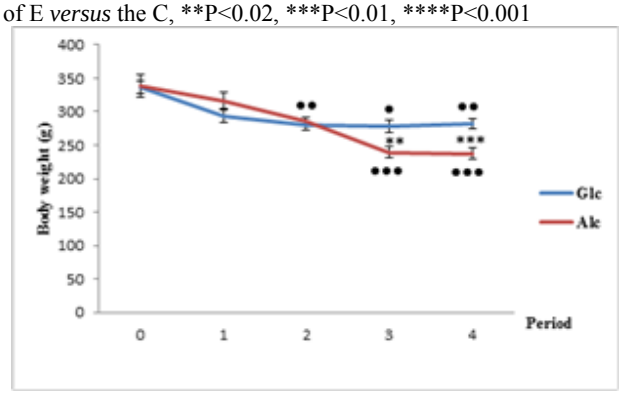
Fatty acids		Quantity in %
saturated fatty acids	lauric acid C12 :0	< 0.5
	myristic acid C14 :0	0.5-2
	palmitic acid C16 :0	39.5-47.5
	stearic acid C18 :0	3.5- 6
Mono-unsaturated fatty acids	oleic acid C18 :1n-9	36-44
Polyunsaturated fatty acids	linoleic acid C18 :2n-6	9-12
	linolenic acid C18 :3n-3	<0.5

treatment (P<0.001). This improvement decreases at the end of treatment and becomes of little significance (P <0.05).

Cholesterolemia gradually decreases in experimental animals treated with colocynth alkaloids. This decrease is significant (P<0.02) in the fourth week of therapy and becomes highly significant (P<0.001) at the end of treatment. It is of 36.51%.



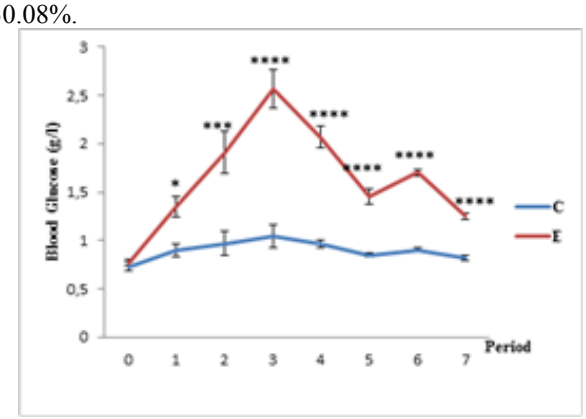
Values are expressed as mean ± SEM 1 period=1 month Control group (C), Experimental group (E), n=6 (C), n=20(E) Difference of E *versus* the C, **P<0.02, ***P<0.01, ****P<0.001



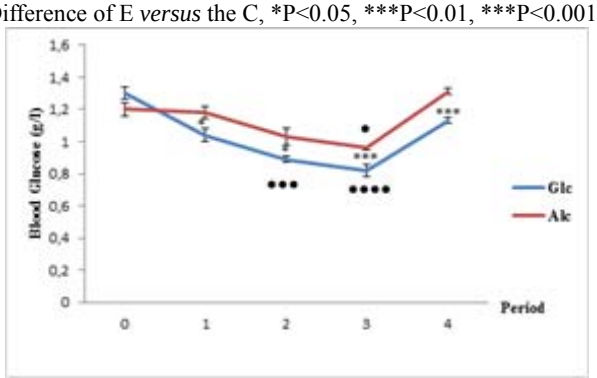
Values are expressed as mean ± SEM 1 period= 2 weeks Glycosides group (Glc), Alcaloids group (Alc) n = 6 (Glc), n=6(Alc) Difference of Alc *versus* the Glc **P<0.02 (Alc_3 Vs Glc_3), ***P<0.01 (Alc_4 Vs Glc_4) Difference of Alc versus Alc and Glc versus Glc ●P<0.05 (Glc_3 Vs Glc_0), ●●P<0.02 (Glc_2 Vs Glc_0), ●●●P<0.01 (Glc_4 Vs Glc_0), ●●●P<0.01 (Alc_3 Vs Alc_0), ●●●P<0.01 (Alc_4 Vs Alc_0)

Fig. 1: Average evolution of body weight in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

Similarly, in experimental animals, treated with glycosides, cholesterolemia gradually decreases but not significantly (P<0.05) in the third week to become very significant (P<0.01) in the fifth week of treatment. It is of 30.08%.



Values are expressed as mean ± SEM, 1 period=1 month Control group (C), Experimental group (E), n = 6 (C), n=20(E) Difference of E *versus* the C, *P<0.05, ***P<0.01, ***P<0.001

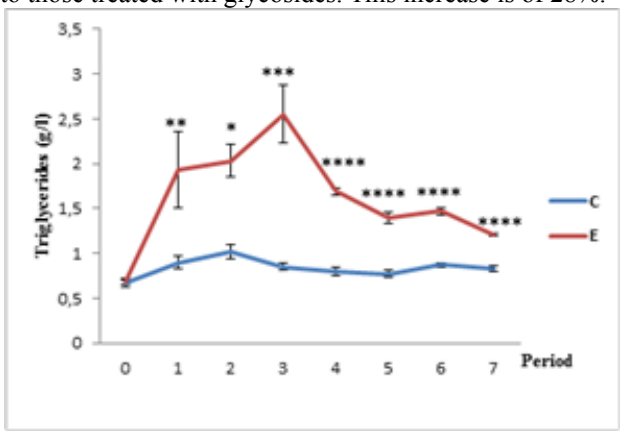


Values are expressed as mean ± SEM 1 period= 2 weeks Glycosides group (Glc), Alcaloids group (Alc) n = 6 (Glc), n=6(Alc) Difference of Alc *versus* the Glc *P<0.05 (Alc_1 Vs Glc_1), *P<0.05 (Alc_2 Vs Glc_2), ***P<0.01 (Alc_3 Vs Glc_3),

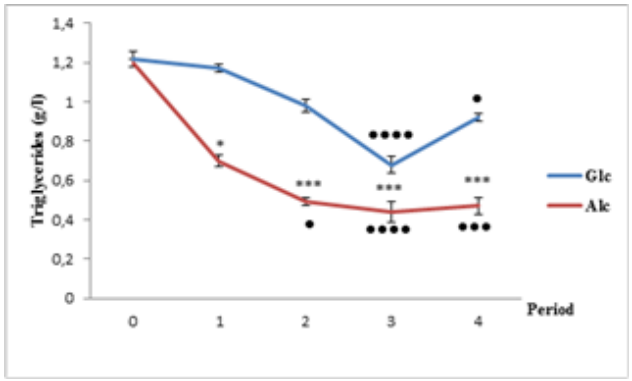
**P<0.01 (Alc_4 Vs Glc_4) Difference of Alc versus Alc and Glc versus Glc ●P<0.05 (Alc_3 Vs Alc_3), ●●●P<0.01 (Glc_2 Vs Glc_0), ●●●●P<0.001 (Glc_4 Vs Glc_0)

Fig. 2: Average evolution of glycemia in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

The change in HDL-c plasma levels, in our treated experimental animals is of low amplitude. We note a significant increase (P<0.02), on the fourth week of treatment, in animals treated with the alkaloids, compared to those treated with glycosides. This increase is of 28%.



Values are expressed as mean ± SEM, 1 period=1 month Control group (C), Experimental group (E), n = 6 (C), n=20(E) Difference of E *versus* the C, *P<0.05, **p<0.02, ***P<0.01, ****P<0.001



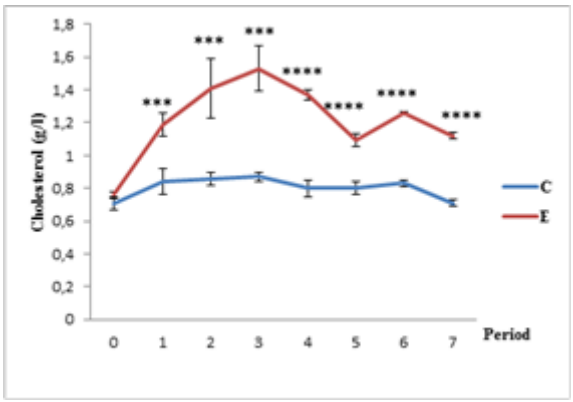
Values are expressed as mean ± SEM, 1 period= 2 weeks Glycosides group (Glc), Alcaloids group (Alc), n = 6 (Glc), n=6 (Alc), Difference of Alc *versus* the Glc, *P<0.05 (Alc_1 Vs Glc_1), ***P<0.01 (Alc_2 Vs Glc_2), ***P<0.01 (Alc_3Vs Glc_3), ***P<0.01 (Alc_4 Vs Glc_4), Difference of Alc versus Alc and Glc versus Glc ●P<0.05 (Alc_2 Vs Alc_0), ●P<0.05 (Glc_4 Vs Glc_0), ●●●P<0.01 (Alc_4 Vs Alc_0), ●●●●P<0.001 (Alc_3 Vs Alc_0) (Glc_3 Vs Glc_0)

Fig. 3: Average evolution of plasma triglycerides in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

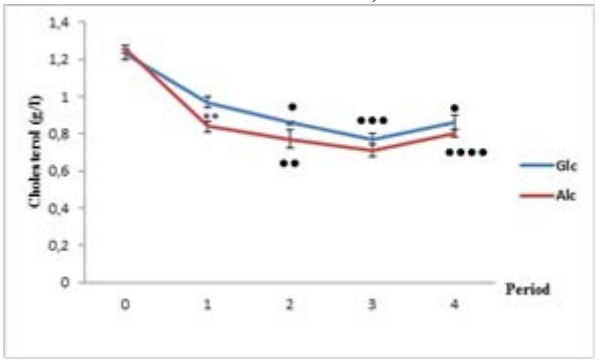
However, the LDL-c plasma levels in treated experimental animals gradually decrease. In those treated with glycosides, this decrease is very significant (P<0.01) in the fourth week of treatment and becomes of little significance (P <0.05) at the end of treatment.

In animals treated with alkaloids, this decrease is very significant (P<0.01) in the sixth week of treatment and becomes of little significance (P<0.05) at the end of treatment. The decrease is respectively of 45.95% and 49.35%.

The plasma levels of aspartate aminotransferase, in experimental animals, treated with colocynth glycosides, show a variation of small amplitude. Indeed, the difference is statistically not significant However, colocynth alkaloids, administered to experimental animals, improve these rates very significantly (P <0.01), in the sixth week of treatment. the decrease reaches 29.68%.



Values are expressed as mean ± SEM, 1 period=1 month Control group (C), Experimental group (E), n = 6 (C), n=20(E) Difference of E *versus* the C ***P<0.01, ****P<0.001



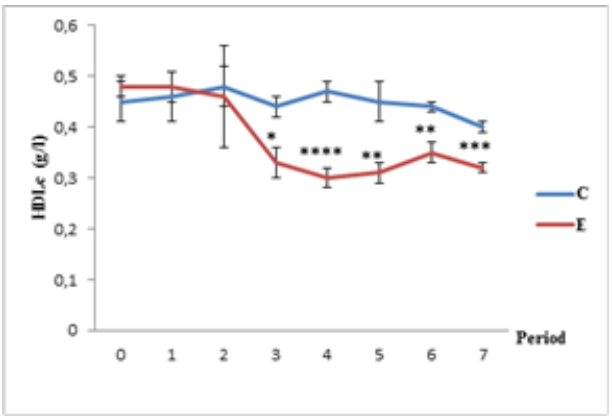
Values are expressed as mean ± SEM, 1 period= 2 weeks Glycosides group (Glc), Alcaloids group (Alc) n = 6 (Glc), n=6 (Alc) Difference of Alc *versus* the Glc **P<0.02 (Alc_1 Vs Glc_1) Difference of Alc versus Alc and Glc versus Glc ●P<0.05 (Glc_2 Vs Glc_0), ●P<0.05 (Glc_4 Vs LGl_0) ,●●P<0.02(Alc_2 Vs Alc_0), ●●●P<0.01 (Glc_3 Vs Glc_0), ●●●●P<0.001 (Alc_4 Vs Alc_0)

Fig. 4: Average evolution of total plasma cholesterol in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

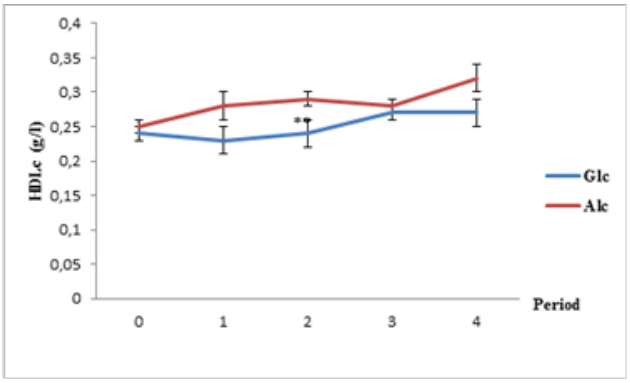
As for alanine aminotransferase rates of experimental animals, treated with alkaloids, compared to those treated colocynth glycosides, a decrease of little significance (P <0.05) is observed in the second week of treatment, this regression becomes very significant (P<0.01) from the fourth week and remains until the end of treatment (P <0.01).

Plasma levels of alkaline phosphatase, in experimental animals, treated with colocynth glycosides, show a variation of small amplitude. Indeed, the difference is statistically not significant.

However, the experimental animals, treated with alkaloids colocynth improve these rates very significantly (P <0.01) from the fourth week of treatment.



Values are expressed as mean ± SEM, 1 period=1 month Control group (C), Experimental group (E) n = 6 (C), n=20(E) Difference of E *versus* the C *P<0.05, **p<0.02, ***P<0.0, ****P<0.001

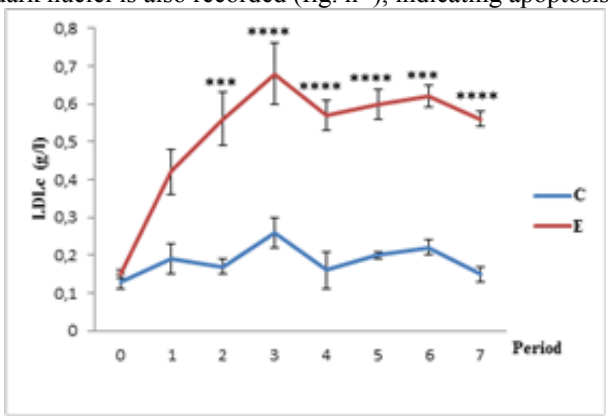


Values are expressed as mean ± SEM, 1 period= 2 weeks Glycosides group (Glc), Alcaloids group (Alc) n = 6 (Glc), n=6 (Alc) Difference of Alc *versus* the Glc **P<0.02 (Alc_2 Vs Glc_2)

Fig. 5: Average evolution of HDLc in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

This decrease continues and becomes of little significance (P<0.05) at the end of the treatment. It reaches 28.03%. Compared to treatment with glycosides, that with alkaloids gives better results. The insulin levels in experimental animals, subject to high-fat diet increase significantly (P<0.001), compared to those of control animals, subject to the standard laboratory diet, from its beginning. This increase is maintained until the end experimentation. It is of 486.45%.

Insulin levels, recorded after treatment administration to experimental animals, show a very significant improvement (P<0 .01) with the alkaloids extract as well as with the glycosides extract. It is respectively of 71.77% and 86.03%. Histological and histochemical test of the liver gland in experimental animals, subject to high- fat diet, compared to those of control animals, subject to standard laboratory diet, shows a deep architectural disorganization; disruption of structural aspects are at inflammatory and necrotic scale. Indeed, an hypertrophy of hepatocyte light nuclei is recorded (fig. ◄g), very likely sign of an over activity ; and at some places dentate dark nuclei is also recorded (fig. h<), indicating apoptosis.



Values are expressed as mean ± SEM, 1 period=1 month Control group (C), Experimental group (E) n = 6 (C), n=20(E) Difference of E *versus* the C ***P<0.01, ****P<0.001

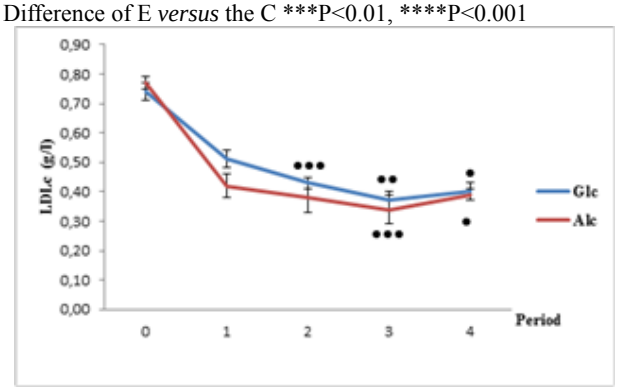


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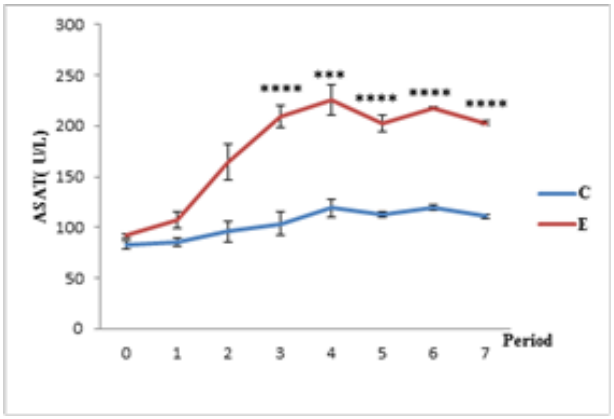
Fig. 6: Average evolution of LDLc in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

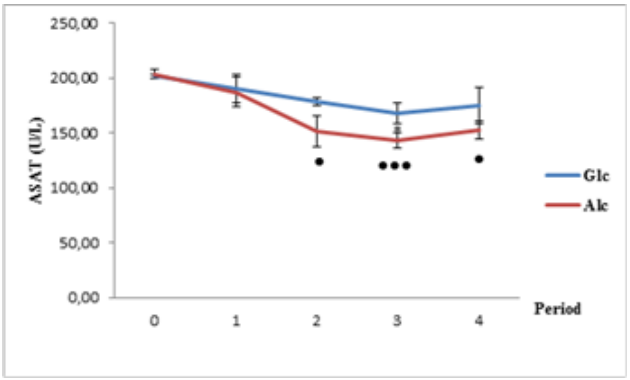
Note the presence of ballooned and vacuolated hepatocytes (fig. f). This state is certainly due to hydropic degeneration. In addition, inflammatory foci - positive PSA - are found in the liver parenchyma (fig. m). The latter reveal a significant recruitment of leukocytes, including neutrophils, lymphocytes and macrophages.

At their vicinity, necrotic areas are also observed (fig. h), showing Councilman bodies which are characterized by retracted and apoptotic hepatocytes. The core of the latter is often dark, dentate and sometimes segmented (fig. g).

In addition to all these observations, we detect on some sections of the examined gland the existence of eosinophilic body, certainly derived from the cytoskeleton, hence the presence of Yalin Mallory bodies (fig. g and h).



Values are expressed as mean ± SEM, 1 period=1 month Control group (C), Experimental group (E) n = 6 (C), n=20(E) Difference of E *versus* the C, ***P<0.01, ****P<0.001



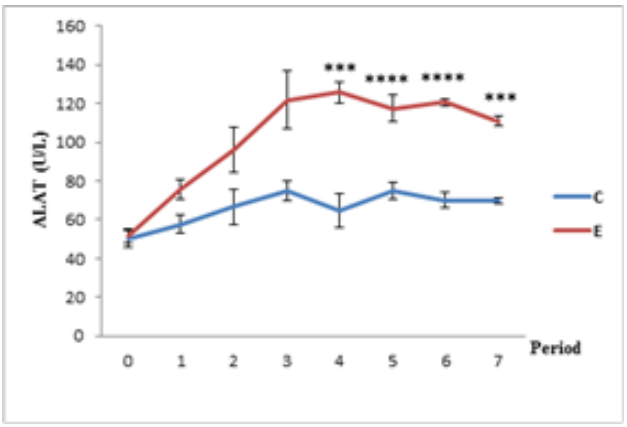
Values are expressed as mean ± SEM, 1 period= 2 weeks Glycosides group (Glc), Alcaloids group (Alc) n = 6 (Glc), n=6 (Alc) Difference of Alc *versus* the Glc *P<0.05 (Alc_3 Vs Glc_3) Difference of Alc versus Alc and Glc versus Glc ●P<0.05 (Alc_2 Vs Alc_0), ●P<0.05 (Alc_4 Vs Alc_0) , ●●●P<0.01 (Alc_3 Vs Alc_0)

Fig. 7: Average evolution of aspartate aminotransferase AST in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

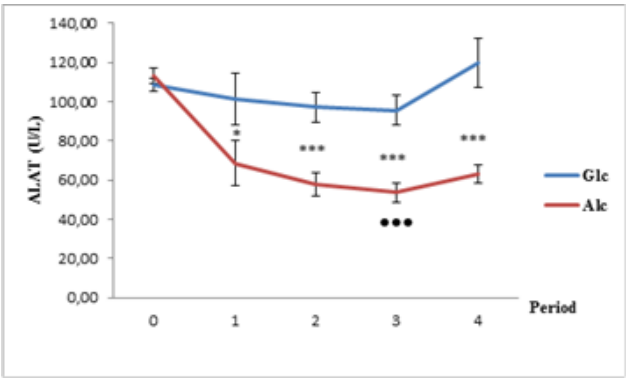
Furthermore, the hepatic infiltration by cells and collagen fibers of connective tissue is noted. The strong presence of the elements of the extra cellular matrix, positive APS (fig. n) characterize the installation of fibrosis (fig. d), indicating inflammation. The significant expansion of the sinusoidal cavities (fig. c and d) is also noted.

This amphicrine gland physiopathology of is also expressed by the metabolic damage, lipid infiltration, revealing a hepatocyte micro-macrovesicular steatosis (fig. e and f). En effet, les animaux traités aux alcaloïdes, leur glande hépatique montre une franche réorganisation cellulaire et tissulaire (fig. j et q).

As for the microscopic analysis of the gland of animals treated with glycosides and alkaloids, compared to that of the experimental group, structural and physiological improvements are revealed. Indeed, the hepatic gland of animals, treated with alkaloids, shows a distinct cellular and tissular reorganization (fig. j and q).



Values are expressed as mean ± SEM, 1 period=1 month Control group (C), Experimental group (E), n = 6 (C), n=20(E) Difference of E *versus* the C, ***P<0.01, ****P<0.001



Values are expressed as mean ± SEM, 1 period= 2 weeks Glycosides group (Glc), Alcaloids group (Alc), n = 6 (Glc), n=6 (Alc) Difference of Alc *versus* the Glc, *P<0.05 (Alc_1 Vs Glc_1), ***P<0.01 (Alc_2 Vs Glc_2), ***P<0.01 (Alc_3 Vs Glc_3), ***P<0.01 (Alc_4 Vs Glc_4) Difference of Alc versus Alc and Glc versus Glc ●●●P<0.01 (Alc_3 Vs Alc_0)

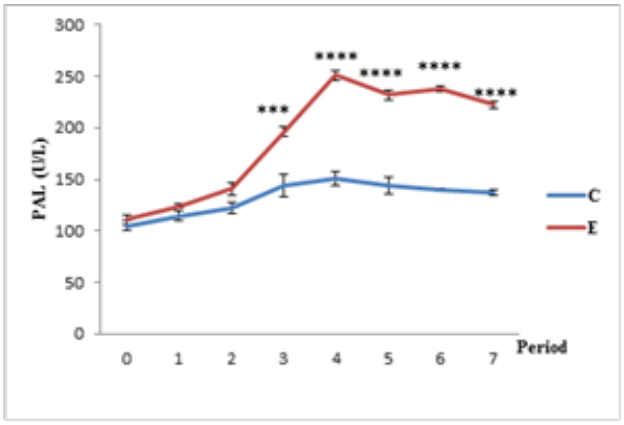
Fig. 8: Average evolution of alanine aminotransferase ALT in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

This treatment seems effective because it corrects almost all of the observed disturbances in experimental group of animals. Nevertheless the micro steatosis and cell infiltration are still present in the gland in the experimental animals, treated with glycosides.

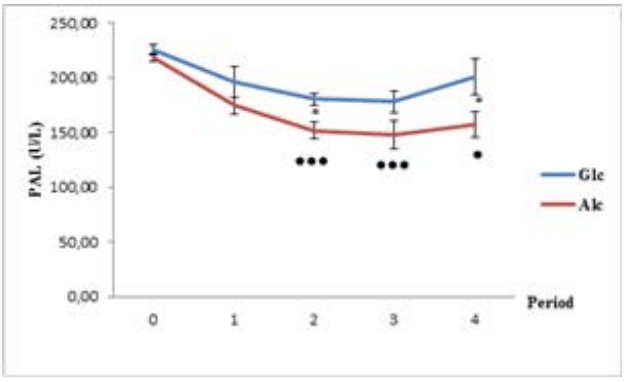
Indeed, following the treatment with alkaloids, we note a reorganization of the liver tissue. However the microstéatose and cell infiltration are still observed, following the treatment with glycosides (fig. i and o).

DISCUSSION

Through administration of a palm oil-based high-fat diet, rich with saturated fatty acids in *Rattus norvegicus*, for seven months leads to visceral obesity and a metabolic dysfunction in particular glucose regulation, essentially marked lipemia by the increase in plasma triglycerides, total cholesterol and dyslipoproteinemia marked by an increase in LDL cholesterol levels and a drop in HDL.



Values are expressed as mean ± SEM, 1 period=1 month Control group (C), Experimental group (E) n = 6 (C), n=20(E) Difference of E *versus* the C ***P<0.01, ****P<0.001

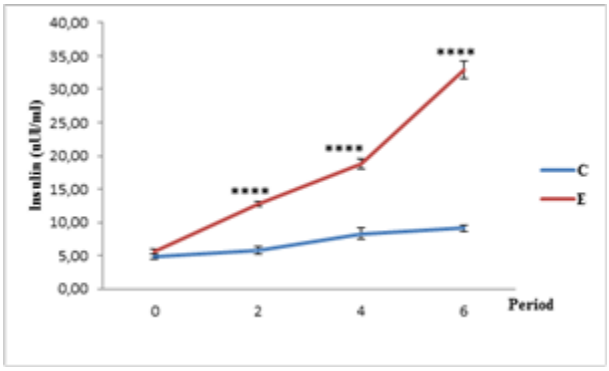


Values are expressed as mean ± SEM, 1 period= 2 weeks Glycosides group (Glc), Alcaloids group (Alc) n = 6 (Glc), n=6 (Alc) Difference of Alc *versus* the Glc *P<0.05 (Alc_2 Vs Glc_2), *P<0.05 (Alc_4 Vs Glc_4) Difference of Alc versus Alc and Glc versus Glc ●P<0.05 (Alc_4 Vs Alc_0) ●●●P<0.01 (Alc_2 Vs LAl_0), ●●●P<0.01 (Alc_3 Vs Alc_0)

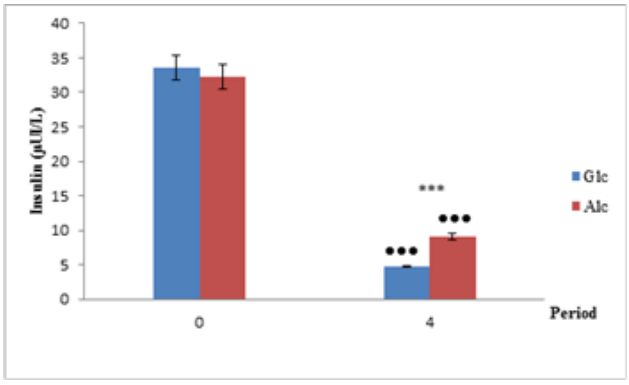
Fig. 9: Average evolution of alkaline phosphatase PAL in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

Body weight

At the end of the experiment, rats subject to our high-fat diet (H-FD) show a 117.18% weight increase. These results match those of Eisinger *et al*, (2014) who fed mice with lipid-enriched diet. According to Niloofar *et al*, (2010), this weight gain is related to dysregulation of the hormones involved in the energy balance, such as hyperleptinemia and hyperinsulinemia, resulting in hyperphagia and therefore a high energy input, due to H-FD overconsumption, because of its low satiaty power, its high energy density and high palatability. According to the same researcher, there is stimulation of nuclear receptors of the PPARs family (Peroxisome proliferator activated receptors) by saturated fatty acids, leading to adipocytes hypertrophy and hyperplasia.



Values are expressed as mean ± SEM, 1 period=2 month Control group (C), Experimental group (E) n = 6 (C), n=20(E) Difference of E *versus* the C ***P<0.01, ****P<0.001



Values are expressed as mean ± SEM, 1 period= 4 weeks Glycosides group (Glc), Alcaloids group (Alc) n = 6 (Glc), n=6 (Alc) Difference of Alc *versus* the Glc ***P<0.05 (Alc_4 Vs Glc_4) Difference of Alc versus Alc and Glc versus Glc ●●●P<0.01 (Alc_4 Vs LAl_0), ●●●P<0.01 (Glc_4 Vs Glc_0)

Fig. 10: Average evolution of insulinemia in Wistar control rats, submitted to high-fat diet and treated with glycosides and alkaloids.

Treatment with colocynth corrects this overweight in our animals, that with alkaloids compared to glycosides. The regression is highly significant (P<0.01). It is of 30%. Choi *et al*, (2015) found this anti-obesity effect, due to the inhibitory action of adipogenesis by alkaloids of Coptis chinensis, administered to of 3T3-L1 adipocytes cultures.

However, the weight regression is explained by a decrease in the differentiation of pre-adipocytes into adipocytes, due to the PPARγ decreased expression as well as a decreased lipoprotein lipase activity and the expression of the synthase fatty acid, deducting a lipogenesis decrease (Niloofar *et al*, 2010)

Glycoregulation Status

Glycemia

The introduction of the diet supplemented with palm oil in our animals induced a highly significant increase of blood glucose levels ($p<0.001$) that reaches 233.77%. Our results confirm those of Zhigang *et al*, (2015). who worked on C57BL / 6J mice, subject to an atherogenic diet.

According to Tony *et al*, (2002), this hyperglycemia is due to the PKC-θ activation (Protein kinase C theta) by fatty acids, inducing a phosphorylation increase of serine / threonine residues of insulin receptor and a phosphorylation reduction of tyrosine residues, necessary for the insulin hypoglycemic action.

These blood glucose high levels are being significantly improved after colocynth treatment. This regulation is highly significant ($p<0.001$) and is carried out particularly with glycosides, compared to alkaloids. This recalls the works of Lahfa *et al*, (2015), who observed the same effects in rats made diabetic with streptozotocin.

Abd El-Baky *et al*, (2009) *Citrullus colocynthis* antidiabetic action is due to improved insulin secretion, increasing peripheral glucose uptake or to gluconeogenesis reduction by inhibiting counter-regulation hormone secretion, namely cortisol, glucagon and growth hormone.

Whereas for Shafaei *et al*, (2014), the colocynth hypoglycaemic effect can be attributed to the activation of the glucokinase enzyme, responsible for the glycogen accumulation in the hepatocytes

Insulinemia

The Insulinemia in animals subject to H-FD increases very significantly ($P<0.001$). The increase is of 486.45%. This hyperinsulinemia is in agreement with that observed by Thiago *et al*, (2013) in Wistar rats subject to enriched fat diet.

The works of Seiji *et al*, (2009) showed that palmitic acid from palm oil could induce insulin resistance by inhibiting the tyrosine residues phosphorylation on both receptor substrates to insulin IRS 1/2 (Insulin Receptor substrate), via the activation of Jun kinase (JNK).

Amanda *et al*, (2012) think of oxygen reactive species that play a role in the JNK activation, induced by palmitate. For this purpose, the insulin pathway, according to Amanda *et al*, can be altered by ROS, inducing the decrease of the phosphorylation of serine / threonine IRS, transcript reduction of GLUT4 gene and regression of the mitochondrial activity.

Furthermore, hyperinsulinemia recorded in our animals is probably due to reduced insulin receptors, decreased glucose transport and a pacification of the activity of glycogen synthase in liver and muscle; The result is a reduction in the storage of glucose as glycogen. These disorders subsequently generate hypertrophy and hyperplasia of fat tissue, resulting in cytokine secretion and decrease of the cellular response to insulin (Niloofar *et al*, 2010).

Treatment with the two colocynth extracts, in experimental rats, significantly improves insulinemia, the drop reaches 71.77% for alkaloids and 86.03% for glycosides. The glucoregulation normalization, after this treatment administration, shows improved insulin sensitivity. However, many authors have shown insulin-stimulating effect of fractions of colocynth seeds (Benariba *et al*, 2013).

The lipid status

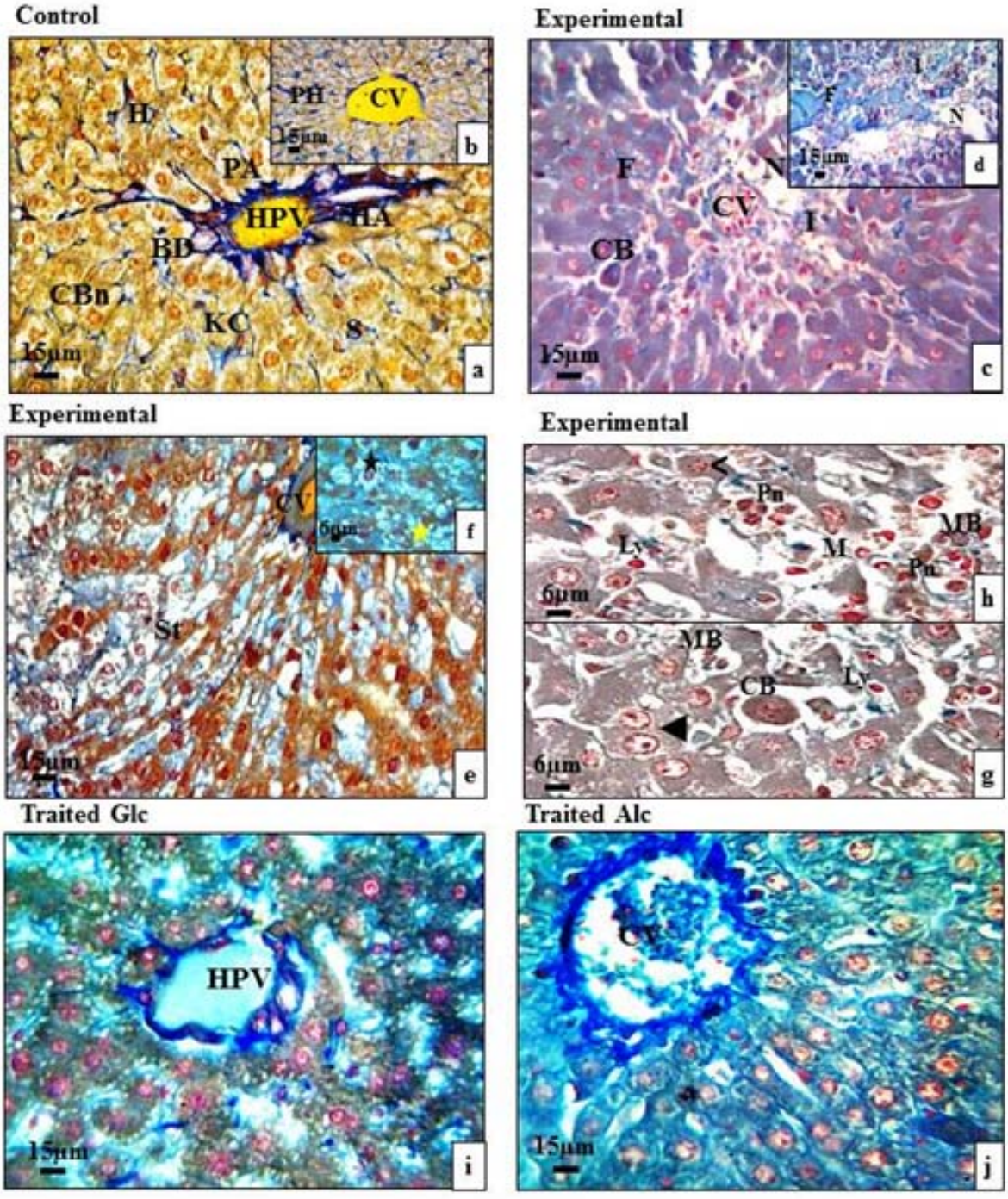
The diet, based on palm oil, generates hypertriglyceridemia, hypercholesterolemia, hyper LDLemia and hypo HDLemia in our animals. This lipid disorder recalls that recorded by Wansi *et al*, (2013) in rats fed with palm oil-enriched diet.

Hypertriglyceridemia in our experimental animals can be attributed either to the increase of hepatic production of very low density VLDL lipoprotein, the resistance to the insulin inhibitory effect on the VLDL production and secretion or to the rise of novo lipogenesis, linked to SREBP-1c increased expression (stearol regulatory element binding protein-1c), responsible for enzyme activation of lipogenesis in the liver (Taskinen *et al*, 2003). As for the recorded hypercholesterolemia, it may be due to the HMG-CoA activation (3-hydroxy -3-methyl-glutaryl-CoA), a key enzyme in cholesterol biosynthesis (Nan Wu *et al*, 2013).

Besides, there is a strong relationship between plasma total cholesterol rate, the HDLc, the LDLc and LCAT (Lecithin cholesterol Acyl Transferase) which is responsible for the esterification of plasma cholesterol allowing the return of cholesterol peripheral tissues to the liver.

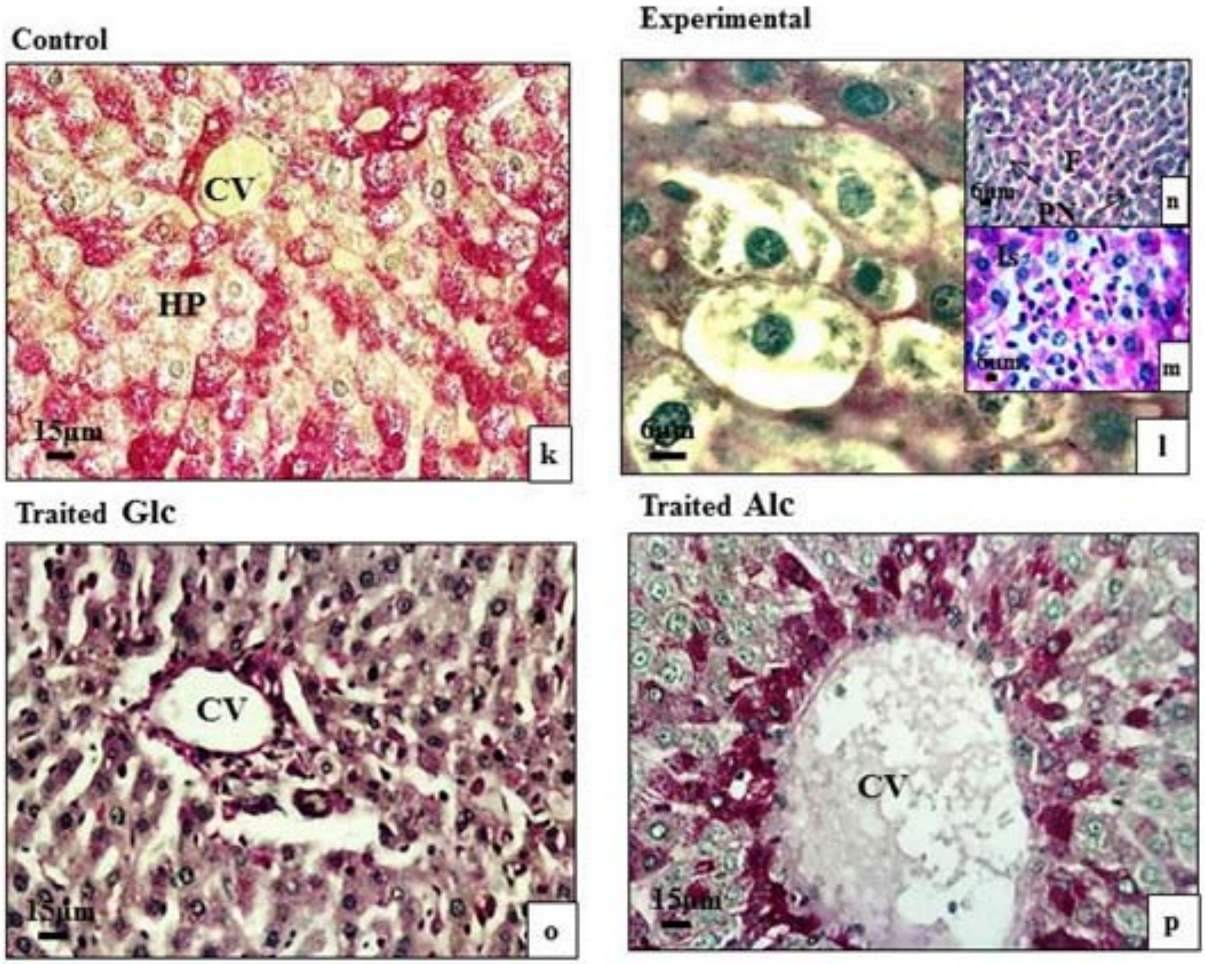
The deficit, in this enzyme, is associated to the cardioprotective fraction regression (Al-Awadi *et al*, 2013). The reduction of HDLc rates, recorded in our animals, can be attributed to the increase in the CETP activity (Cholesterol Ester Transfer Protéine) which is responsible for the transfer of esterified cholesterol of high density "cardioprotective"lipoprotein to the "atherogenic" low-density lipoprotein (Clandinin *et al*, 1999).

Liver tissue histophysiopathology in experimental Wistar rats, treated with colocynth compared to that of control rats



Control : CV (Central Vein) ; HP (Hepatic Parennchyma) ; HPV (Hepatic Portal Vein) ; HA (Hepatic Artery) ; BD (Bile Ductule) ; PA (Portal Area) ; S (Sinusoid) ; H (Hepatocyte) ; CBn (Cellule Binuclees) ; KC (Kupffer Cell)
Experimental : CV (Central Vein) ; CB (Councilman ody) ; I (Infiltration) ; F(Fibrosis) ; N (Necrosis), St (Steatosis) ; M (Monocyte) ; Pn (Polynucleaires) ; LY(Lymphocyte) ; MB (Mallory Body).
Experimental traited with glycosides HPV (Hepatic Portal Vein).
Experimental traited with alkaloids : CV (Central Vein).

Fig. 11: Portion of liver in control, experimental *Rattus norvegicus* and experimental ones treated glycosides and alkaloids. Staining with heidenhein. Azan



Control : CV (Central Vein) ; HP (Hepatic Parenchyma)
Experimental : Is (Inflammatory site) ; F (Fibrosis), PN(Pyknotic Nucleus).
Experimental traited with glycosides : CV (Central Vein)
Experimental traited with alkaloids : CV (Central Vein)

Fig. 12: Portion of liver in control, experimental *Rattus norvegicus* and experimental ones treated glycoside and alkaloids. PAS (Periodic Acid Schiff) Staining

Treatment with alkaloids proves to be a better way, compared to that with colocynth glycosides. The latter shows a reliable action on serum lipid parameters. This positive response is similar to that registered by Rahbar *et al*, (2010) in patients with hyperlipaemia and treated with *Citrullus colocynthis*.

The hypocholesterolemic effect of this plant may be due either to the improvement of the cholesterol secretion in the bile, inhibition of hepatic cholesterol biosynthesis or to the inhibition of intestinal cholesterol absorption.

As for the hypotriglyceridemic effect, it may be attributed to improved catabolism of hepatic lipoprotein or to the inhibition of the liver VLDL secretion. Improved HDLc levels may be explained by the increase in the apolipoprotein A-1 concentration, cofactor that activates the enzyme responsible for the cholesterol reverse transport.

Indeed, LCAT plays an important role in the antiatherogenic pathway of the cholesterol transport, so that excess cholesterol from peripheral tissues is transported from the plasma to the liver and excreted in the bile: this would explain the hypocholesterolemia and increase in HDLc

However, lower LDLc levels can be explained by the increased number of LDL receptors, fixing LDLc and removal of cholesterol via the 7α-hydroxylase cholesterol. The normalization of lipid parameters rates looks like/resembles to that reported by Xiao Yang *et al*, (2015), who studied Coptis chinensis alkaloids, in hamsters subject to lipid-rich diet.

Hepatic status

In conjunction with these metabolic disorders, our results show a significant increase in markers rates of ASAT ALT and PAL functions, in our experimental batch. These

results agree with those of Gloria *et al*, (2010), which showed a rise in AST and ALT plasma rates in Wistar rats subject to a high-fat diet. According to the same researcher, the release of these enzymes in serum shows severe hepatocellular injury.

The plasma levels of hepatic transaminase and alkaline phosphatase, recorded after treatment, show a specific positive improvement by alkaloids compared to those with glycosides. These results agree with those of Agarwal *et al*, (2012), who noted an improvement in liver function levels in rats made diabetic by alloxan and treated with *Citrullus colocynthis*.

The improvement rate of these parameters can cause a stabilization of the hepatocyte plasma membrane and a damage repair of liver tissue (Agarwal *et al*, 2012). Liver tissue histophysiopathology in experimental Wistar rats and experimental ones, treated with colocynth compared to that of control rats. Liver tissue histophysiopathology in experimental Wistar rats, treated with colocynth compared to that of control rats Histology and histochemical liver gland test in experimental rats subject to high-fat diet, compared to that in control animals subject to standard laboratory diet shows a deep architectural disorganization.

Indeed we recorded steatosis, hepatocellular ballooning, the presence of Mallory hyaline bodies, inflammation, necrosis and a fibrosis setting. These alterations recall those of the metabolic stéatopathy. Our results join those of Azeemuddin *et al*, (2016), who showed the development of steatopathy in Wistar rats, subject to a 15-week high-fat diet; the spread of this disease may be attributed to the genes upregulation, involved in lipogenesis, inflammation and fibrogenesis.

According to Seoyoon *et al*, (2013), a high-fat diet causes hepatic steatosis via increased novo lipogenesis associated with an increase in the SREBP- 1c expression (stearol regulatory element binding protein-1c) and the reduction of the fatty acid oxidation promoting lipid accumulation in hepatocytes.

According to Carmiel *et al*, (2005), liver damage during a high-fat diet may be associated with an increased production of cytokines and oxidative stress. This is due to increased ROS production, associated with an increase of the activity of the NADPH oxidase. This latter is coupled with a decrease in antioxidant reserves. A disequilibrium in the prooxidant / antioxidant balance, promoting ROS, causes an increase in lipid peroxidation.

The products of lipid peroxidation and ROS attack the cell membrane and cause hepatocyte ballooning and necrosis. They can also activate the hepatic stellate cells which synthesize proteins of the extracellular matrix and bind cytokeratin proteins to form the Mallory bodies They also induce an inflammatory response by recruiting, by chemotaxis, various populations of immune cells, such as neutrophils and monocytes.

Moreover, ROS highly contribute to the immune response by activating the production of proinflammatory cytokines (TNF, TGF- ß, IL-8...), accompanied by increased collagen synthesis and necrosis.

In addition, the products of lipid peroxidation could also participate to fibrogenesis, catalyzing the production of TGF- ß (Transforming Growth Factor-β) by the Kupffer cells and stimulating the excessive production of collagen by activated stellate cells, thus favoring the impairment of a progressive liver fibrosis Haddad, (2008).

According to Wang *et al*, (2009), a high-fat diet leads to an induction of the expression of the cytochrome P450 (CYP 2E1) and therefore increased oxidative stress, associated with JNK activation and an imbalance of proteins pro - and anti-apoptotic of the Bcl-2 family. To this end, there results an increase of hepatocyte apoptosis that could play an important part in the NASH pathogenesis.

The set of all these deleterious tissular onsets are being significantly improved following treatment with both extracts of colocynth. Syed *et al*, (2015) also revealed the colocynth hepatoprotective effect in rats made hepatotoxic by paracetamol.

For our investigation, we believe that the introduction of colocynth treatment, in our experimental rats, fights against oxidative stress, lipid per oxidation, excessive release of cytokines, the increase of microsomal cytochrome expression (CYP2E1) of the liver and the mitochondrial dysfunction.

CONCLUSION

We need to emphasize the sensitivity of the laboratory rat, athero resistant species, to the action of the standard laboratory diet, enriched with palm oil that makes it particularly fragile and ready to develop a metabolic syndrome correlated to major inflammatory damage of NASH.

The treatment by the two colocynth extracts demonstrated an hypoglycemic effect for glycosides and hypolipedemic for alkaloids as well as for cell and tissue reorganization of hepatic gland. However, it would correct the factors causing disturbance levels and give back the gland the ability to self-regulate through control mechanisms in return, characteristics of all neuroendocrine system, running smoothly.

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