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Histopathological Alterations in Liver, Kidney, and Testis of Obese Male Albino Rats Following Thunder God Vine Roots Extract Treatment.

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ABSTRACT

Obesity is a multi-factor chronic condition caused by an amalgamation of genetics, environment, and an energy intake/expansion imbalance. Modern civilization is seeing a significant growth in obesity, as well as an increased risk of metabolic syndromes in the general population due to lifestyle changes. Even though obesity is a medical problem, maintaining an individual's quality of life will require weight control. Weight loss goal is to reduce health risks, promote weight loss, and prevent weight gain. Weight management herbs include thunder god vine (roots). Herbs like thunder god vine (roots) are used for weight management. Various laboratory studies proved that using thunder god vine extract might cause damage to multiple organs and tissues, and is fatal in some cases. The goal of this study is to measure the toxicity of two doses of thunder god vine root extract, low 5mg/kg and high 7.5mg/kg, on the liver, kidney, and testis tissues of fat diet-induced obese male rats. Histological examination of the obtained sections of all animal groups revealed that treatment with two different doses of the extract in obese rats showed no changes in the seminiferous tubules and different stages of spermatogenesis in testis. Liver injury and nephrotoxic effects were reported after treatment with the plant extract with two doses.

Keywords: *Tripterygium wilfordii*, Toxicity, Obesity, Liver, Kidney, Testis, Histopathology

1.INTRODUCTION

Obesity is a serious global epidemic for its high prevalence and high morbidity and mortality rates. According to reports, 39.8% of Egyptian adults suffer from obesity [1]**.** Obesity, particularly abdominal obesity, has been associated with a higher risk of hyperglycemia, heart disease, dyslipidemia, breathing problems, and cancer [2].

The prevalence of obesity has risen dramatically owing primarily to social and cultural environments. Obesity is related to increased dietary habits, larger portion sizes, limited physical exercise, and inactivity, and also eating disorders [3]. Changes in adipose tissue structure and secretion are caused by these behavioral and environmental aspects [4].

Since obesity is a serious illness, living a healthy lifestyle will be necessary for the rest of one's life. Weight management objectives include an accurate weight loss regime to reduce health risks [5].

Excessive intake of vegetables, beans, legumes, lentils, grain, unsweetened cereals, and fibers, along with substitution of low-fat dairy products and meats for high-fat alternatives should be in the diet plan [6].

Exercise regards as an important part of a weight-loss scheme when merged with caloric restriction [7].
In the short and medium- term, bariatric surgery is said to help with type 2 diabetes recovery [8].
The drug should indeed be construed as part of a weight management strategy. Medication can help patients stay on track, help decrease sedentary lifestyles health risks, and improve people's lives. [9].The disadvantage of synthetic drugs is that they are relatively expensive, and there are side effects because of long period of use. Alternative therapies based on naturally occurring compounds have been proposed as a consequence. [10].
Medical plants have several bioactive chemicals that could be used as a disinfectant, as they have antioxidant activities, antifungal effects, and antiviral activities. Because of rising food safety awareness and their low cost and accessibility, medicinal plants (spice, fresh herbs, extracts and essential oils) have become increasingly popular as organic options. [11].
The Chinese herb lei gong teng is *Tripterygium wilfordii* (TW), a member of the Celastraceae family. The plant is poisonous, but the root pulp is thought to have anti-inflammatory and anti-autoimmune characteristics [12]. More than 300 components have been identified from TW, celastrol and triptolide are the main bioactive components [13]. The roots of this plant have many therapeutic properties, and are widely used in traditional medicine showing several potent anti-inflammatory and cytotoxic activities [14].

2. MATERIALS AND PROCEDURES

2.1 Extract from plant root

One kilogram of TW debarked root was delivered by Taobao, China, and was identified with the assistance of a taxonomist from the Botany Department, Faculty of Science, Port Said University. The roots were chopped into small pieces using an electric mill., and immersed in a 70% ethanol solution three times for two hours, with occasional shaking, before filtering through four sheets of gauze, twice on filter paper, and drying by rotatory evaporation at 60±5° C.
under reduced pressure . The crude extract was then stored at – 20 C^{o} in sterile bottles until it was used [15].

2.2 Animals of experiment

The experiment began with thirty-two male rats weighing 70-80 g provided by Egypt's National Research Center in Cairo. The animals were housed in plastic cages at the Animal House, Faculty of Science, Port Said University in a well-ventilated room (26± 2 °C) with a relative humidity of (40± 2 percent), a 12 hours light/12 hours night cycle, fresh tap water ,and *ad* libitium through a glass bottle with capillary dropper fixed to the cage and available for all rats. Every day, water was changed as well as cages were cleaned. The tests were approved by state authorities and carried out in accordance with Egyptian animal protection regulations as well as specific local institutional animal protection legislation under the observation of proper authorization. [16].

2.3.Models assessment and grouping of diet-induced obese rats:

Only twenty four rats were subjected to a high-fat diet (Naami feed, margarine and soybeans) [17], for two months until they reached the weight of 300-400 grams. The animals were divided into four groups, with each group of eight rats receiving intraperitoneal injections daily for 45 days

Group I: Normal group injected with 0.2 mL saline.

Group II: Positive control group of obese rats injected with 0.2 ml saline.

Group III: Low dose treated group of obese rats injected with 5 mg/kg of TW [18].

Group IV: High dose treated group of obese rats injected with 7.5 mg/kg of TW [18].

Doses were meticulously prepared in the laboratory, and rats were injected daily for 45 days. At the end of the experimental period, the animals were sacrified and abdominally dissected. To remove any blood that might obstruct the fixation process, the liver, kidney, and testis of each animal were immediately

removed and washed in a physiological saline solution (0.9 percent NaCl). The specimens were soaked in a fixative (10% Neutral buffered formalin) for 24 hours. Before being embedded in paraffin wax, the tissues were routinely dehydrated and cleared. Microtome sections were prepared and mounted on clean glass slides before being de-paraffinized in xylene twice for 5 minutes, rehydrated with graded alcohol and stained with hematoxylin and eosin stain. The stained sections were examined and photographed using a camera microscope. The sections were compared to others from different groups for histopathological changes.

2.4. Histopathological examination:

Nominated sections were obtained from liver, kidney, and testis of each group and examined for potential histopathological changes induced by the administration of low dose and high dose of TW.

Liver examination; the sections from liver were assessed regarding portal tract inflammation, bile ductular proliferation, hepatic lobular necro-inflammatory response, cholestasis, hepatocytes hydropic degeneration and steatosis (microvesicular and macrovesicular), and vascular changes.

Kidney examination; the sections from kidney were assessed regarding glomerular size, tubular injury & interstitial chronic inflammation.

Testis examination: the sections from testis were assessed regarding seminiferous tubules (outlines & spermatogenesis maturation) and interstitial Leydig cells.

A calibrated digital microscope camera (Tucsen® ISH1000 digital microscope camera) and an Olympus® CX21 microscope with a resolution of 10 MP (megapixels) was used to capture tissue images (3656 x 2740 pixels per image). For image capture and enhancement, "IS Capture" software was used. The UIS optical system (Universal Infinity System, Olympus®, Japan) was used to capture all cuts at magnifications of 400x and 100x, with objectives of 40x and 10x. These images were depicted in plates as group I, II, III ,and group IV.

3. Results

3.1.Histopathological examination of liver sections:

Liver sections from **group I (negative control)** revealed intact normal liver architecture, intact hepatic lobules, hepatocytes arranged in a plate and intact portal tract (Figure 1, A). Liver sections from **group II** (positive control) revealed no portal inflammation or bile ductules proliferation focal lobular inflammation (lymphocytes within the hepatic lobules). Cholestasis, micro ,and macro- vesicular steatosis could be seen. There are localities in which hepatocytes have dropped out. Regarding vascular changes, congestion of central veins was detected (Figure 1, B &B*). Liver sections from **group III** (low dose TW) revealed moderate portal tracts inflammation in all portal tracts (enlargement by chronic inflammatory cells) and bile ductules proliferation with moderate can be seen in portal tracts. No steatosis or cholestasis were found . Vascular changes include dilated sinusoids with lymphocytes within them (Figure 1 C). Liver sections from **group IV** (high dose TW) revealed intact portal tracts. No bile ductules proliferation. Hepatocytes showed significant hydropic degeneration. Cholestasis was detected. Regarding vascular changes; congestion of central veins and congestion of hepatic sinusoids were detected (Figure 1 D).

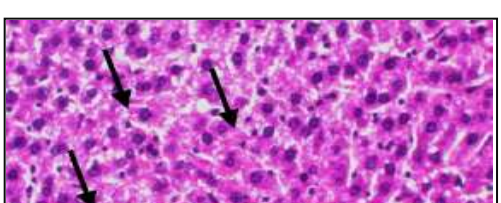

Figure 1, Liver sections of different obese rat groups after treatment with TW extract. **A. group I (normal control)**: uniform hepatocytes (Black arrows) and portal tract (Red arrow). **B ,and B*group II (positive control):** hepatocytes showed both micro (Black arrows) and macro (Arrowheads) vesicular steatosis. Some hepatocytes showed cholestasis (Red arrows). There are areas of a drop out hepatocytes (Blue arrow). There are areas of lymphocyte infiltrate within hepatocytes (focal lobulitis) (Yellow arrow). Some areas show congestion in central veins (Red arrows). **C. group III (low dose TW)**: portal tracts show proliferated bile ductules (Black arrows) with mild expansion by chronic inflammatory cells (Red arrow). Dilated sinusoids with lymphocytes are seen (Arrowheads). **D. group IV (high dose TW):** hydropic changes (ballooning degeneration) in hepatocytes (Black arrows). Congestion of sinusoids is seen (Yellow arrows). (H&E, 40x).

3.2. Histopathological examination of kidney sections:

kidney sections from **group I (negative control)** revealed intact normal kidney architecture (uniform glomeruli and tubules). Tubules are lined by uniform epithelial cells with intact well defined brush borders. The interstitium has no pathological changes (Figure 2 A).
kidney sections from **group II (positive control)** revealed glomerular enlargement (glomerulomegaly), acute tubular injury with focal areas of epithelial cells necrosis in the form of loss of epithelial cells and brush borders (Figure 2 B).
kidney sections from **group III (low dose TW)** revealed that all glomeruli were reduced in size. Extensive acute tubular necrosis in the form of disruption of epithelial cells lining, loss of epithelial cells nuclei and degenerated cells within tubular lumens (Figure 2 C).
kidney sections from **group IV (high dose TW)** revealed that all glomeruli were reduced in size. Extensive acute tubular necrosis with disrupted epithelial cells lining with cytoplasmic plebs, loss of their nuclei and degenerated cells within tubular lumens were detected. Interstitial chronic inflammation was detected (Figure 2 D).

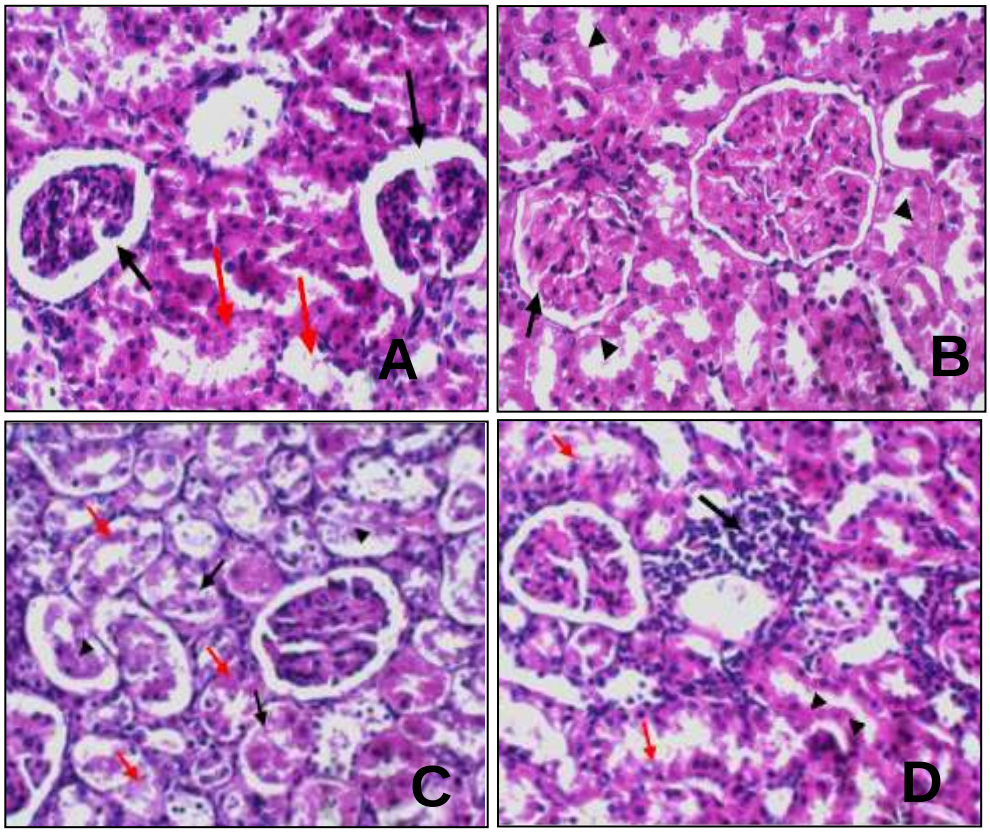

Figure 2, Different sections in kidneys of obese rat groups after treatment with TW extract. **A, group I (negative control):** Renal glomeruli are uniform in size (Black arrows) tubules have intact epithelial cells with brush borders (Red arrows). **B, group II (positive control):** Glomeruli are enlarged in size (Glomerulomegaly) (Black arrows). Tubules showed focal areas of epithelial necrosis (loss of epithelial cells and brush border) (Arrowheads). **C, group III (low dose TW):** All glomeruli are reduced in size. Almost all tubules show evidence of acute tubular necrosis and disruption of cell lining (Black arrows), loss of some cell nuclei (Red arrows) and degenerated cells within the lumen (Arrow heads). **D, group IV (high dose TW):** Tubules showed evidence of acute tubular injury and cytoplasmic plebs (Red arrows), loss of epithelial cells nuclei and brush border (Arrowheads). There are areas of interstitial chronic inflammation (Black arrows). (H&E, 40x).

3.3.Histopathological examination of testicular sections:

Sections from testis obtained from **group I** (negative control) displayed that all seminiferous tubules have uniform regular outlines with complete stages of spermatogenesis and visible spermatids within the lumens. Intact Leydig cells within the interstitium (Figure 3A). Sections from testis obtained from **group II** (positive control) showed about 50% of seminiferous tubules have irregular outlines, reduced spermatogenic cells and absence of spermatids. There is a mild increase in Leydig cells. However, other tubules exhibit regular outline with complete stages of spermatogenesis and visible spermatids within the lumens (Figure 3B).

Sections from testis obtained from **group III** (low dose TW) presented that all seminiferous tubules showed uniform regular outline with complete stages of spermatogenesis and visible spermatids within the lumens. (Figure3C).

Sections from testis obtained from **group IV** (high dose TW) illustrated that all seminiferous tubules have uniform regular outline with complete stages of spermatogenesis and visible spermatids within the lumens. Leydig cells within the interstitium are increased in number (Figure 3 D).

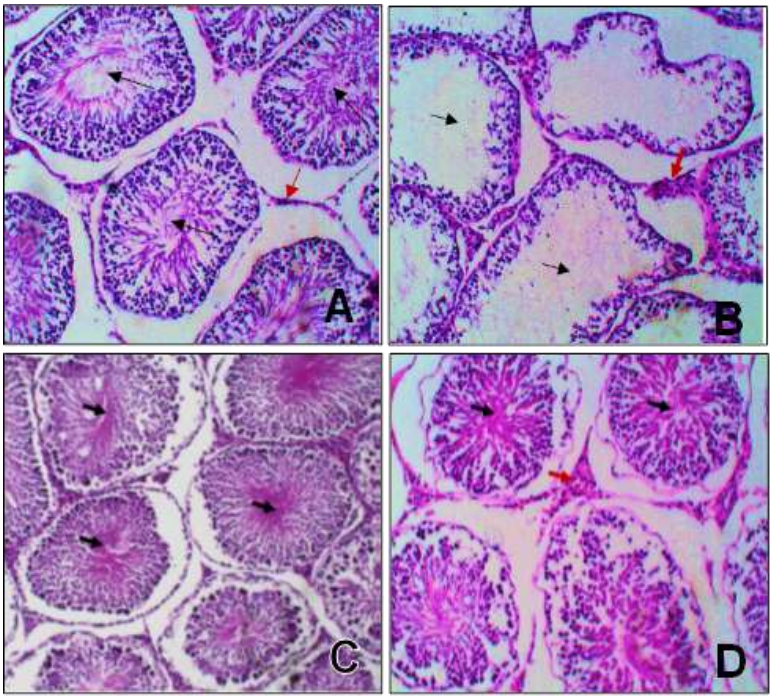

Figure 3: Different sections in the testis of obese rat groups after treatment with TW extract **A, group I (normal control group),** All seminiferous tubules showing uniform outlines, with complete stages of spermatogenesis. Lumens showing spermatids (Black arrows). Leydig cells are seen within interstetium (Red arrows). **B, group II (positive control group):** Most of seminiferous tubules showing reduced spermatogenic cells with absent spermatids (Black arrows). Mild increase in Leydig cells is seen (Red arrows). **C, group III (low dose TW)**: All seminiferous tubules show uniform outlines, with complete stages of spermatogenesis. The lumen shows spermatids (Black arrows). **D, group IV (high dose TW):** All seminiferous tubules showing uniform outlines, with complete stages of spermatogenesis. The lumen shows spermatids (Black arrows). There is an increase in Leydig cells number within interstitium (Red arrows) (H& E, 10x).

4. Discussion

Thunder god vine, also identified as "*Tripterygium wilfordii,"* is a classical Chinese plant found in China's east and south, and also Korea and Japan. It has been used in traditional Chinese medicine for a long time,

most notably for rheumatoid arthritis. It has been shown to possess anti-inflammatory, immune modulation, antitumor, and antifertility properties [19].

Clinical application of thunder god vine is restricted due to its potential toxicity to several organs, including liver and kidney. The most common side effects of thunder god vine are gastrointestinal tract disturbances (particularly diarrhea), leukopenia, thrombocytopenia, rash, skin pigmentation, and male and female reproductive dysfunctions [20]. Animal studies have shown that administering thunder god vine extract causes damage to multiple organs and tissues, even leading to death. One of the most important organs in plant-induced toxicity is the liver [21].

In the present study, the histopathological examination of liver tissues revealed that normal liver architecture was observed in the negative control group in the form of plates of normal uniform hepatocytes and normal portal tract. The liver architecture was disturbed in the obese group and hepatocytes showed both micro and macro vesicular steatosis. Some hepatocytes showed cholestasis. In terms of the drug's toxic effect on the liver, the current study found that both low and high dose administration were toxic and this was supported by numerous studies [22].

In liver of rats given the root extract 1mg\ kg orally, researchers discovered focal necrosis with inflammatory cell infiltration. They also observed a change in serum concentrations of glutamyl transpeptidase, (ALT\ GPT), and (AST\GOT), all of which are important biochemical parameters of liver injury.[23] Microvesicular steatosis and increased oxidative stress were discovered to be associated with liver injury. This is due to mitochondrial activity inhibition in the respiratory chain and secondary oxidative damage. According to researchers [24], the plant's oxidative stress is linked to the activation of inflammatory molecules, which results in the release of cytokines and the start of an inflammatory response, with the resulting amplification of hepatic damage. Hepatocytes in the low dose group are uniform, with no evidence of steatosis, cholestasis, or other pathological changes. Nonetheless, sinusoids were dilated with few lymphocytes, and portal tracts had bile ductules proliferated with mild expansion by chronic inflammatory cells. Dilated sinusoids contain lymphocytes [25]. After administering 1 mg/kg extract orally, researchers observed that in rat hepatocytes, there is focal necrosis with inflammatory cell infiltration.

Histopathological examination of kidney sections revealed that the plant extract has a nephrotoxic effect, which manifests as a decrease in glomerular size, acute tubular necrosis, epithelial cell separation and detachment, and loss of brush borders. [26] After treatment with the plant extract, proximal convoluted tubular epithelial cell separation is caused by the destruction of intercellular junctions and changes in paracellular permeability in proximal tubules. [27] revealed that a single dose of the plant extract resulted in severe renal injury, including brush border injury and tubular obstruction. .According to[28] TNF- release in the blood has been linked to upregulation of organic cation transporter 2 in the kidney, which transports excess extract into the kidney, exacerbating the drug's nephrotoxicity after long-term administration. The reported renal damage could have been caused by oxidative stress as a result of single dose intraperitoneal injection of the plant extract.

In terms of the drug's toxic effect on the testis, the current study found that it has a negative toxic effect on the size of the seminiferous tubules. This finding was in contrary with that of [29] who found distinct change in seminiferous tubules [30] reported that 8 weeks later of administration of (0, 100, 200, 400 µg/kg) orally once daily of the root extract, testis weight , sperm mortility , content and sperm quality were significantly reduced. The impairment in spermatogenesis was attributed to abnormal lipid and energy metabolism in the testis caused by stimulant down-regulation of peroxisome proliferator-activated receptor. [31] The weights of the testis as well as sperm content and motility were significantly reduced in male rats after 8 weeks of oral dose of thunder god vine extract. It has also been proposed that abnormal lipid and energy metabolism in the testis impairs spermatogenesis via thunder god vine extract-mediated down-regulation of peroxisome proliferator-activated receptor.

Thunder god vine extract treatment is said to reduce the expression of breast cancer resistance protein in the testis while increasing testicular content, contributing to the drug's cumulative testicular toxicity [32].

The current study showed that significant changes in seminiferous tubules and epididymides were observed in male reproductive toxicity, In addition, almost all tubules show signs of acute tubular necrosis, including disruption of cell lining, loss of cell nuclei, and degenerated cells within the lumen. The glomeruli in the treated high dose are smaller, the tubules still show evidence of acute tubular injury ,and there are areas of interstitial chronic inflammation.

5.CONCLUSION

Although thunder god vine extract is used as a traditional treatment, its toxicity affects tissues even when used in low doses. To avoid organ toxic side effects, the extract should be used with warning and for a short period.

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