

Effect of Apple- Lite Contained of Apple Fibers and Apple Gel Pectin on Body Weight, Lipid Profiles, Kidney Function and Histological Structure of Kidney in Male Albino Rats.

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Abstract

In this study, we evaluated the effect of Apple- lite for 4 weeks on body weight, lipid profiles, kidney functions and histological structure of kidney in male rats. Twenty adult male albino rats (240 - 250 gm) were divided into 2 groups: The first group was considered as control group. The second group was treated orally with Apple- lite (55 mg/kg b.w) by use of intragastric tube. Different physiological parameters were performed including recording of the body weight and measuring lipid profiles, creatinine and urea levels. Body weight gain, total cholesterol, triglyceride, LDL cholesterol, and VLDL cholesterol levels were significantly ($p < 0.05$) reduced in Apple- lite treated rats when compared with the control rats. Urea level was significantly ($p < 0.05$) increased in Apple- lite treated rats, but HDL cholesterol and creatinine levels were none significantly ($P > 0.05$) increased when compared with the control rats. Histological examination of Apple- lite treated rat's kidney showed aggregation of inflammatory cells (monocytes) around glomerulus, fibrosis around Bowman's capsule, and congestion of blood vessels. From these results it can be conclude that the treatment with Applelite produced a significant reduction in body weight and lipid profiles, but it is incapable of improving the kidney functions. Also there are histopathological effects on kidney tissue in treated rats.

Key Words: Apple- lite, Apple fibers, Lipid profile, Kidney Function, Rats.

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**تأثير المستحضر ابل لايت الحاوي على اليف التفاح وبكتين تفاح جيلاتيني في وزن الجسم ومستويات
الدهون ووظائف الكلى والتركيب النسيجي للكلى في ذكور الجرذان البيض**

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الخلاصة

في هذه الدراسة تم تقييم تأثير تناول مستحضر الابل لايت لمدة اربعة اسابيع في وزن الجسم ومستويات الدهون ووظائف الكلى، وفي التركيب النسيجي للكلى في ذكور الجرذان البيض. استعملت عشرون من ذكور الجرذان البيض البالغة تراوحت اوزانها ما بين 240 الى 250 غرام قسمت الى مجموعتين؛ الأولى عوملت كمجموعة سيطرة، وتلقت المجموعة الثانية جرعة من مستحضر الأبل لايت مقدارها 55 ملغرام / لكل كيلوغرام من وزن الجسم عن طريق الفم باستخدام انبوب المعدة. تم قياس عدة معايير فسلجية تضمنت وزن الجسم ومستويات الدهون ومستوى اليوريا والكرياتنين. اظهرت النتائج وجود انخفاض معنوي في معدل اكتساب وزن الجسم ومستويات الكوليستيرول والدهون الثلاثية والكوليستيرول واطى الكثافة والكوليستيرول واطى الكثافة جدا في دم الجرذان المعاملة بمستحضر الأبل لايت عند المقارنة بالسيطرة، في حين لوحظ ارتفاع معنوي في مستوى اليوريا وارتفاع غير معنوي في مستويات الكوليستيرول عالي الكثافة والكرياتنين عند المقارنة بالسيطرة. اظهرت نتائج الفحص النسيجي لنسيج كلى الجرذان المعاملة بمستحضر الأبل لايت تجمع الخلايا الأحادية النواة حول اللمة الكبيبية، التهاب النسيج الليفي حول محفظة بومان، كما اظهرت أحتقان في الأوعية الدموية. ومن نتائج الدراسة يتضح ان مستحضر الأبل لايت له تأثير واضح في خفض وزن الجسم ومستويات الدهون، لكن ليس له القدرة على المحافظة على وظائف الكلى، كذلك ظهرت له تأثيرات امراضيه نسيجية في نسيج الكلى في الجرذان.

كلمات مفتاحية: ابل لايت، اليف التفاح، مستويات الدهون، وظائف الكلى، الجرذان.

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Introduction

The use of medicinal plants for treatment and management of diseases has been gaining prominence worldwide especially in the developing countries where 80 % of the population still depends on traditional healing methods [1]. Apple lite is food supplement during weight loss programs; it's composed of Apple fibers and Pure Apple Gel Pectin. Apple reduces inflammation of gut and is useful in children and infant's diarrhea, enterocolitis, dysentria, chronic gastroenteritis and Typhoidal fever. Alpona (apple powder) has pectin and reduces blood cholesterol, defecate harmful bacteria from gut (desinfecting effect). Among fruit apples are an excellent source of soluble fiber, pectin and polyphylic compounds [2]. Pectin is a soluble fiber found in the cell walls of many plants. Some studies describe the healthy properties of this fiber. Apple pectin has demonstrated a better cholesterol-lowering effect than other pectins including orange pectin. Pectins are a family of complex polysaccharides that contain 1, 4-linked α -Dgalactosyluronic residues [3].

Three pectic polysaccharides, homogalacturonan, rhamnogalacturonan-I and substituted galacturonans, have been isolated from primary plant cell walls. Pectins also carry nonsugar substituents essentially methanol, acetic acid, phenolic acids and occasionally amide groups.[4]. High methoxy pectin have the ability to form gels with sugar and acid, so-called low water activity gels or sugar-acid-pectin gels. Such a gel is considered a 2-dimensional network of pectin molecules in which the solvent (water) with the co-solutes sugar and acid are immobilized. Several physiological responses, such as lowering of plasma cholesterol level, modification of the glycemic response, improving large bowel function, and lowering nutrient availability, have been associated with isolated fiber fractions or diets rich in fiber-containing foods. In mediating these responses, it is clear that the physical properties of dietary fibers affect the function of gastrointestinal tract and influence the rate and site of nutrient absorption [5]. The fiber in apples, which is thought to play a major role in its lipidlowering capacities, is not found in especially high concentration (2–3 g/100 g), and soluble fibers such as pectin represent 50% of the fiber in apples. Nevertheless, it has been reported that this fraction probably contributes to the effects of apples on lipid metabolism [6]. Several dietary fibers significantly decrease

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serum cholesterol concentration in human and thereby reduce the risk for coronary heart disease [7]. Apple dietary fiber and rice bran, a predominantly insoluble fiber source was reported to show hypocholesterolic effect in hamsters [8]. Apple fiber is among top five high fiber diets rich in cellulose, hemicellulose, lignin, pectin contents with potentials of hypcholesterolemic effect [2]. For example, eating two apples a day contributes 2 g of pectin to the diet, which can reduce blood cholesterol by 0.10 mmol/l during the period of consumption. [9]. The lower energy intake associated with the consumption of fiber leads to a modest reduction in body weight. Howarth et al., [10] concluded that the intake of approximately 12 g fiber per day for 4 months in an ad libitum diet is associated with a decrease of 10% in energy consumption and a weight loss of approximately 2 kg. The aim of this study was to investigate the effects of daily oral consumption of Apple-lite on body weight, some kidney functions, lipid profiles and histological structures of kidney in rats.

Materials and methods

Animals and experimental design

Twenty adult 16 weeks old, male albino rats (*Rattus norvegicus*), weighing 240-250 gm were obtained from animal's house of the College of Science, University of Baghdad, Iraq. They were housed in standard plastic cages. The animals were kept in a well ventilated room, temperature of 24-28°C with 12 hrs natural light and 12 hrs darkness. The rats had free access to tap water and dry rat pellets obtained from local market *ad libitum*. The rats were allowed to acclimatize for ten days and then divided into 2 groups; (10 per group) as follows: untreated control group, and Apple- lite treated group which received 55 mg/kg b.w. Apple- lite orally by use of intragastric tube for 4 weeks. (Applelite produced by the Arab Company for Pharmaceuticals and Medical plants MEPACO-Egypt), compositions: Apple fibers (from Apple cuticle) 500mg and pure Apple gel pectin (from apple pulp) 50mg.

Collection of blood samples and biochemical analysis

At the end of the experiment, blood samples were taken by cardiac puncture and blood was collected in clean EDTA tubes, then plasma was separated by centrifugation (3000 rpm for 15

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min.) and stored at -20°C . Triglycerides level was estimated by use of Randox kit according to [11]. Cholesterol level was estimated by Randox kit according to [11]. The HDL Cholesterol (HDL-C) was estimated by BioMaghreb kit according to [12]. VLDL composition and LDL cholesterol can be calculated with reasonable accuracy by the Friedewald formula [13]. Kits of creatinine, and urea were purchased from Spinreact, S.A. Ctra. Spain. Creatinine was determined by kinetic method described by [14], determination of urea was according to the enzymatic method of [15].

Histological examination

Animals were killed and small piece of kidney tissue taken from experimental animals were fixed in 10% neutral formalin, alcohol-dehydrated, paraffin-embedded and then sectioned to mean thickness of $4\ \mu\text{m}$. The histological examination was evaluated by assessing the morphological changes with Hematoxylin and Eosin (H&E) stains [16].

Statistical analysis

Data are expressed as the mean \pm SE. The statistical significance was carried out using one-way analysis of variance test followed by Duncan's Multiple Range Test (SPSS statistical software package) [17]. A possibility of P value ($p < 0.05$) was considered as significant difference between means.

Results and Discussion: Changes in body weight.

Table 1, shows a significant ($p < 0.05$) decrease in the mean body weight of the Apple-lite treated rats (from 244 ± 3.80 g to 262 ± 7.61 g) when compared with normal control group (from 245.8 ± 4.26 g to 297.8 ± 4.65 g). According to Adams et al., [18] the reduction in body weight observed in animals fed highly methoxylated pectins suggests that these fibers can be used as aids in weight management, helping to control obesity. The rationale for the potential role of an increased consumption of fruit in the prevention of overweight and obesity is related to relatively high content of dietary fibers. Dietary fibers and, in particular, viscous dietary fiber, which are present in fruit in considerable amounts, have been shown to increase postprandial satiety and to decrease subsequent hunger [10]. In the long term, this may lead to decreases in energy intake and, thereby, in body weight. The delay in gastric emptying and unstirred layer thickness formed in the intestine due to pectin viscosity are the causes of a decreased intestinal

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absorption of glucose [19]. Most soluble fibers create a sense of fullness in the stomach and may therefore result in lesser intake of calories thus helping weight management and obesity. Fiber can affect energy balance and, as a result, body weight through a variety of mechanisms that lead to both decreased energy intake and increased energy losses. It has been shown that the intake of sufficient amounts of fiber reduces the sensation of hunger. In fact, the presence of fiber in the diet effectively decreases its energy density, and short-term studies show that a lower energy density increases satiety and decreases intake [20]. Additionally, high-fiber foods require greater mastication, which can contribute to the feeling of satiety because the speed of intake is reduced. Likewise, the intake of viscous fiber or foods that are rich in this sort of fiber forms a gel that increases gastric distension and reduces the rate at which the stomach is emptied. This mechanism has been proposed as an explanation for the sensation of fullness and increase in satiety after viscous fiber intake [21]. In turn, soluble fiber decreases the postprandial secretion of insulin [22], which may contribute to satiety. Soluble fiber can also delay or reduce the intestinal digestion and absorption of macronutrients, thus increasing fecal energy losses. In fact, there is a negative relationship between fiber intake and the digestibility of fats and proteins [10]. Finally, fiber may also affect the energy balance through its effects on release of intestinal hormones, such as Glucagon-Like Peptide 1, which favors satiety and weight loss, although the mechanisms are only theoretical [23].

Lipid profiles:

Table 1, shows the effect of Apple-lite on the levels of plasma lipid profile. Apple-lite produced a significant ($p < 0.05$) decrease in plasma levels of cholesterol (76.2 ± 2.03 mg /dl), triglycerides (61.2 ± 2.86 mg /dl), VLDL (12.04 ± 0.38 mg /dl) and LDL-cholesterol (17.94 ± 2.10 mg /dl) when compared with normal rats plasma levels of cholesterol (91.2 ± 4.49 mg /dl), triglycerides (77.2 ± 2.94 mg /dl), VLDL (15.44 ± 0.58 mg /dl) and LDL-cholesterol (32.14 ± 4.32 mg /dl). But plasma HDLcholesterol level shows non significant ($P > 0.05$) increase (46.22 ± 0.76 mg /dl) when compared with normal group (43.62 ± 2.05 mg /dl). The lipid-lowering capacity of pectin has been reported previously in rats [24]. Similar effects were observed when apple supplemented diets were used [25]. It has been recently proposed that polygalacturonic acid in the pectin molecule is responsible for the cholesterol lowering

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properties of pectin, and that viscosity could be an important factor in determining the lipidlowering potency of pectin [26] The metabolic effects of fiber (reduction of blood cholesterol and postprandial glucose peaks) can be attributed solely to soluble fiber and, more specifically, to viscous fiber [21], Viscosity is the ability to form gels.

It is clear that the physical properties of dietary fibers affect the function of gastrointestinal tract and influence the rate and site of nutrient absorption [6]. In regard to the mechanisms of action of dietary fibers, in vitro studies have demonstrated that some but not all types of dietary fiber bind bile acids [27], leading to the hypothesis that bile acid binding, interference with absorption of bile acids or nutrients in the intestine and excretion of bile acids are mechanisms whereby certain types of dietary fiber lower plasma cholesterol. One possibility is that the soluble dietary fibers are producing short chain fatty acids. Short chain fatty acids serve as a source of metabolizable nutrients for the rat and it has been proposed that the sustained production and delivery of short chain fatty acids to the rat liver and systemic circulation in some way modulates blood levels of cholesterol, triacylglycerol and glucose [28]. The production of short chain fatty acids from fermentation of soluble dietary fiber over time might also explain the observed lower energy intake of the rats fed high levels of soluble dietary fiber perhaps by suppression of a hunger sensation. Because the distal ileum is the absorptive site for bile acids, increasing viscosity as water is progressively removed from the luminal contents will hamper bile acid absorption. This effect, together with physical binding of bile acids to the fiber, causes increased faecal bile acid loss and is the principle mechanism by which fiber may reduce serum cholesterol [27].

Creatinine and urea:

Apple-lite produced a significant ($p < 0.05$) increase in plasma levels of urea (37.2 ± 1.30 mg /dl) and a non significant ($P > 0.05$) increase in plasma levels of creatinine (0.584 ± 0.05 mg /dl), when compared with normal control group (urea: 29.8 ± 2.38 mg /dl and creatinine: 0.502 ± 0.04 mg /dl) and as shown in Table 1. No available study has reported the effect of Apple-lite on kidney function parameters, but the results of histological examination in this study may support these results because they showed negative effects on kidney tissue, and this may affect kidney functions. [29] Goetz, showed degenerative changes in the renal tubules of mice treated with

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pectin. It has been said that pectin, a mixture of large carbohydrate molecules of different constitutions, is probably not metabolized by the mammalian organism, since a large proportion of the injected material is excreted unchanged by the kidneys. The presence of casts of various kinds within the tubules can lead to degenerative changes in the tubular epithelium is a well known fact.

Table 1: Body weight and levels of plasma lipids, creatinine, and urea in rats treated with 55 mg/kg b.w. Apple- Lite.

Parameters	Normal control group	Apple-Lite group (treated with Apple- Lite 55 mg/kg b.w.)
Initial body weight. g	245.8 ± 4.26	244±3.80
Final body weight. g	297.8±4.65	262±7.61*
Cholesterol mg /dl	91.2±4.49	76.2±2.03*
Triglyceride mg /dl	77.2±2.94	61.2±2.86*
HDL cholesterol mg /dl	43.62±2.05	46.22±0.76
LDL cholesterol mg /dl	32.14±4.32	17.94±2.10*
VLDL cholesterol mg /dl	15.44±0.58	12.04±0.38*
Creatinine mg /dl	0.502±0.04	0.584±0.05
Urea mg /dl	29.8±2.38	37.2±1.30*

Values are expressed as mean ± S.E of 10 animals.

* Values are statistically significant $P < 0.05$ when compared with normal control.

Histological examination

The present study shows some histopathological findings of kidney tissues. Histological examination of both Apple-lite- treated and non- treated rat group shows tissues with normal histology (Fig. 1). Renal tissues of Apple-lite treated rat shows aggregation of inflammatory cells (monocytes) around glomerulus, fibrosis around Bowman's capsule and congestion of blood vessels (Fig. 2) and (Fig.3). Consistent with the result of Goetz [29], this showed degenerative changes in the renal tubules of mice. It has been said that pectin, a mixture of large

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carbohydrate molecules of different constitutions, is probably not metabolized by the mammalian organism, since a large proportion of the injected material is excreted unchanged by the kidneys. As to the changes in the renal tubules, the anatomical evidence points to stasis of pectin may be as a cause. That the presence of casts of various kinds within the tubules can lead to degenerative changes in the tubular epithelium is a well known fact. Our results may be due to the effect of secondary metabolites result from biodegradation of pectin which may have side effects on kidney tissue. From these results it can be concluded that there are negative side effects of Apple-lite on histology of kidney, so as a further study is needed in this respect.

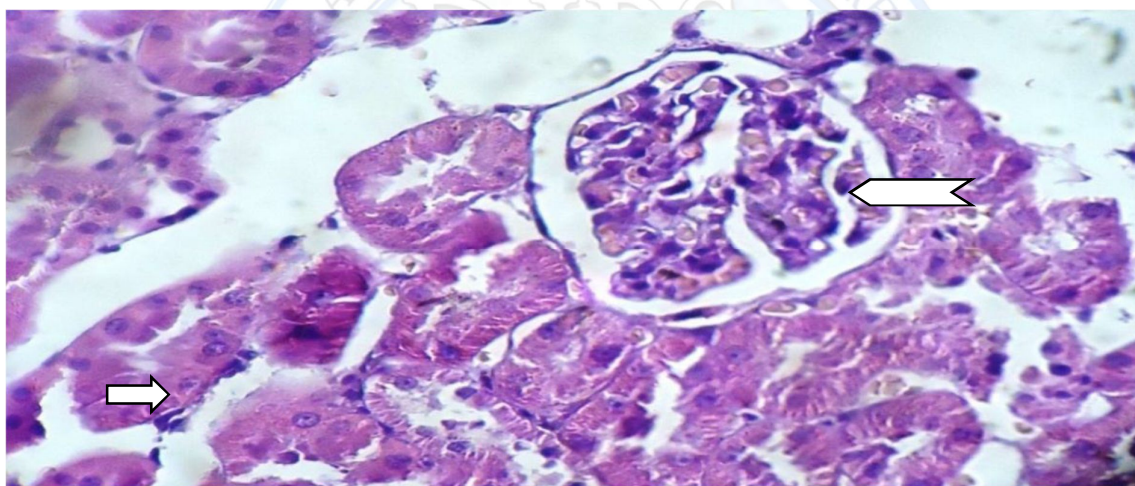
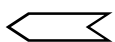



Figure (1): Section in kidney tissue belongs to normal control rat showing normal Bowman's capsule () normal convoluted tubules () (H&E) 400X.

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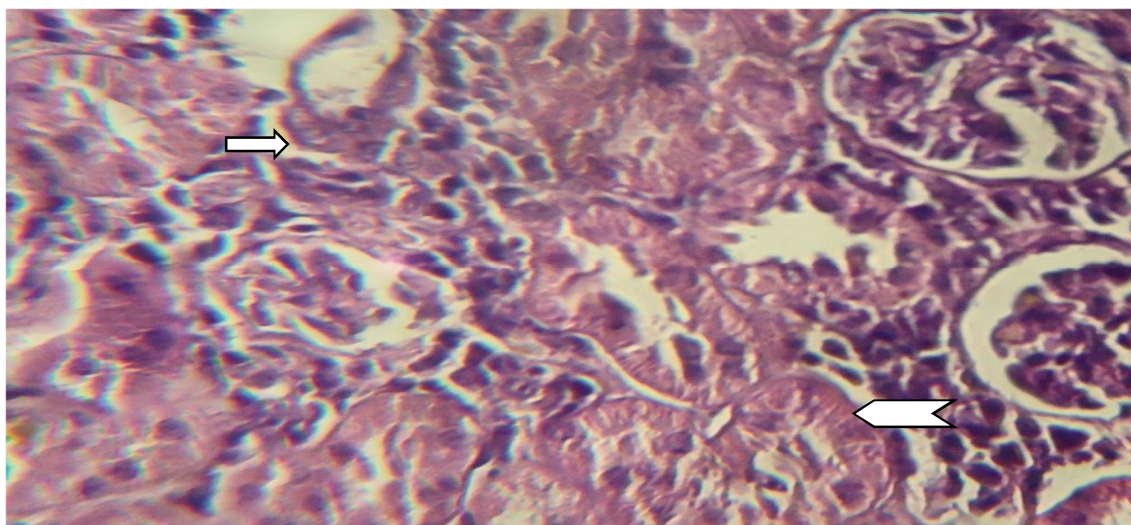


Figure (2): Section in kidney tissue belongs to rat treated with Apple-Lite showing aggregation of inflammatory cells (monocytes) around glomerulus (\Rightarrow) fibrosis around Bowman's capsule (\Leftarrow) (H&E) 400X.

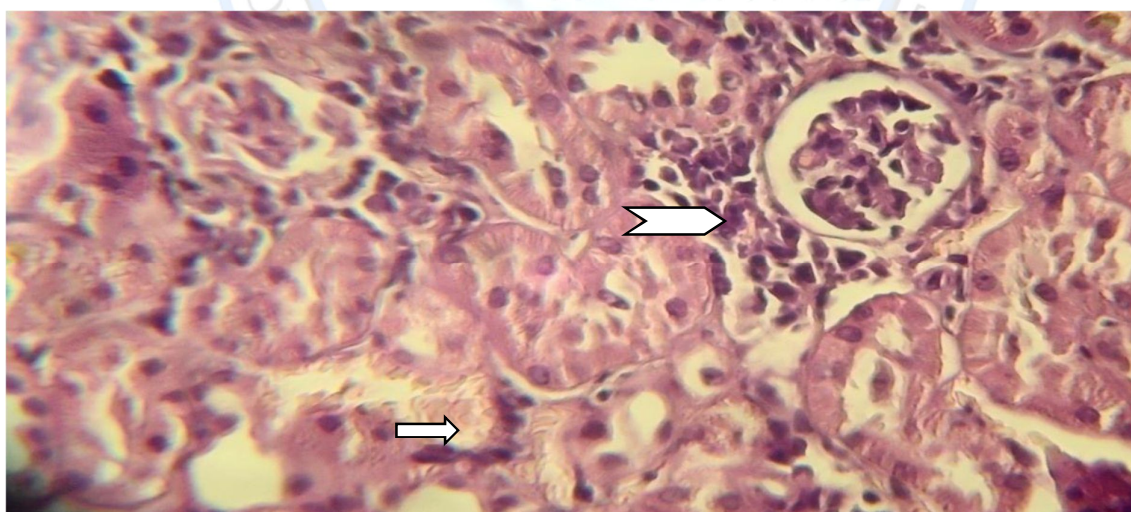


Figure (3): Section in kidney tissue belongs to rat treated with Apple-Lite showing aggregation of inflammatory cells (monocytes) around glomerulus (\Rightarrow) congestion of blood vessels (\Rightarrow) (H&E) 400X.

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