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The effect of *Euphorbia resinifera* propolis on obesity induced by High Fructose diet in rats during prepuberty and adolescence

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Abstract

Propolis is a substance produced by the hive, and whose therapeutic properties are very varied and potentially useful to humans. This study aims to evaluate the effect of *Euphorbia resinifera* propolis supplementation on weight status and biochemical blood parameters in High Fructose treated Wistar rats during prepuberty and adolescence. 24 rats distributed into 3 groups of 8 rats for each, control group (C): receiving normal diet, group 2: fructose (F), treated with fructose 23% /day for 6 weeks and group 3: Fructose + (MEP), treated in 6 weeks fructose feeding combined to 2 weeks of 200 mg/kg/day of methanolic extract of propolis (MEP) for 15 days. Administration by gavage of MEP solutions significantly reduced the weight and biochemical parameters compared to the negative control, as well as the healing of MEP. The measured organ weights in all animal groups revealed a significant decrease in liver weight for the F group compared to the control group ($p=0.0315$). However, it was observed that the relative liver weight significantly increased in the groups treated with propolis, reaching levels comparable to the control group. Notably, this effect was not observed in the heart and, adrenal gland. Fructose diet alters body and organs weight and impairs biochemical parameters, MEP supplementation decreased body weight and regulated biochemical parameters mainly glucose and HDL.

Keywords: *Euphorbia resinifera* propolis, fructose, blood sugar, cholesterol, prepuberty, adolescence.

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1. Introduction

Body weight gain arises from a chronic imbalance between the anabolism and catabolism of carbohydrates and lipids, stemming from a high intake of calorically dense nutrients and a decrease in energy expenditure. This imbalance leads to the excessive accumulation of body fat in white adipose tissue [1-5]. Overweight and obese become deleterious for metabolic health when this excessive storage leads to an expansion of visceral adipose tissue and the accumulation of lipids in non-adipose tissues such as the muscles, the liver, and the pancreas [6–8]. In addition, tissues affected by ectopic lipid accumulation potentially undergo major damage that defines insulin resistance, type 2 diabetes and impaired muscle function. Obesity is often associated with dyslipidemia combining an increase in triglycerides, sometimes LDL cholesterol, as well as a decrease in HDL cholesterol [7].

The management of dyslipidemia linked to obesity is close to the situation with normal weight; it mainly includes nutritional advice and physical activity, while medication is less often necessary.

Metabolic syndrome is a set of cardiometabolic risk factors associating abdominal obesity, glucose intolerance, even type 2 diabetes, hypertension, hypertriglyceridemia and reduction in HDL cholesterol. Patients with metabolic syndrome often have other lipid disturbances, namely an excess of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL), commonly grouped under the name of lipid-related dyslipidemia obesity.

This dyslipidemia is found in 30-60% of cases of overweight or obesity according to studies, partly explaining the increased risk of cardio-metabolic complications in obesity, combined with the low-level inflammatory state and insulin resistance [9,10].

Interestingly, not all obese patients experience dyslipidemia, a phenomenon known as the "obesity paradox." Some individuals fall into the category of metabolically healthy obesity. However, this concept is debated, as certain studies indicate that this state, initially characterized by metabolic parameters within normal ranges, may later progress to cardiometabolic complications. While modern medicine has made strides in treating obesity with synthetic hypoglycemic drugs, these chemicals come with limitations and side effects, prompting the exploration of new drugs. [11,12].

Traditional medicine based on herbal medicine to prevent or treat diabetes has been used for millennia by various populations and civilizations. Apitherapy is another approach which is widely practiced using bee products:

honey, royal jelly, pollen, propolis to treat several pathologies such as diabetes and obesity [13].

Plant secondary metabolites, especially polyphenols like natural phenols, are believed to contribute significantly to the therapeutic effects of medicinal plants. They play a crucial role in safeguarding against various disorders such as urolithiasis, atherosclerosis, brain dysfunction, and obesity [14–20].

Bee products (wax, honey, venom, royal jelly, pollen, propolis) have this particularity that they all have therapeutic utility for humans. Among these products, propolis seems remarkably promising. In fact, it has been discovered to have antibacterial properties, antiviral, antifungal, antiparasitic, analgesic, antioxidant, anti-inflammatory, immunostimulating, healing and even anticotic. [20-23].

Studies are aimed at researching the hypoglycemic activities of medicinal plants as well as bee products used traditionally. This work is established to discover new natural remedies that are more effective, less toxic and with fewer side effects. However, this area remains very little exploited in Morocco. In this context, considering the prepubertal and adolescence period vulnerability, our work consists of evaluating the effect of *Euphorbia resinifera* propolis on a weight status and some biological parameters including glucose, lipids and liver enzymes in high fructose treated rats during prepuberty and adolescence.

2. Materials and methods

2.1 Honey

In current study, the used *Euphorbia resinifera* propolis produced in the region of Drâa Tafilalet, on Tinghir city (Morocco), known for its therapeutic properties and potentially useful to humans (figure1) [20,21].

2.2 Animals

The experiments carried out in this work were carried out on male Wistar strain laboratory rats, born and raised in the animal facility of the Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra (Morocco). Three groups of male Wistar rats aged 21 days were used for this study. The animals were separated randomly into one of three pre-established groups and put on a diet upon their arrival. All animals had free access to water and food, subject to a photoperiod of 12/12 (12 light/12 dark) and an ambient temperature of 22°C. They were regularly monitored by an increase in body weight during their breeding. The cages were regularly cleaned. The animals were distributed into 3 groups of eight rats each:

- Group 1: control group (C): consisting of 8 rats received normal diet.
- Group 2: fructose (F): made up of 8 rats treated with fructose 23% of fructose/day for 6 weeks during prepuberty and adolescence.
- Group 3: Fructose + methanolic extract of propolis (MEP) includes 8 rats treating in 6 weeks fructose feeding combined to 2 weeks of 200 mg/kg/day of methanolic extract of propolis (MEP).

2.3 Biochemical parameters

After completion of the experiment, the rats were kept fasting for 18 hours, then they are sacrificed. The blood taken from the portal vein of each rat was placed in heparin tubes, centrifugation was carried out to recover the plasma necessary for carry out the requested biochemical analyses. These analyzes allowed us to obtain the values of serum glucose, total cholesterol, triglycerides, low density lipids (LDL), high density lipids (HDL) and liver enzymes including alanine aminotransferase (ALAT) and aspartate aminotransferase (ASP).

2.4 Statistical analyses

Values were presented as mean ± standard error. As for the statistical tests, we first performed a one-way ANOVA analysis which made it possible to evaluate the effect of the diet and that of MEP supplementation separately. When there was a significant interaction ($p<0.05$) we carried out an additional statistical test, Tukey Post-Hoc Test. This method allows you to observe significant differences between different pairs of data. Statistical analyzes were performed by using GraphPad Prism 7 software.

3. Results and Discussions

In Figure 2, it's evident that all groups of rats on a high-sugar diet (F) exhibited a significant increase in body weight over the 12-week study period compared to the control group (F: $n=8$, $p<0.001$). This weight gain in F rats was attributed to increased food intake (hyperphagia). The control group followed a normal growth pattern, reaching a final weight of 165% of the initial baseline, while the F group reached 180%. However, rats treated with MEP showed a substantial decrease in body weight ($p<0.0001$) compared to the F group (figure2).

In our research, the recorded organ weights in all animal groups revealed a significant decrease in liver weight for the F group compared to the control group ($p=0.05$). However, it was observed that the relative liver weight significantly increased in the groups treated with propolis, reaching levels comparable to the control group. Notably, this effect was not observed in the heart and, adrenal gland, where differences remained non-significant across all groups (figure3).

The effects of the extract on the biochemical parameters of the males showed no statistically significant value between the group taking distilled water and those treated with our extract for ALT and AST (figure 4).

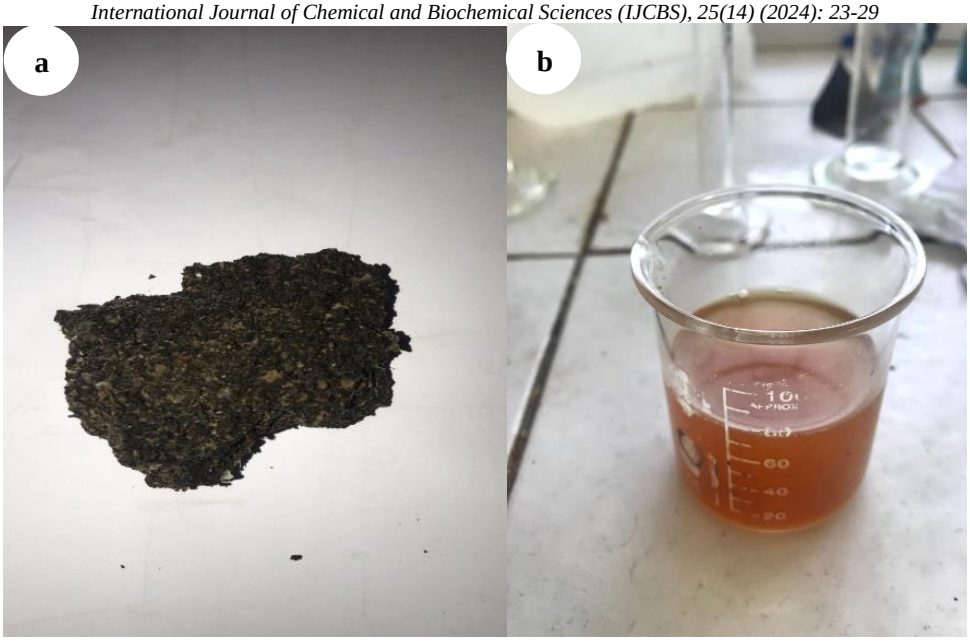

Figure 1: *Euphorbia resinifera* propolis (a) and methanolic extract of *Euphorbia resinifera* propolis (b).

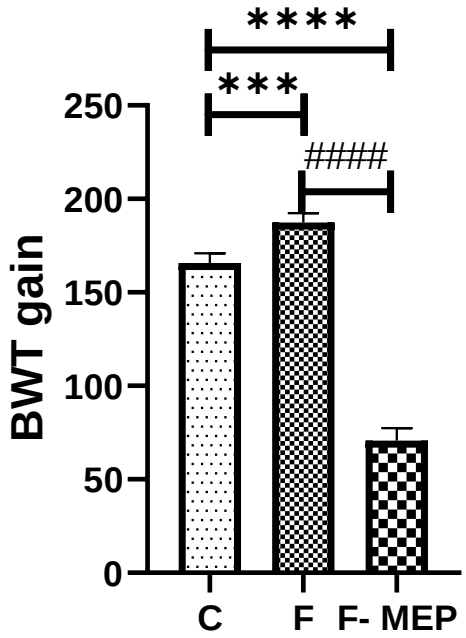

Figure 2. Effects of 6 weeks fructose feeding combined or not to 2 weeks orally administration of *Euphorbia resinifera* propolis on Body weight gain (BWT). * p < .05, ** p < .01, *** p < .001. Values are mean ± SEM of seven rats per group (Control (C), Fructose (F) and fructose + MEP (F-MEP)). ***P<0.001 vs C, ****P<0.0001 vs C, #### P < 0.0001 vs F.

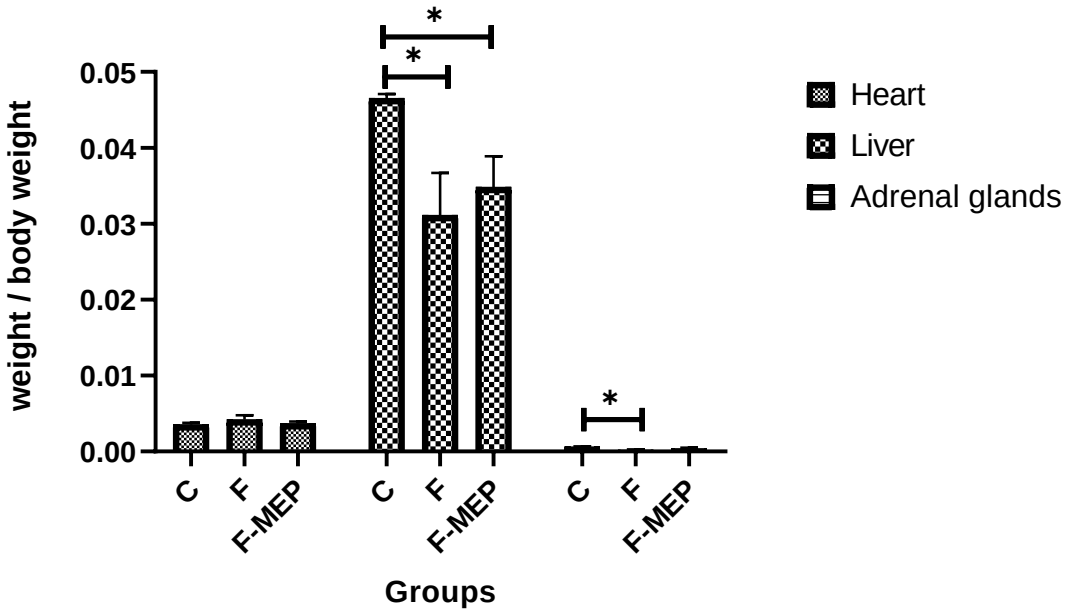

Figure 3. Effects of 6 weeks fructose feeding combined or not to 2 weeks orally administration of *Euphorbia resinifera* propolis on heart, liver and adrenal glands weight gain. * p < .05, ** p < .01, *** p < .001. The results are expressed as Means ± SEM of seven rats per group (Control (C), Fructose (F) and fructose + MEP (F-MEP)). * p <0 .05 vs C.

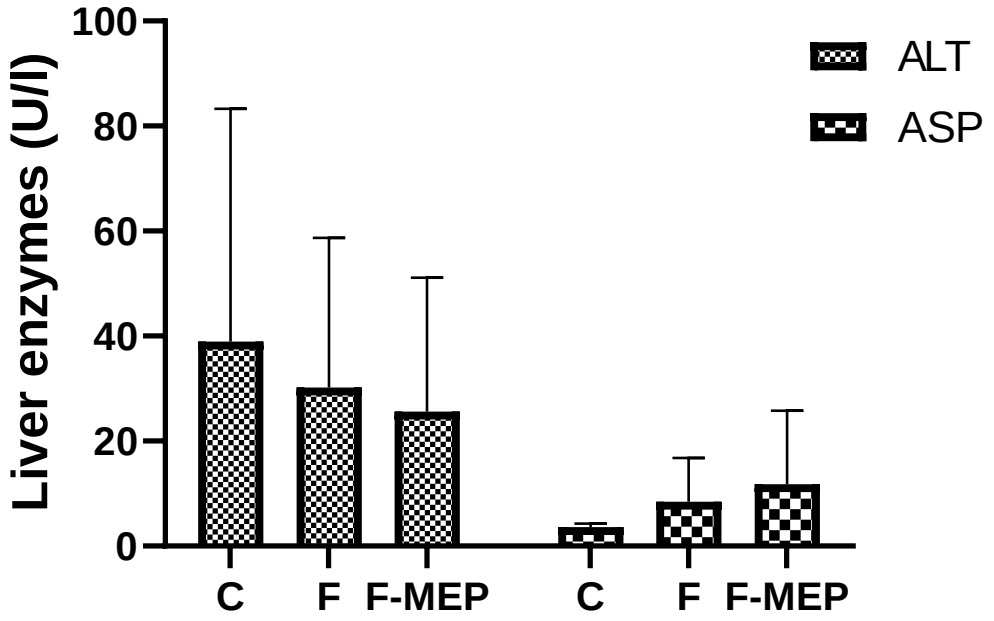

Figure 4. Effects of 6 weeks fructose feeding combined or not to 2 weeks orally administration of *Euphorbia resinifera* propolis on liver enzymes concentration using One-Way ANOVA followed by Tukey's multiple comparisons. Values are mean ± SEM of seven rats per group (Control (C), Fructose (F) and fructose + methanolic extract of propolis (F-MEP)). ASP (Aspartate aminotransferase). ALT (alanine aminotransferase).

Table 1: Effects of 6 weeks fructose feeding combined or not to 2 weeks orally administration of *Euphorbia resinifera* propolis on Biochemical parameters.

Parameters mg/dl	Control	Fructose	Fructose + MEP
Glucose	54.88± 20.78	445.8 ± 16.51***	126.2± 26.09**##
Total cholesterol	771.0± 24.65	1091 ± 435.3	603.3 ± 82.80
Triglyceride	247.8± 43.38	250.7 ± 69.2	248.7 ± 23.23
LDL	545.6 ± 7.763	1007 ± 290.2	408.8± 69.23#
HDL	186.3 ± 30.09	80.62 ± 3.421*	188.3 ± 25.99#

*P<0.05 vs C, **P<0.01 vs C, ***P<0.001 vs C, # P<0.05 vs F, ## P<0.01 vs F. (MEP: methanolic extract of propolis)

The high fructose diet led to a notable rise in blood sugar, serum triglycerides, total cholesterol, serum LDL, in comparison to rats drinking water. Additionally, there was a significant decrease in serum HDL cholesterol. Notably, MEP exhibited a similar lipid profile-reducing effect as fibrate class substances when compared to groups consuming the high fructose diet (table 1).

The objective of current study was to assess the effect of *Euphorbia resinifera* propolis supplementation on weight status, blood sugar and biochemical parameters in high fructose treated rats during prepuberty and adolescence.

Fructose diet alters body and organs weight, the results represented in figure confirm the gain weight effect of fructose diet in rats.

According to our results, fructose feeding impairs biochemical parameters, a significant increase in glucose and in LDL. However, MEP supplementation decreased body weight and the biochemical parameters.

According to previous studies, there's a growing interest in understanding the potential role of fructose in the development of obesity and metabolic diseases. The study specifically concentrates on dietary carbohydrates, highlighting fructose as a tool employed by scientists to induce diabetes and/or obesity in animal models within certain research contexts. Ongoing research in both humans and animals seeks to uncover the implications of fructose in the etiology of obesity. Some scientists propose that the observed quick metabolic changes may play a significant role. The provided link directs to a research paper on the effects of peroxisome proliferator-activated receptors alpha agonist and cinnamon oil on obesity induced by a high-fructose diet [24,26].

Studies have shown that indigestible dietary oligosaccharides like fructo-oligosaccharides, galacto-oligosaccharides and lactulose have a preventive effect against obesity, insulin resistance, and diabetes mellitus by acting as prebiotics on the intestinal flora. The antioxidant potent substances contained in propolis may be linked to the antidiabetic, antihyperlipidemic, and hepatoprotective effect [27,28].

Minimal amounts of fructose reduced blood glucose by increasing hepatic glucose through the activation of glucokinase. The beneficial effect of this molecule on glycemic control has been proven in patients with type 2 diabetes [29-34].

Indeed, various studies indicate a dual impact of propolis. Firstly, its antioxidant properties and free radical scavenging activity have been observed to inhibit the oxidation of LDL, potentially delaying or preventing the development of atherosclerosis. Secondly, the antioxidants in propolis extract have shown a beneficial effect on controlling blood sugar levels, impacting tissues or organs vulnerable to oxidative stress induced by diabetes. This, in turn, contributes to a reduction in diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiomyopathy [35,36].

The study enabled the observation of the impact of a high-sugar diet on the weight status and lipid levels in rats. Additionally, the administration of MEP (assuming a substance or treatment) was associated with reduced blood sugar, LDL, and triglyceride levels. This aligns with findings from other studies [37,38]. Notably, the administration of MEP showed a highly significant effect in lowering blood glucose levels ($p<0.01$).

In our attempt to understand the increased liver function, we investigated the levels of liver enzymes (ALT and ASP) in various tissues. However, the results obtained were insufficient to explain the observed phenomenon. Additionally, at this stage of the disease, there were no observed changes in the activity of energy metabolism enzymes.

4. Conclusions

In summary, this study indicates that a subchronic fructose diet is linked to body weight gain, elevated glucose, lipids, and liver enzymes in adult Wistar rats. Additionally, the administration of *Euphorbia resinifera* propolis shows promise as a potential therapeutic intervention for mitigating body weight gain induced by a fructose diet. Further research is necessary to uncover the mechanisms underlying this therapeutic effect.

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