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Protective effect of *Eurycoma Longifolia* Extract on High-Fat Diet- induced histopathological changes in the Islets of Langerhans and Kidneys of Rats

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Abstract

Obesity has emerged as public health problems, potentially guiding to many diseases such as diabetes mellitus type 2 and chronic kidney disease (CKD). The aims of the present investigation were to explore possible protective effect of *Eurycoma longifolia* (EL) extract on the islets of Langerhans and kidneys of high-fat diet (HFD) fed rats. Twenty four healthy adult male Sprague Dawley (SD) rats were randomized into four groups (n=6) and treated for 12 weeks; normal control (ND) group received only normal diet, Group NDEL was received normal diet and EL, Group HFD received HFD only, Group HFDEL received HFD and EL extracts. The animals were scarified at the end of the 12th week; the kidneys and pancreatic tissues were fixed in 10% formal saline and processed for histological studies. The HFD group revealed hypertrophy of the pancreatic islets with congested blood vessels, vacuolization and injured cells. The kidney sections from the HFD treated group exhibited sever damage in the renal corpuscle and tubules. Treatment with EL extract produced an enhancement in tissue damage produced by HFD feeding in the kidneys and pancreatic islets. These observations confirmed positive valuable outcomes of EL extract against the HFD induced-cellular oxidative injury in the pancreatic islets and the kidneys.

Keywords: *Eurycoma longifolia*, High fat diet, Islets of Langerhans, Kidney, obesity, Rats

تأثير مستخلص عكازة علي *Eurycoma Longifolia* على التغيرات النسيجية في الكلية و جزرلانجرهانز في الفئران المعامله بالنظام الغذائي العالي بالدهون المشبعة.

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الخلاصة

السمنة من الأمراض التي اصبحت تنتشر في كثيرمن المجتمعات، و تزيد من الاضطرابات الصحية وتؤدي الى حدوث امراض عديدة مثل مرض السكري وامراض الكلية المزمنه وارتفاع ضغط الدَّم وأمراض القلب وبعض أنواع السرطان .

عكازة علي *Eurycoma Longifolia* شجرة تنمو بصورة طبيعية في غابات جنوب شرق اسيا: لها خواص مضادة للجراثيم و للملاريا، خافضة للحرارة، ومضادات الأكسدة.

ركزت هذه الدراسة على تأثير" عكازة على " على المعلمات المتعلقة بالتركيب النسيجي للكلية و جزرلانجرهانز في الفئران المعاملة بالنظام الغذائي العالي بالدهون المشبعة. تم تقسيم أربعة وعشرين من الفئران الذكور الأصحاء إلى أربع مجموعات (6 جرذان في كل مجموعة). المجموعة الاولى(ND) : غذيت بالنظام الغذائي العادي ، المجموعة الثانية(NDEL): غذيت بالنظام الغذائي العادي و عولجت بعشبه عكازة علي عن طريق الفم لمدة 12 أسبوعا. المجموعة الثالثه(HFD): غذيت بالنظام الغذائي العالي بالدهون المشبعة . المجموعة الرابعة (HFDEL) غذيت بالنظام الغذائي العالي بالدهون المشبعة العادي و عولجت بعشبه عكازة علي) 15ملغ / كلغ) عن طريق الفم لمدة 12 أسبوعا.

اوضحت الدراسة النسيجية لشرائح البنكرياس لمجموعة الجرذان التي غذيت بالنظام الغذائي العالي بالدهون المشبعة انكماش في جزرلانجرهانزمع تلف وفقدان التحبب في خلايا بيتا كما لوحظ ظهور فجوات وترسب زجاجي مع احتقان في الاوعية الدموية الشعريه, بينما اظهرت شرائح الكلى انكماش في بعض الكبيبات وانحلال في البعض الاخرمع توسع في المساحة البولية المحيطة بالكبيبات كما لوحظ انحلال مائي في بعض الخلايا الطلائية الانبوبية مع توسع الانابيب وتحطم الخلايا المنتشرفي التجويف الانبوبي. لقد تحسنت هذه التغييرات المرضية في الجرذان المعالجة بعشبه عكازة علي.

كشفت هذه الدراسه بان لعشبة (عكازة علي) صفات وقائيه على التأثيرات السلبيه النسيجيه التي يحدثها النظام الغذائي العالي بالدهون المشبعة في الكلية و جزرلانجرهانز .

1. Introduction

Obesity has rapidly become a crucial public health issue it forms challenge to health care systems throughout the world, it is associated with several important health complications. Among the complications associated with the pathological aspects of obesity, is the development hypertension, diabetes, and other pathological conditions that involved to development of chronic kidney disease "CKD" [1, 2]. There is evidence that the firm rise in onset CKD incidence may be closely associated with high body mass index [3]. Several studies have demonstrated that chronic consumption of high-fat diet (HFD) lead to type 2 diabetes and insulin resistance, and the development of CKD [4,5]. HFD has been used to induce obesity in experimental animals. Rats treated with HFD showed enlargement of the pancreatic islets and wide intercellular spaces associated with significant increase in fasting glucose levels, and triglyceride [6]. Nontypical islets, distinguished by nonuniform shape, large size, inflammation and marked fibrosis were observed in rats pups treated with HFD after weaning for 23 weeks [7]. HFD also induces structural changes in the kidneys such as expansion of the Bowman's space, enlargement of mesangial matrix and thickening of the glomerular and tubular basal lamina accompanied by greatly elevation of plasma renin activity and insulin concentrations in dogs fed HFD [8]. Elevated lipid and renal profiles associated with mesangial hypercellularity and segmental mesangial matrix expansion of the glomeruli were detected in Sprague-Dawley rats fed on high cholesterol diet for 6 weeks [9].

So far, no pharmacological drug offers continuous weight loss is obtainable without disadvantageous side effects [10]. In recent decades, there has been a tremendous increase in the use of traditional natural therapies for management of several diseases. Numerous oriental medicinal plants are described to have biological effectiveness and compound-based therapeutics have been discovered [11]. In recent years, various herbal medicines have been

recognized as source of many phytochemical activities, and possess powerful antioxidants,; therefor recent investigation on medicinal plants extract revealed that they might prevent endocrine pancreas and kidney injury induced by HFD, and displayed antioxidant and anti-inflammatory effectiveness [12, 13].

Tongkat ali (EL) "*Eurycoma longifolia"* is a flowering plant that is domestic in Southeast Asian countries. The roots of EL, possess a variety of medicinal properties and has been used for the treatment of various diseases such as diarrhea, ulcer, tuberculosis, jaundice and dysentery, it is also used as an aphrodisiac, antibiotic, and appetite stimulant [14]. Recently, the authors investigated the effects of EL on atherosclerosis-related parameters and testicular injury in HFD consumed rats; EL remarkably reduced triglyceride increased by HFD and weakened the development of atherosclerotic damage in the aorta and prevent the testicular damage [15, 16]. The current study was conducted to investigate the potential protective effects of the aqueous crude extract of EL on the morphological injury induced in the islets of Langerhans and the kidney of HFD fed rats.

2. Materials and Methods

2.1. High-Fat Diet

High-Fat Diet (HFD) Pellets were purchased from MP Biomedical, USA. (Table 1 shows the composition of the HFD).

Table 1: The composition of HFD

INGREDIENT	AMOUNT
Casein Purified High Nitrogen	4000 g
DL-Methionine	60 g
Sucrose	6116 g
Corn Starch	4000 g
Coconut Oil Hydrogenated	4000 g
Alphacel, Non-Nutritive Bulk	1000 g
DL-a-Tocopherol Powder (250 IU/gm.)	24 g
AIN-76 Mineral Mix	800 g
Plus MP Vitamin Diet Fortification Mixture 1.2 x Normal	

2.2. Eurycoma Longifolia Extract

The powder of EL extract PHYSTA®, was purchased from Biotropics Malaysia Berhad. The aqueous extract was prepared and administrated using oral gavage as described previously [17, 18].

2.3. *Animals and experimental design*

Twenty four young, adult male Sprague-Dawley (SD) rats (10-12 weeks old), weighing 250–350 gm each, were used in the present study. The rats were housed in plastic cages and kept under suitable environmental conditions such as a room temperature (22–24°C) and exposed to 12 hour/day light program. The schedule of treatment consisted of giving the drug for 12 weeks. Following one week of adaptation, the rats were randomly allocated into four groups of 6 rats each. The control rats (ND group) were fed a commercial rodent chow diet containing 5% fat, group NDEL received normal commercial rat diet and EL extracts "15mg/kg" dissolved in distilled water, HFD group received HFD only, and group HFDEL received HFD and EL extracts "15mg/kg" (Table 2).

Table:.Animal grouping 2

Group initial	Group definition	Treatment
ND	Normal diet control rats	- Pellets and water
NDEL	Normal diet rats treated with EL	- Pellets and water - 15mg/kg EL aqueous extracts
HFD	HFD treated rats	- High fat diet and water
HFDEL	HFD and EL treated rats	- High fat diet and water - 15mg/kg EL aqueous extracts

At the end of the 12th week, the rats were killed by cervical dislocation. Tissue samples of pancreas and kidney were rapidly removed, and processed for light microscopic examination as previously described [15, 16]. The current investigation was conducted in compliance with the instructions and approval of IIUM moral committee (IIUM/519/14/4/IACUC).

3. Results

Sections of pancreas from ND control group showed organized pattern, normal appearance of small to medium size pancreatic islets characterized by large pale cells with pale nuclei in the centre (Figure1 a). The pancreas of the EL treated group revealed no obvious changes in the structure with milled increase in the islet size (Figure1 b). The pancreas of HFD-treated rats showed hypertrophy of islets size with increased cell population; many of these cells process small darkly stained nuclei with acidophilic cytoplasm in its center, congested blood vessels, vacuolization as well as damaged of the pancreatic islet cells (Figure1 c & d). The pancreas of the HFDEL group showed rounded and small islet with evidently normal shaped cells and narrowing intercellular spaces with few congested blood vessels with contrast to HFD-groups (Figure1 e & f).

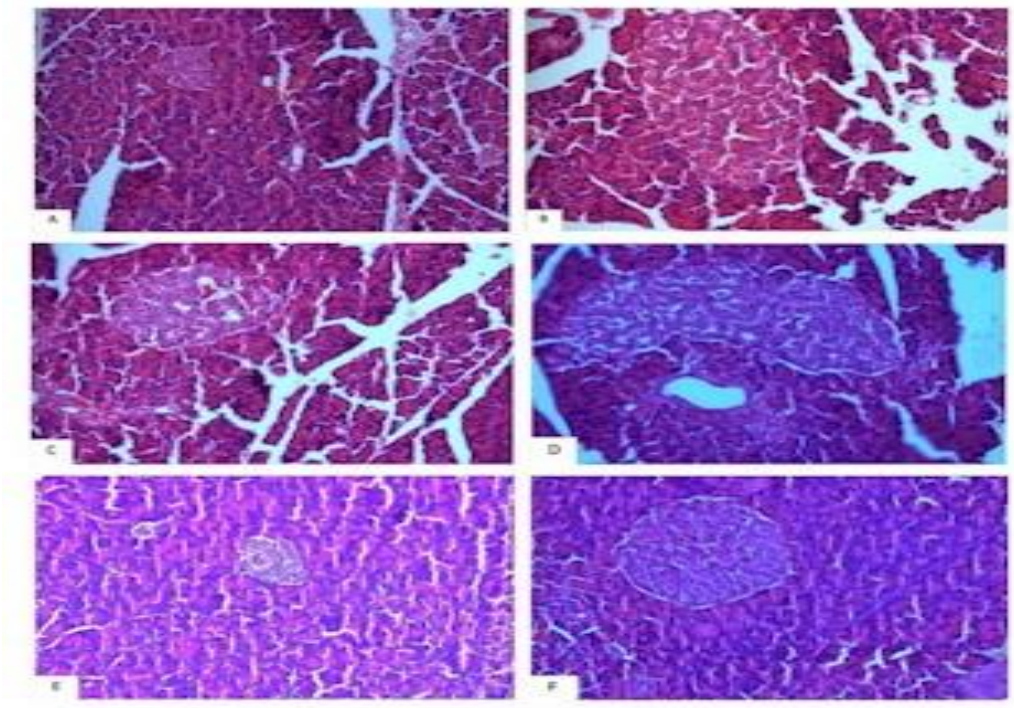

Figure 1: Photomicrographs of pancreatic Islets sections from: (A); ND control rat showing normal and small islet. (B); EL treated rat revealed normal and milled increased size islet. (C&D); HFD-treated rats showing hypertrophied and damaged islets with vacuolization as well as congested blood vessels. (E&F); HFDEL-treated rats showing normal pancreatic islets size and decreased vacuolization.

The ND control kidney sections revealed normal architecture of the renal parenchyma with well-defined renal corpuscles and tubules (Figure2 a). Treatment with EL alone displayed normal structure of the renal tissues and showed no histopathological effects (Figure2 b). The kidney sections from the HFD treated group exhibited intensive deterioration in the renal corpuscle; such as necrosis of glomeruli, atrophic and shrinkage of glomeruli, dilatation of Bowman's capsule with widening of Bowman's spaces, there were many areas of tubular damages characterized by destroying of epithelial cells of proximal convoluted tubules with fragmented and exfoliated epithelial cells in their widened lumina. Moreover, blood vessel congestion and mononuclear cells infiltration was also observed in the renal cortices (Figure2 c & d). Treatment of rats with combination of HFD and EL (HFDEL group) revealed improvement in the glomeruli and renal tubular epithelium compared with HFD group, but blood vessel congestion and miner tubular changes still observed (Figure2 e & f).

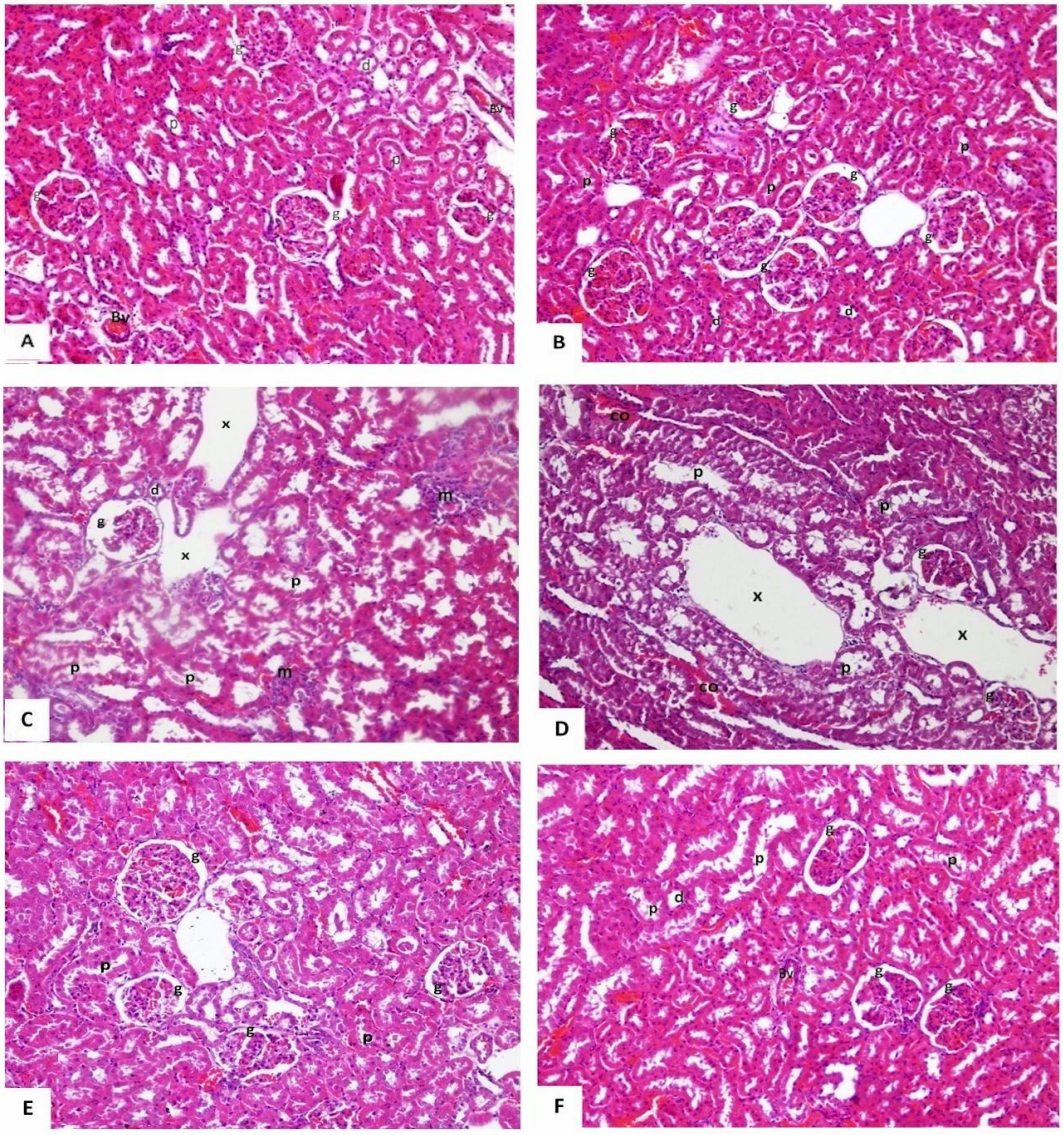

Figure 2: Photomicrographs of kidney sections from: (A); ND control rat showing normal and small islet normal histological architecture. (B); EL treated rat exhibited normal structure of the renal tissues. (C&D); HFD-treated rats showing atrophy and shrinkage of glomeruli, dilatation of Bowman's capsule, tubular damages with degenerated epithelial cells, congested blood vessel and mononuclear cells infiltration. (E&F); HFDEL-treated rats showing exhibited improvement in the glomeruli and renal tubular epithelium.

4. Discussion.

Obesity is abnormal or excessive fat accumulation that contributes to cluster of disorders known as the metabolic syndrome, which is characterized by hyperglycaemia, hyperlipidaemia, insulin resistance, and abnormally high blood pressure [19]. Obesity is associated with many diseases including diabetes mellitus type 2, cardiovascular disease, hepatic steatosis, and CKD [20].

HFD has been demonstrated to promote hyperglycaemia and insulin resistance, and motivate disorder of renal lipid metabolism, and consequent pancreatic islets and kidney damages in animal models. Rats fed on HFD developed an elevation in blood glucose levels associated with beta cells atrophy and reduced beta cell mass of the pancreatic islets [21]. Mice fed on HFD for twelve weeks, developed hypertension, elevated plasma insulin, hyperglycaemia, albuminuria, and renal injuries including increment in glomerular tuft, mesangial enlargement and aggregation of neutral lipid in the proximal convoluted tubules and renal corpuscles [22]. Sprague Dawley rats fed on (30%) HFD for twelve weeks developed increased kidney mass, accompanied by substantial kidney histological changes revealing renal disease [23].

Nowadays, many herbal medicines are extensively been recommended by the public as being natural, healthy, and with minimal side effects, besides being relatively low coast and locally available. Okra addition remarkably reduced the increased FBS levels, total cholesterol, and improved the histological deteriorations in pancreatic islets of HFD fed rats [13]. Administration of barley brans in hypercholesterolemic rats demonstrated a substantial reduction in lipid parameters, enhanced antioxidant enzyme and prevent the histopathological changes in the uriniferous tubules and glomeruli and decreased the deposition of cholesterol in the kidney [24]. *Aster tataricus* "a traditionally Chinese herbal medicine" significantly reduced the levels of creatinine, lipid profile, and serum urea and increased Nitric Oxide levels in rat fed HFD for 4 weeks [25]. Administration of herbal combination extracts of pumpkin seed oil, peanuts shell and Orlistat decreased the circulating lipids level and improved the degenerative change in the renal tubules and the glomerular tuft in HFD fed rats [26]. Treatment of mice with resveratrol "a natural plant polyphenol", prevents renal failure and kidney pathological injury induced in a rat treated with HFD [27].

The present study has investigated the ameliorating effect of EL against the histopathological changes induced by HFD feeding. Rats fed with HFD revealed several morphological changes in the pancreatic islets such as hypertrophy, degenerated islet cells, vacuolization and congested blood vessels. The HFD treated rats also demonstrated many renal histological changes for instance, degenerated glomeruli and renal tubules, congested blood vessel and mononuclear cells infiltration; these observation are associated with significant elevation of serum total cholesterol (TC) and triglyceride (TG) levels, in HFD fed rats observed by the authors in previous study [19]. These detected histopathological changes are similar to those observed in earlier investigations [13, 22, 24]. The administration of EL in "HFDEL group" of the present investigation, evidently enhanced the improvement of the histological deterioration of the kidney and the pancreatic islets in HFD group rats, and restored their normal histological architecture.

Several studies have presented evidences both in humans and experimental animal models in support of that oxidative stress plays decisive mission in the beginning and advancement of CKD. An elevation in kidney and serum content of oxidation products, together with reactive oxygen species (ROS) production and lower levels of antioxidant in

plasma and failure of ROS clearance with decline in renal function have been documented in CKD patients [28, 29]. Experimental data in animal models of renal disease have led to the impression that supplementation with antioxidant-rich diet may protect against the injury effects on the kidney, corrected the oxidative imbalance, decreasing albuminuria and diminished the severity of kidney injuries [30].

It has been established that EL possesses antioxidant and anti-inflammatory activities and potential activities towards cancerous cells [31]. The anti-tumor activity of EL probably has been contributed to the free radical scavenging property of the plant and this property has been contributed to its entire phenolic and flavonoid substances [32]. Phytochemical investigations showed that EL has antioxidant, antiulcer, anticancer, antitumor features and has possibility for controlling of numerous illnesses [33]. In the present investigation, the extract of EL supplement confirmed positive pancreatic islets and kidney defense alongside HFD-induced injury, as documented by the observation of overwhelming normal pancreatic islets and kidney architecture and nonexistence of histological injury, indicating that it has renal-protective activities. Whether the protecting impact of El is direct on the pancreatic islets and kidney cells or it is secondary to the metabolic changes is still to be investigated.

5. Conclusions

The present investigation affirmed positive advantageous influences of *Eurycoma longifolia* aqueous extract against the HFD produced pathological damage in the pancreatic islets and the kidneys. Thus, the protecting effect of *Eurycoma longifolia* against HFD-induced oxidative stress is valuable in evolving applicable adjuvant therapy in the management of oxidative stress-mediated complications developed in the primary phases of obesity.

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