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Effects of ethanolic extract of *Ficus umbellata* leaves on lipid parameters and liver, kidney and heart function in obese Wistar rats

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***ABSTRACT**: The present study aims to evaluate the effect of the ethanolic extract of the leaves of Ficus umbellata on lipid parameters and on hepatic, renal and cardiac function in obese Wistar rats. The result of the phytochemical screening carried out on this extract from the leaves of Ficus umbellata showed the presence of various compounds, in particular phenolic compounds, flavonoids, catechin tannins, leuco-anthocyanins, alkaloids, saponosides, anthraquinones, mucilages and sterols and terpenes. A significant decrease in body weight, plasma and tissue lipids was noted; in addition, the level of parameters such as urea, uric acid, AST, ALT and creatinine also decreased in rats treated with doses of 100, 300 and 500 mg/kg BW. Histologically, this study did not reveal any hepatic, renal or cardiac damage for the doses used.*

***KEYWORDS:** Ficus umbellata, ethanolic extract, lipid parameters, hepatic, renal, cardiac.*

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I. INTRODUCTION

Medicinal plants represent a precious resource for the majority of populations in Africa and represent the main means by which individuals heal themselves (Badiaga, 2011). With the progress of pharmacology, the traditional therapeutic use of medicinal plants is very present in several countries of the world and especially in developing countries (Akouègninou et *al*., 2006). These medicinal plants, widely distributed in African forests and savannahs, have for several millennia been used as the only means of treatment by African peoples (Tabuti et *al*,.2003). The knowledge of the species because of their use in traditional phytotherapy is a big step towards a better exploitation of these resources (Tamboura et *al*., 1998). It makes it possible to know which species should be given priority in research for the discovery of new molecules (Okou, 2009). Today, according to the World Health Organization (OMS, 2003), nearly 80% of populations depend on traditional medicine for primary health care. Considerable economic benefits in the development of traditional medicine have been found (Tossou, 1998). The study of plant chemistry is still topical despite its age. This is mainly due to the fact that the plant kingdom represents an important source of an immense variety of bioactive molecules (Ferrari, 2008). The use of medicinal plants as a second resort to the discovery of new compounds by modern medicine must be taken into account (Zerbo et *al.*, 2011). Knowing, improving and promoting the use of medicinal plants is an urgent and permanent need. It is with this in mind that the fig tree, used both in traditional medicine and in the food sector, has been the subject of several studies.

Ficus umbellata belongs to the genus Ficus and the family Moraceae (Acacha-agody, 2007). It is a plant that can reach 6 to 10 m in height, the different parts of which are used in traditional medicine to treat various diseases such as ulcers, diabetes, jaundice, cancer and warts (Acacha-agody, 2007). The leaves are boiled and used to treat painful or swollen hemorrhoids. The concentrate has an effect on the clarification of the liver and kidneys (Bellakhdar, 2004). The objective of the present study is to evaluate the effect of the ethanolic extract of the leaves of Ficus Umbellata on certain biochemical parameters and on the structure of the kidneys and liver of obese Wistar rats.

II. MATERIALS AND METHOD

Collection, identification and preparation of plant material

The plant material used consists of leaves of *Ficus Umbellata* which were harvested in May 2022 in the town of Abomey-Calavi and then identified in the national herbarium of the University of Abomey-Calavi (UAC). They were ground and the powder obtained was used for the various tests.

Preparation of the ethanolic extract

The extraction is carried out in 3 stages: maceration, filtration, evaporation. 50 g of F. umbellata leaf powder was put in 500 ml of ethanol. After maceration (72 hours), the product was filtered with filter paper then with absorbent cotton placed in a funnel connected to a suction pump in order to accelerate the filtration. After a few minutes, an exclusively liquid solution was obtained (the operation was repeated three times in a row). The filtrate obtained was put in an oven at 45°C to evaporate the ethanol. Then the dried extract was scraped, weighed and the yield was calculated.

Phytochemical screening

The phytochemical screening of the plant was carried out according to the methods described by Bruneton for the detection of secondary plant metabolites (Bruneton, 2009).

Animal material

The animal material was constituted:

- Normal Wistar strain rats with a weight between 100 g and 120 g. They were fed pelleted feed and water.
- Obese Wistar rats with a weight between 317,8 g and 392,45 g. They were fed with "fattening" granulated feed and a mixture of sausage, biscuits, cheese, chips, chocolate, peanuts in the proportions 2:2:2:1:1:1 and sugar water (Darimont et al., 2004).

Animal treatment

These different were divided into five (5) groups and kept under the same conditions.
Lot1: Healthy controls (RS) force-fed with distilled water
Lot2: Obese Controls (RO) force-fed with distilled water
Lot3: Obese rats (RO) force-fed with 100 mg/kg body weight of the extract
Lot4: Obese rats (RO) force-fed with 300 mg/kg body weight of the extract
Lot5: Obese rat (RO) force-fed at 500 mg/kg body weight of the extract
Blood samples were taken on the days (J) indicated (on J0, J 14, J21, and J30). At the end of the experiment, the animals were dissected. The kidneys, liver and heart of the rats were removed, then ground using a homogenization ultrathurax in 10 mMTris base (TBS) buffer, pH 7.4. The ground material was centrifuged at 3000 rpm for 20 min, and the supernatants stored at -20°C. The removed organs were also the subject of a histological study (Folch et al.,1957).

Parameters evaluated

The biochemical examinations (Triglycerides, Total cholesterol, HDL, LDL, AST, ALT, urea, creatinine, uric acid) were carried out by the kinetic method using the "Mindray-BS 240" automaton. Tissue lipids were assayed after extraction in the presence of a chloroform-methanol (2:1, v/v) extraction solvent according to the method of Folch and Porter (Folch et *al*., 1957). After centrifugation at 3000t/min, the lower chloroform phase containing the lipids is recovered using a Pasteur pipette, which was then dried in a water bath at 50°C.

Histological analyzes

Liver, kidney and heart were removed, fixed in 10% buffered formalin solution and embedded in paraffin. Sample sections (5 µm) are mounted on glass slides, deparaffinized and hydrated. For histological analysis, the sections were stained with hematoxylin and eosin (H&E), according to a standard protocol (Senou et al., 2010). Photos were taken at 400X magnification.

Statistical analyzes

The data collected was entered into Excel 2013 software. Normality and homogeneity of variances were checked using R Studio software using the Shapiro test and Levene test respectively. Data comparison was performed using the paired two-sample parametric test with R Studio software. Significance is declared when the P-value is less than 0.05.

III. RESULT

Extraction yield

The yield of the extraction is obtained by calculating the ratio between the mass of the extract and the mass of the leaf powder.

$$R = (\text{Mass of extract}/\text{Mass of dry leaf powder}) \times 100$$

Table No.1- Yield of Ethanolic Extract of*Ficus Umbellata*

Extract	Yield	Color	Aspect
Ethanolic	R=20.37%	Black	Granular

Chemical group detection test

Phytochemical screening allowed us to highlight the various secondary metabolites contained in the powder of dry leaves ofF. *Umbellata*. The results of the tests for detecting the chemical groups responsible for the therapeutic effects, carried out on the ethanolic extract of the leaves of *F. umbellata* are collated in Table No.2.

Table No.2:- Phytochemical screening

Metabolites	Characterization
Alkaloids	(+)
Flavonoids	(+)
Phenoliccompounds	(+)
Anthocyanins	(+)
Leucoanthocyanins	(+)
Anthraquinones	(+)
Reducing compounds	(+)
Cyanogenicderivatives	(-)
Saponosides	(+)
Mucilage	(+)
Gallic tannins	(-)
Catechic tannins	(+)
Sterols and terpenes	(+)
Coumarins	(-)

Legend: Presence (+) Absence (-)

Change in body weight of rats (g)

Figure 1 shows the variation in the weight of rats treated with different doses (100, 300 and 500 mg/Kg BW) of the ethanolic extract of *F.Umbellata*leaves between J0 and J30.

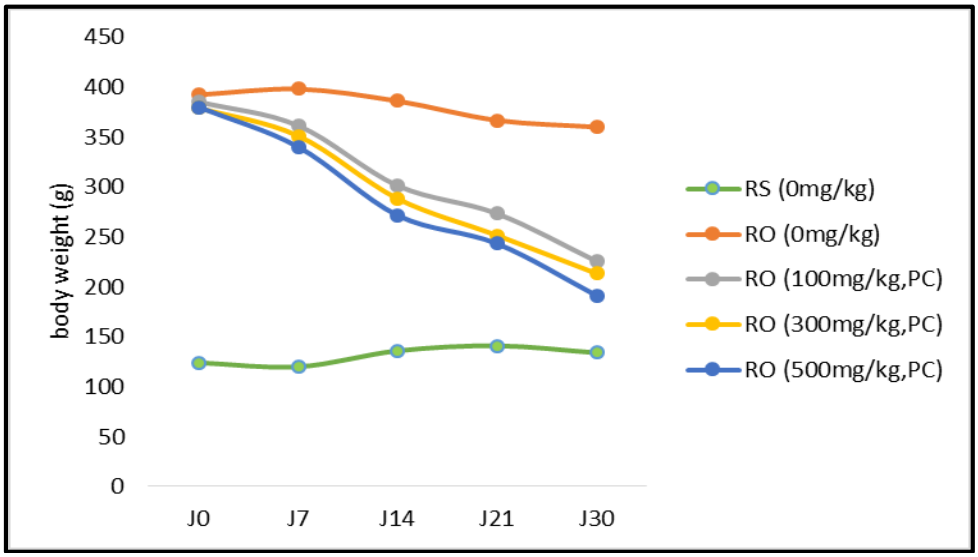
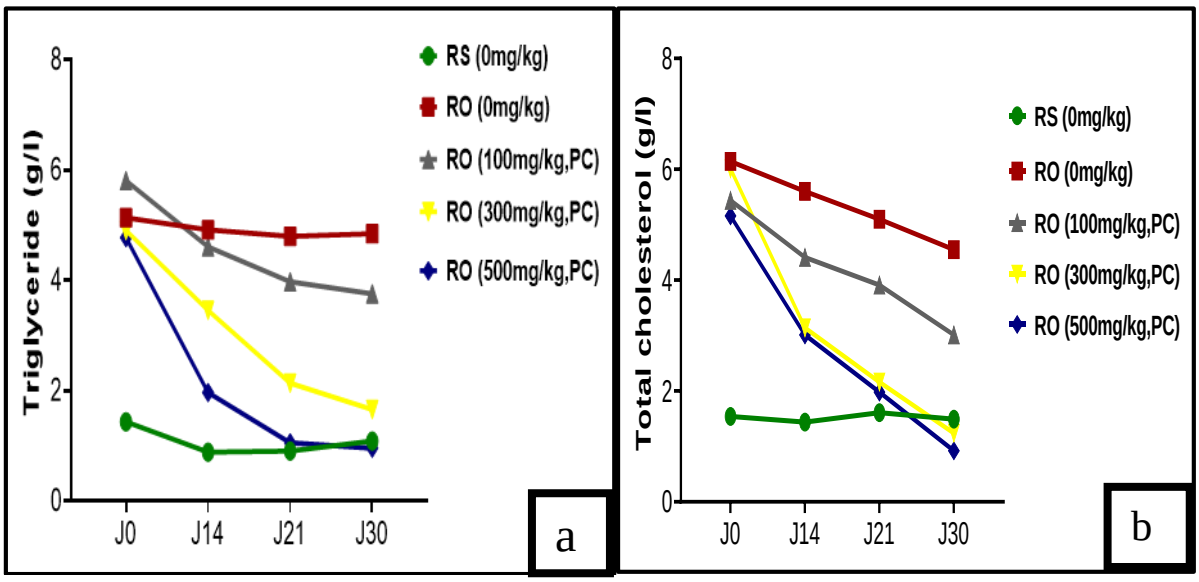


Figure No. -1: Curve showing the variation in weight of rats treated with different doses of the ethanolic extract of *F. umbellata* leaves between J0 and J30

A statistically significant reduction in the body weight of the obese rats treated at 100, 300 and 500 mg/Kg compared with the untreated obese rats is noted. This decrease is observed from the seventh day of force-feeding.

Effects of Ethanolic Extract of*Ficus Umbellata*Leaves on Plasma Lipid Parameters of Obese Rats
Variation in triglyceride and cholesterol levels

Figure 2 shows the variation in the level of triglycerides (a), Total cholesterol (b), HDL (c) and LDL (d) in the blood of rats treated at different doses (100, 300 and 500 mg/Kg BW) of the ethanolic extract of the leaves of *F. umbellata* between J0 and J30.



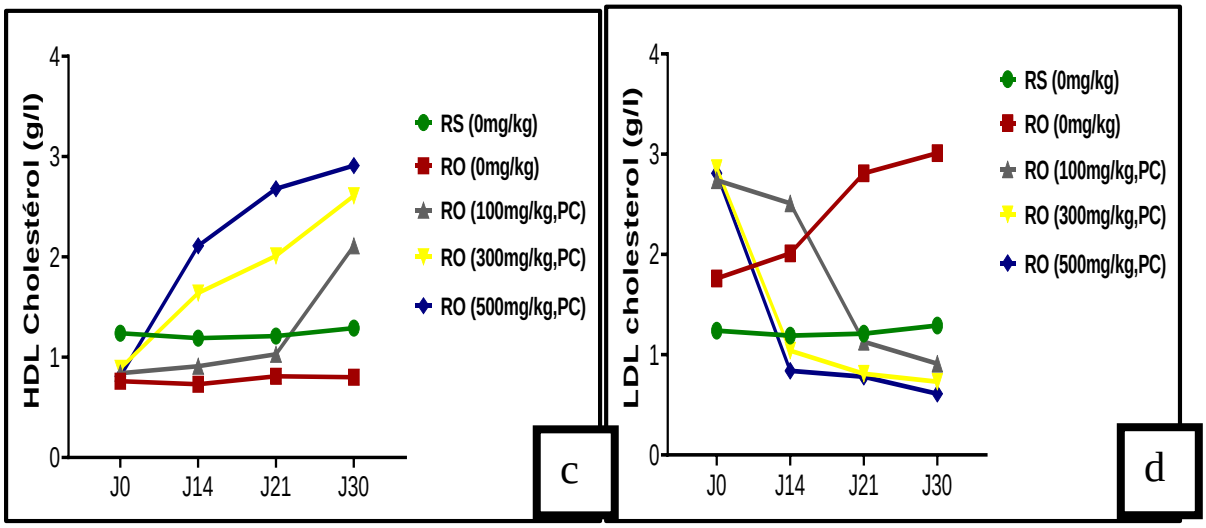


Figure No. -2: Curves showing the variation in the blood level of triglycerides and cholesterol in obese rats treated with different doses of the ethanolic extract of *F. umbellata* leaves between J0 and J30

The level of blood triglycerides (a) of obese animals treated (having received different doses of the ethanolic extract of F. Umbellata orally) fell from J0 to J30 compared to the obese control (RO).

- For a dose of 100 mg/Kg of the extract, a significant difference ($\rho<0.05$) is noted on the 30th day.
- For the doses of 300 and 500 mg/Kg BW of this extract, the difference is very significant ($\rho<0.01$) from the 21st day and more accentuated ($\rho<0.001$) on the 30th day.

Analysis of this same figure 2 shows that the total cholesterol (b) and LDL (d) levels of the obese animals treated decreased from J0 to J30 compared to the obese control (RO) unlike the HDL cholesterol level (c) increased blood.

The results of analysis of variance showed that:

- For a dose of 100 mg/Kg BW of the extract, there is a very significant difference ($\rho<0.01$) on the 14th day and more accentuated ($\rho<0.001$) on the 30th day.
- For the doses of 300 and 500 mg/Kg, the difference is highly significant ($P<0.001$) from the 14th day.

Effects of ethanolic extract of*Ficus Umbellata* leaves on fat content in the liver and kidneys of obese rats

Figure 3 shows the variation in fat content in the liver (a) and kidneys (b) of obese rats treated with different doses (100, 300 and 500 mg/Kg BW) of the extract for 30 days.

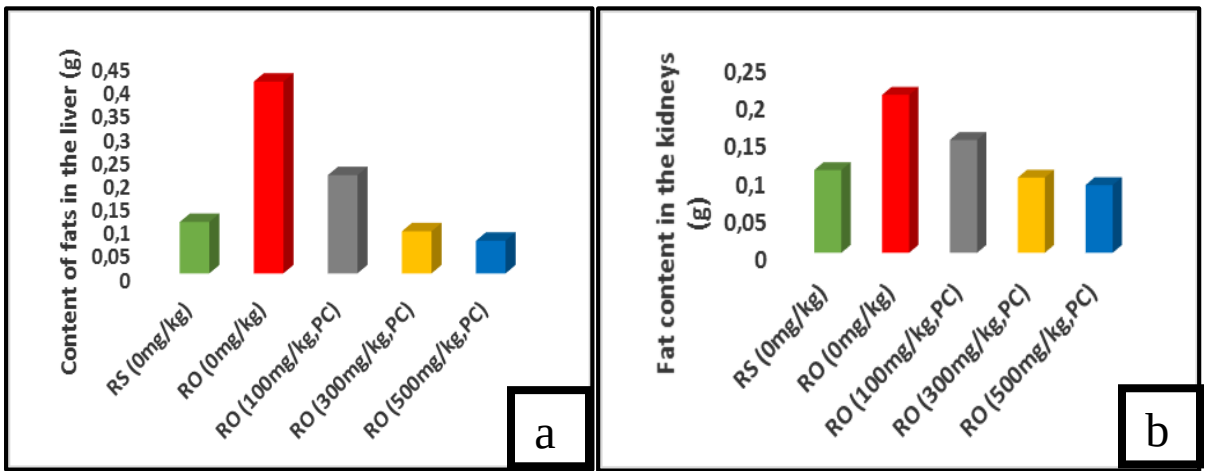


Figure No. -3: Effect of extract on fat content in liver and kidney of obese rats.

Whatever the dose administered, there is a statistically significant decrease in fats in the liver (a) and kidneys (b) of the treated animals. This decrease was accentuated ($\rho<0.01$) for the doses of 300 and 500 mg/Kg BW.

Variation in AST(a) and ALT(b) levels

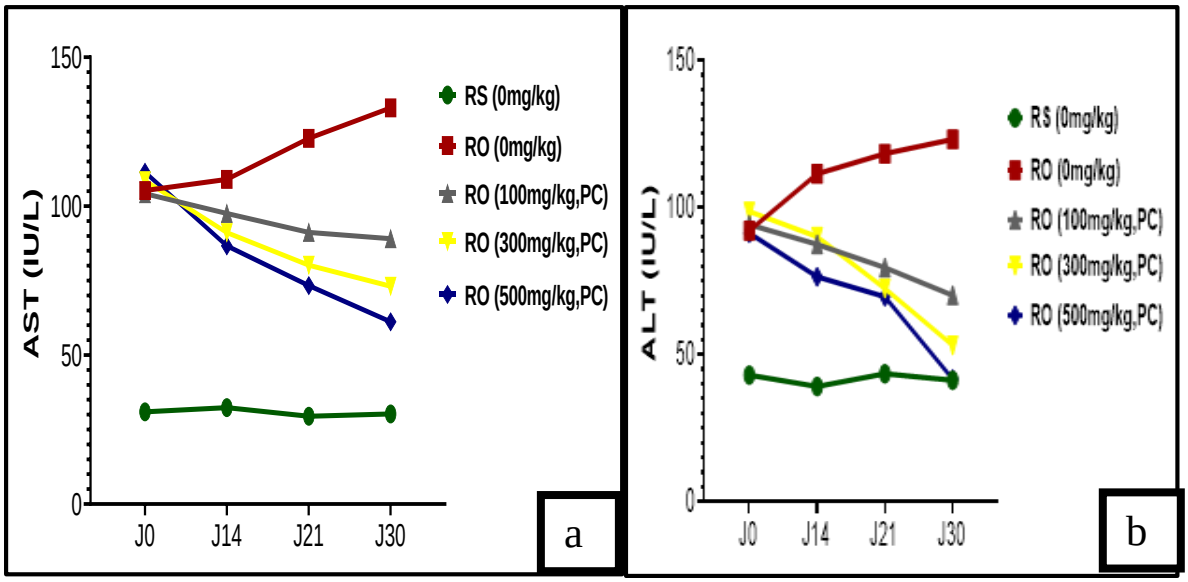
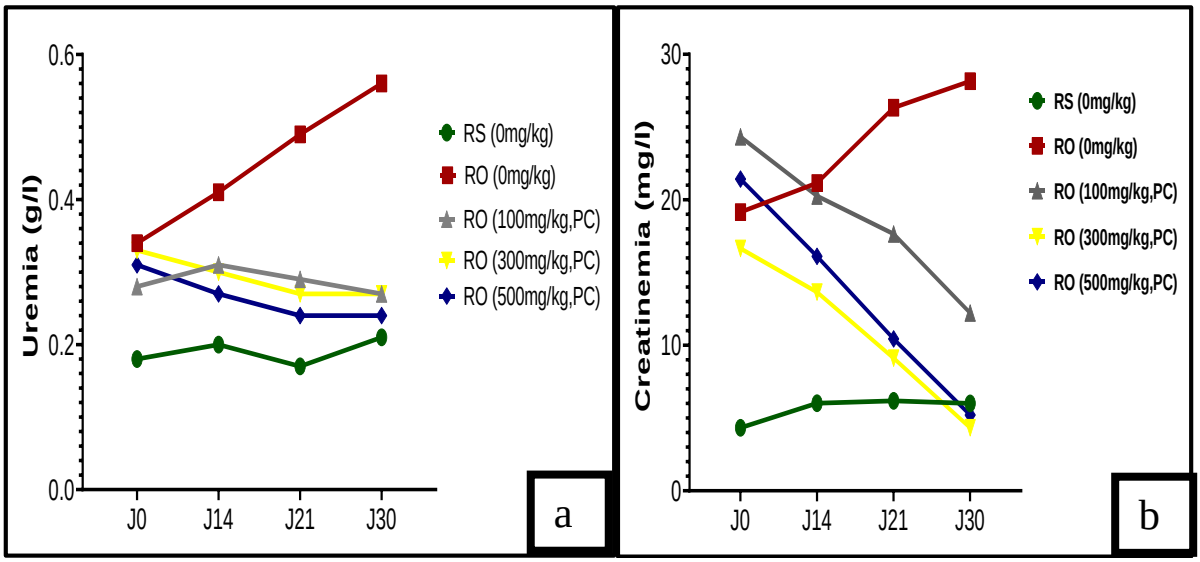


Figure No. -4: Effect of the ethanolic extract of *F. Umbellata* on the level of AST (a) and ALT (b) of obese Wistar rats

Serum analyzes carried out on obese rats treated with the ethanolic extract of *F. umbellata* leaves show a dose-dependent decrease in transaminases ($\rho < 0.01$ and $\rho < 0{,}001$ for the doses used) for 30 days of experimentation.

Change in uraemia (a), creatinine (b) and uricemia (c)

According to Figure 5(a), gavage with ethanolic extract of *F. umbellata* leaves for 30 days of obese Wistar rats caused a tendency to decrease blood urea level.



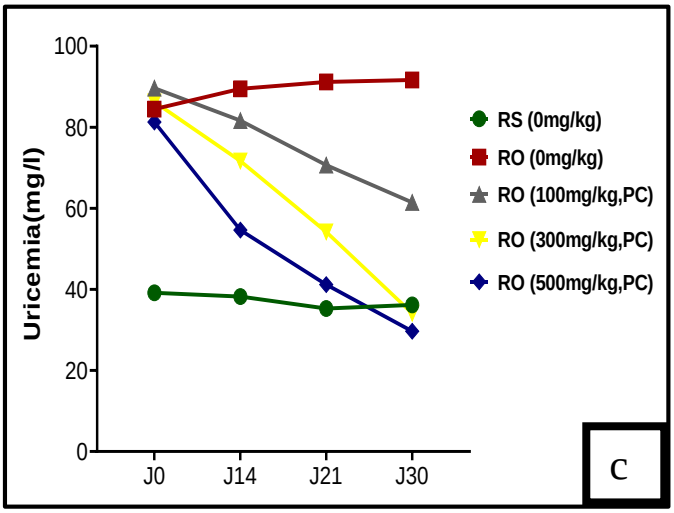


Figure No. -5: Effect of ethanolic extract of *F. Umbellata* on uremia (a), creatinine (b) and uricemia (c) in obese Wistar rats

This extract, whatever the dose administered, induced in these obese animals, a decrease in serum creatinine (b) and serum uric acid (c). The variance shows that with the duration of exposure to the extract and the increase in dose, there is persistence of this decrease.

Histological study of the liver, kidneys and heart of obese rats treated with different doses of the extract.

- **Liver histology**

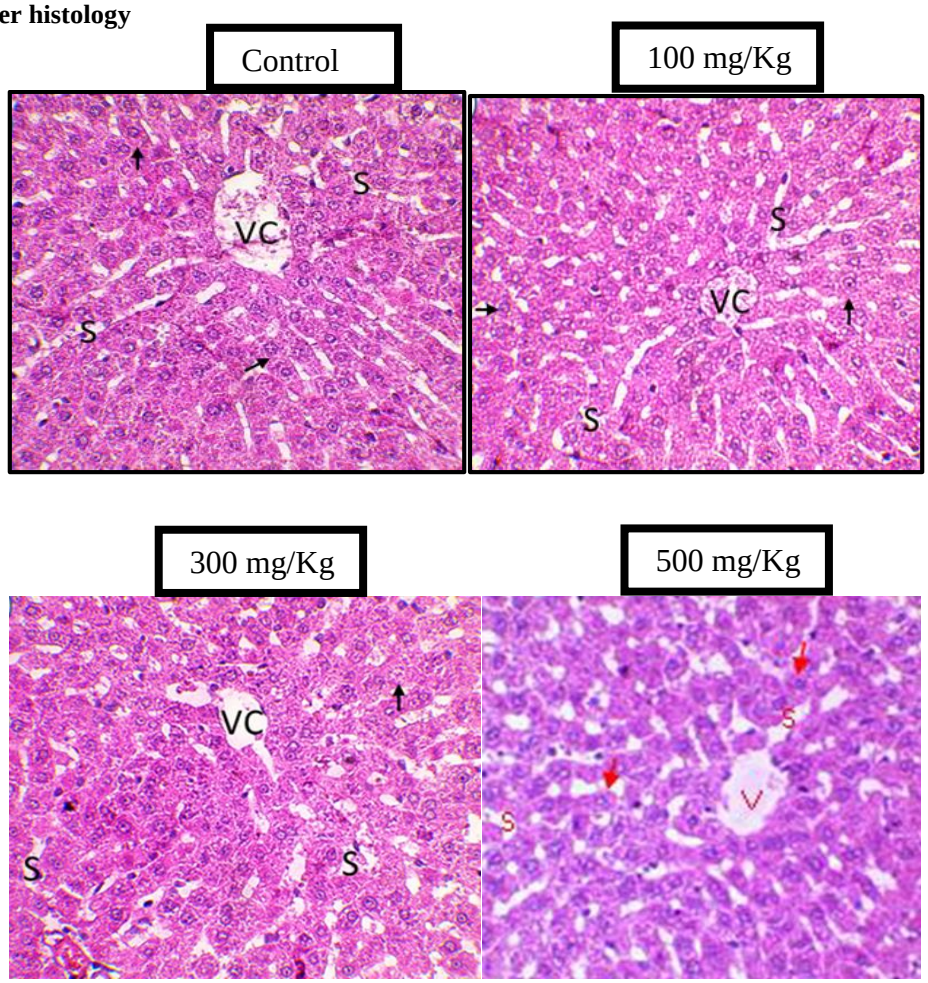


Figure No. -6: Liver histology of obese Wistar rats treated with 100, 300 and 500 mg/kg BW of the ethanolic extract of *F. Umbellata* leaves. (400X)

The liver of these rats treated with different doses of our extract showed no visible atypia. Normal-looking hepatocytes (arrows) are neatly arranged in radial cords around the centrilobular vein (CV). The venous sinusoids (S) are clearly visible as observed in the control rats.

- **Kidney histology**

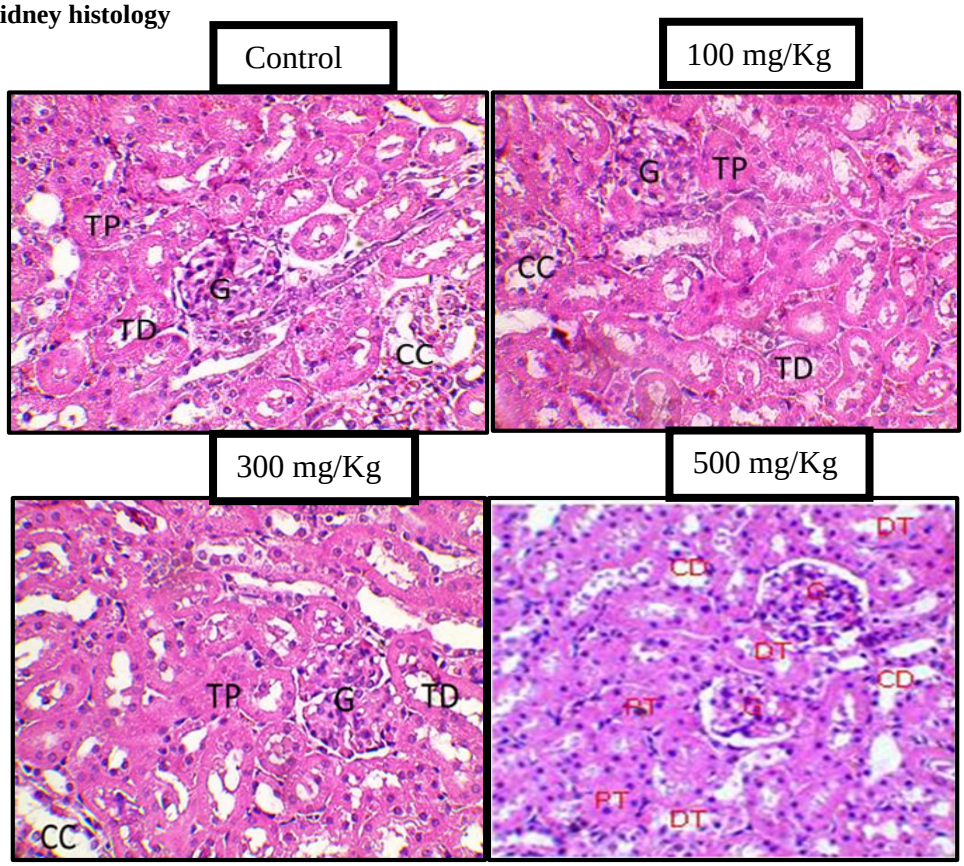


Figure No. -7: Renal histology of obese rats treated with 100, 300 and 500 mg/kg BW of the ethanolic extract of the leaves of *F. Umbellata*. (400X)

The renal parenchyma of the treated obese rats retained its typical appearance as observed in the control rats. The glomeruli (G), the proximal tubes (TP), the distal tubes (TD) and the collecting ducts (CC) showed no visible atypia.

- **Cardiac histology**

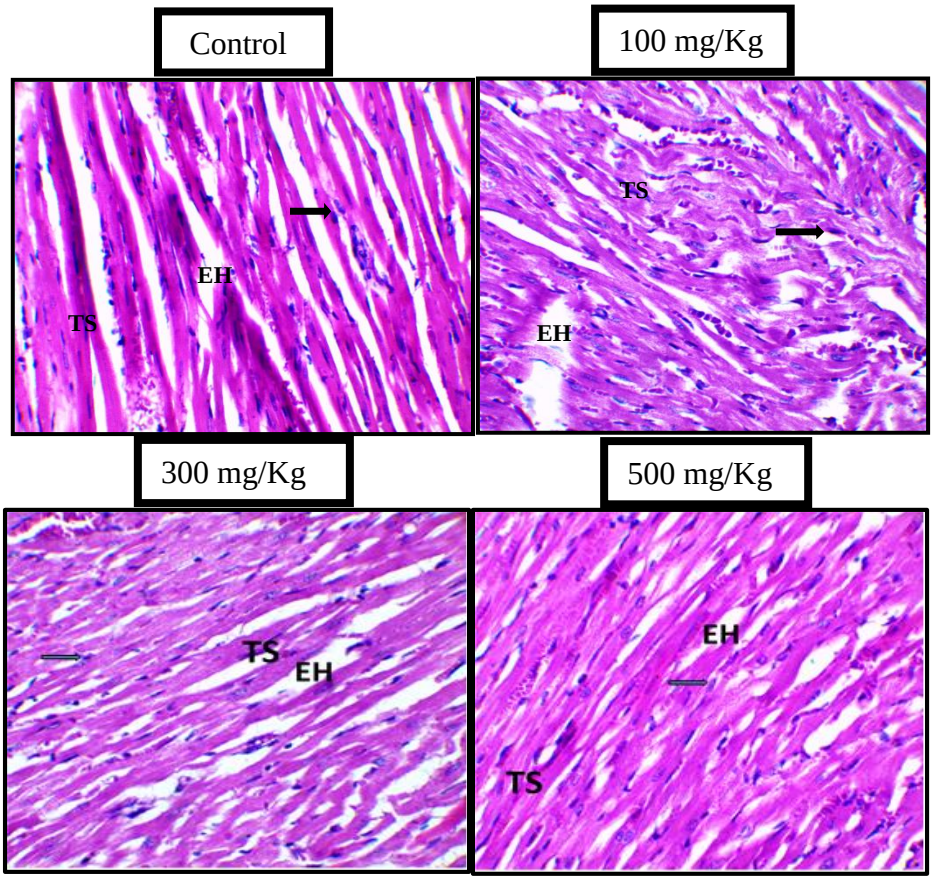


Figure No. -8: Cardiac histology of obese rats treated with 100, 300 and 500 mg/kg BW of the ethanolic extract of the leaves of *F. Umbellata.* (400X)

The henna spaces (EH) of obese rats treated with 100, 300 and 500 mg/kg BW of the ethanolic extract of F. umbellata leaves retained their typical appearance as observed in control rats. Normal-appearing cardiac striated muscle cell nuclei (arrows) are well arranged. The scalariform features (TS) or the intercalated discs showed no visible atypia.

IV. DISCUSSION

The total yield of the extraction is 20.37±2.22% with a blackish-looking extract. Phytochemical exploration revealed the presence of several secondary metabolites in the leaf. These include phenolic compounds, flavonoids, catechin tannins, leucoanthocyanins, alkaloids, saponosides, anthraquinones, mucilages and sterols and terpenes. Polyphenols, flavonoids and tannins are metabolites whose pharmacological activities have been demonstrated by several studies. Effect polyphenols can cause the breakdown of fat from adipose tissue by inhibiting the development of cells specialized in fat metabolism and storage (Williams et *al*., 2013). This would explain the decrease in liver and kidney fats in obese rats treated with different doses (100, 300 and 500 mg/Kg BW) of the ethanolic extract of *F. umbellata* leaves for 30 days. The decrease in body weight in rats is certainly related to the breakdown of fat from adipose tissue as shown by Parrish (1990). Moreover, the presence of saponins and flavonoids in the extract could explain the drop in total cholesterol, LDL and triglyceride levels, because these metabolites have hypolipidic properties (Özlem et *al*., 2007). Recent studies have revealed a strong correlation between elevated plasma lipid levels and impaired vascular relaxation (Stroes et *al*., 1997). Lowering triglyceride levels has been shown to correct hypertension (Bobryshev et *al.*, 2006). You would think that our extract would regulate blood pressure. In our study, AST and ALT activities were used in the evaluation of hepatic disorders (Achliya et al., 2004). When the liver is damaged for various reasons including cirrhosis, hepatic cell necrosis, hepatitis and hepatotoxicity (Al-Habori et *al*., 2002), these enzymes are released into the circulating blood most often before the appearance of clinical signs (Pratt et *al*., 2000). However, the decrease in the level of transaminases (ALT and AST) following treatment with the extract of animals made obese could suggest the correction of a hepatic functional anomaly (Okeke et *al.*, 2014).

Furthermore, the histological study did not reveal any atypical aspect in terms of liver function during the treatment. This drop in transaminase levels could be understood as the correction of the effects of the obesity induced in these animals; effects that could promote the onset of hepatic steatosis due to the very high level of fat in the liver of these obese rats (Recknagel et *al.*, 1989). The flavonoids contained in our extract, thanks to their antioxidant and therefore hepatoprotective activity (Saxenaet *al*., 2004), could inhibit liver damage caused by obesity. Rudenskaya, showed that alkaloids decrease the activity of AST and ALT as well as the level of triglycerides (TG), which gives them hepatoprotective effects (Rudenskaya et al., 1998). In the body, the kidney plays an important role, ensuring the filtration of the blood and the elimination of toxic waste resulting from the functioning of the organs and their excretion in the urine (Alvarez-Llamas et *al*., 2012). Renal nitrogen constituents including urea, uric acid and creatinine were also assessed (Jaballi et al., 2017). Their high level in the blood usually indicates kidney failure (Pritchard et *al*., 2009). The high level of uric acid in the blood of control rats made obese compared to healthy control rats (non-obese), suggests hyperuricemia (Hua et *al*., 2012) and could cause gout disease (Huang et al., 2011). The decrease in uricemia during the treatment of obese animals independently of the dose can be explained by the presence of polyphenols (Hua et *al.*, 2012). It has been reported that some phenolic compounds existing in different plant species have high antioxidant potential that can inhibit xanthine oxidase (Cos et *a*l., 1998), moreover the flavonoids contained in the extract exert anti-hyperuricemic activity (Chen et *al.*, 2011), by inhibiting xanthine oxidase and by increasing the absorption of uric acid (Kostova et *al*., 2007). Blood creatinine levels vary according to a number of factors including diet and muscle mass (Arafat et *al*., 2008). It also depends on the ability of the kidneys to eliminate creatinine, hence its use as an indicator of renal failure (Boumaza et *al*., 2009). The significant decrease in the level of this indicator in rats made obese and treated orally with the extract, leads us to think of the decrease in the catabolism of creatine and phosphocreatine (Boubchir, 2002) in the muscles through the flavonoids present in the extract. Urea comes from the destruction of proteins and is completely filtered by the glomeruli (Ben et *al*., 2017). Its blood level reflects the overall functioning of the kidneys (Feki et *al*., 2021). The histological study showed that at concentrations of 100, 300, 500 mg/kg body weight, the extract has no impact on renal function. The decrease in serum creatinine and serum uric acid could be linked to the fall in the level of fat in the kidneys of the treated rats. In fact, the level of fat in the kidneys of obese rats treated has decreased and is close to that recorded in healthy controls. This result means that our extract has corrected the damage that induced obesity would have caused on the kidneys. Cardiac histology showed that the impact of our extract is not felt on the heart for the duration of the experiment.

V. CONCLUSION

The phytochemical exploration carried out on the powder of dried leaves of *Ficus umbellata* revealed the presence of phenolic compounds, flavonoids, catechic tannins, leucoanthocyanins, alkaloids, saponosides, anthraquinones, mucilages and sterols and terpenes. Our results reported a dose-dependent decrease in body weight and plasma lipid parameters in obese rats. In addition, a decrease in the fat content in the liver and kidneys has been recorded and is accompanied by a dose-dependent decrease in transaminases as well as that of uric acid and creatinine. The extract would thus have corrected liver and kidney damage caused by induced obesity. Histological examinations have shown that this extract has no direct impact on the functional structure of the vital organs of the liver, kidneys and heart.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest

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