Role of Proplis administration in boldenone-induced oxidative stress, Ki-67 protein alterations and toxicity in rat liver and kidney

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Abstract: Boldenone is a derivative of testosterone. The aim of this study, the effect of propolis as co-administrated in liver and kidney toxicity induced with boldenone. Sixty albino rats were divided into 4 groups; 1st control group were rats receive olive oil, the 2nd group were rats receive propolis, 3rd experimental group include animals that receive intramuscular injected with boldenone and 4th group were co- administrated group were rats receive boldenone and propolis at the same time. Ki-67 expression in liver and kidney sections were a significant increase in boldenone group when compared with the control group. Intramuscular injection of rats with boldenone showed a marked disturbance of the hepatocytes with multifocal hepatocellular vacuolations in the liver and marked glomerulus mass reduction with multifocal glomerular injury in the kidney. Co-administration of boldenone with propolis a moderate improved the renal and hepatic injuries induced by boldenone and decrease Ki-67 expression.

Keywords- Boldenone, Propolis, Ki-67 immunoreactivity, Liver; Kidney..

I. INTRODUCTION

Boldenone applied as a growth promoter on meat production farms; in order to increase the productivity and to reduce breeding expense, therefore might be abused to achieve more efficient meat production [1]. Also it is used to improve athletic, body builder and racing horse performance in sports [2]. The abuse of anabolic androgenic steroids can lead to serious and irreversible organ damage [3]. Liver as structure and function of primary site of anabolic androgenic steroids clearance may be altered. These alterations include intrahepatic cholestasis, jaundice, hyperplasia, benign (adenoma's) and malign (hepatocellular carcinoma) tumors and Pelosi's hepatic [4], also; renal side effects are contradictory. High levels of serum urea, serum uric acid and hypophosphatemia and possible nephrosclerosis with obstructive glomerulosclerosis have been reported [5]. Anabolic androgenic steroids have also been associated with suppression of immune function, changes of hemostatic system, occurrence of Wilms' tumor [6].

Propolis is a resinous substance collected by worker bees (Apis mellifera) from the bark of trees and leaves of plants [7]. Propolis has been mainly used as home remedies and a personal product since 300 BC [8]. Propolis is a honeybee product with a broad spectrum of biological properties [9]. Propolis shows a complex chemical composition [10]. Biological properties and chemical compositions of propolis may vary according to different plant sources that bees could visit, collecting time and geographic locations [11]. Propolis also contains more than 300 biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, vitamins, amino acids [12]. Propolis possesses several biological properties such as anti-inflammatory, anticancer, antioxidant, antibiotic and antifungal activities [13].

Because Ki-67 is nuclear only in proliferating cells, it is widely used as a marker to assess cell proliferation. For example, immunohistochemical assessment of the proportion of cells staining for nuclear Ki-67 is used to predict the responsiveness or resistance of tumors to therapy [14]. Ki-67 protein is predominantly localized in the cortex and dense fibrillar components of the nucleolus during interphase [15]. During mitosis it relocates to the periphery of the condensed chromosomes. It has been reported that Ki-67 is associated with the nuclear matrix, preribosomes, satellite DNA in G1 and with the chromosome scaffold of mitotic cells [16].

II. MATERIALS AND METHODS

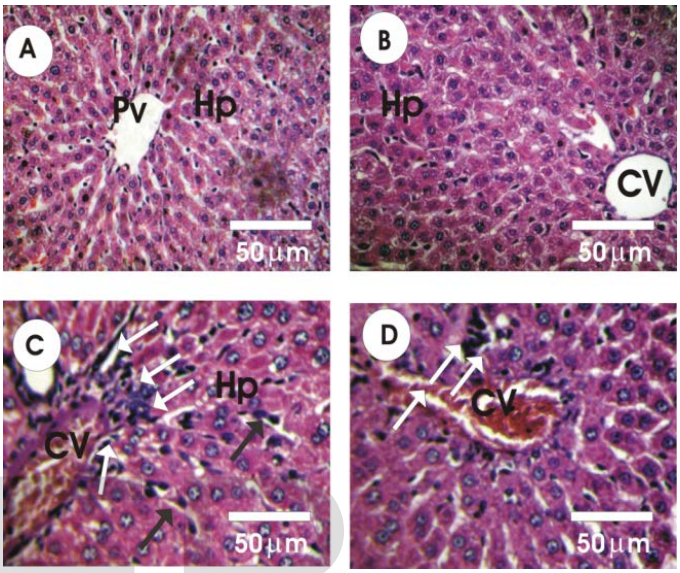
The experiments were performed on 16 male albino rats (Wistar albino) weighing 100-120g supplied from the animal house of National Research Center (Dokki, Giza, Egypt) were used for this study. Animals were housed in cages under proper environmental conditions at room temperature 22-24°C and 12h light/dark cycle and fed with a commercial pellet diet (Wadi El Kabda Co., Cairo, Egypt). The animals had free access to water. The animals were acclimatized to the laboratory conditions for two weeks before beginning the experiment. The experiment continued for 12 weeks on which constant weight of diet was given for

each rat. All the experiments were designed and conducted according to the ethical norms approved by the Ethical Committee of National Research Center. The extraction procedure for propolis leaves was carried out as reported by Yonar et al. [17]. Total of 16 rats were randomly and equally divided into four groups (10 animals each). 1st control group includes animals that injected intramuscularly with olive oil for 10 weeks. 2nd group were rats receive propolis (intragastrically, 400 mg/kg body weight). 3rd experimental group include animals that receive intramuscular injections of boldenone (5 mg/Kg body weight) for 10 weeks. 4th group were co-administrated group where rats receive boldenone (Intramuscular injections, 5 mg/Kg body weight) and propolis (Intragastrically, 400 mg/kg body weight). At the end of the experimental period, rats were fasted overnight and for clinical chemistry. Rats were euthanized with intravenous injection with sodium pentobarbital and subjected to a complete necropsy. Tissue homogenates were prepared as reported by Lahouel et al. [18]. Briefly, specimens were separated into two parts. Each piece was weighed and homogenized separately with a Potter Elvenhjem tissue homogenizer. The crude tissue homogenate was centrifuged at 11,739 rcf, for 15 min in a cold centrifuge, and the resultant supernatant was used for the different estimations. Immediately after decapitation animals were dissected, liver and kidney from different groups were quickly removed and fixed in 10 % neutral buffered formalin. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin (mp. 50–58°C). Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. Sections were stained with Ehrlich's haematoxylin and counterstained with eosin as a routine method after Bancroft and Stevens [19]. All stained slides were viewed by using Olympus microscope and images were captured by a digital camera (Cannon 620). The rest of liver and kidney sections in different groups under study were deparaffinized with xylene and dehydrated with ethanol. The slides were then immersed in water for 10 min. For antigen retrieval, the slides were boiled in citrate buffer, pH 6.0, for 15 min in a microwave oven and subsequently cooled for 20 min. Next, the slides were washed in TBS and endogenous peroxidase was blocked with 0.3% hydrogen peroxide for 15 min. After washing with TBS, the sections were incubated overnight at 4ºC with mouse antibodies to Ki-67 at 1:50 concentration [20]. After 24 h, the slides were washed and incubated with a multilink antibody for 20 min, washed in TBS and incubated for 20 min with the avidin-biotin-peroxidase complex. After washing with TBS, the slides were incubated for 3 min with diaminobenzidine, and finally counter-stained with hematoxylin prior to mounting. The percentage of proliferating neoplastic cells was evaluated directly by light microscopy.

III. RESULTS

Effect of propolis on liver histopathology

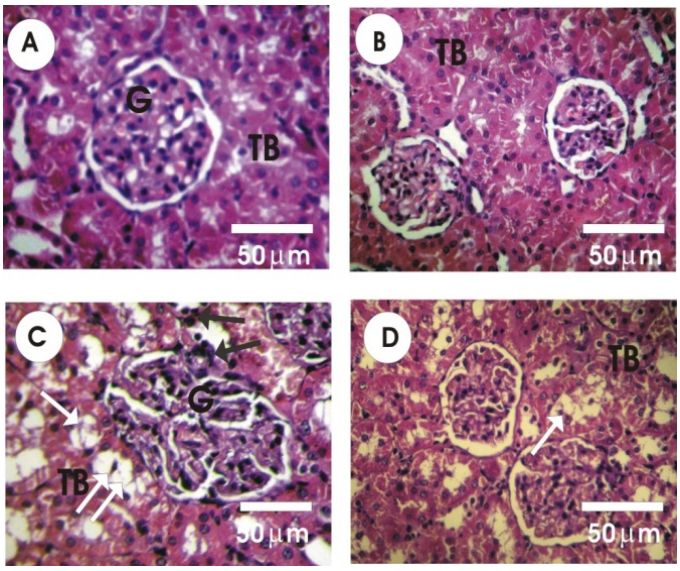
Rat liver sections in control and propolis groups showing normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords (Figures 1A & 1B). Rat liver sections in boldenone group showed a disturbance in the hepatocytes with moderate vacuolated hepatocytes, mild atrophy and congestion in central veins (Figure 1C). Liver sections in co-administration of propolis with boldenone showed a few vacuolated hepatocytes; mild cellular infiltrations and mild congestion in central veins (Figure 1D).



Figures (1A-1D): Photomicrographs of rat liver sections in the different experimental groups stained with Haematoxylin & Eosin. A: Rat liver sections in control group revealed normal structure of hepatocytes. B: Rat Liver section in propolis group revealed normal structure of hepatocytes (hp). C: Liver sections of boldenone group showed congestion (White arrows) in central veins (CV), atrophied, and vacuolated hepatocytes (White arrows). D: Liver sections in co-treated rat with propolis groups revealed a moderate to mild degree of improvement in hepatocytes where a few vacuolated hepatocytes (White arrows).

Effect of propolis on kidney histopathology

The rat kidney of control and propolis groups revealed a normal structure of glomeruli and renal tubules (Figures 2A & 2B). Kidney sections in boldenone group showed marked glomerular damage and marked tubular necrosis with invading inflammatory cells (Figure 2C). Kidney sections in co-administration of propolis with boldenone revealed a good degree of improvement, where the glomerular and the tubular cells appeared with minimal vacuolization and mild congestion in blood vessels (Figure 2D).



Figures (2A-2D): Photomicrographs of rat kidney sections in the different groups stained with H& E. A&B: kidney sections in control and propolis groups respectively showed normal structure of renal cortex (White arrows), proximal and distal convoluted tubules. C: kidney sections in boldenone group showed vacuolization in tubular cells (White arrows), focal necrosis and cell infiltration. D: Kidney section of co-treated group with propolis revealed a good degree of improvement glomerular damage with minimal vacuolization (Arrow heads) in tubular cells.

Effect of propolis on liver and kidney Ki-67 immunoreactivity:

The detection and distribution of Ki-67 immunoreactivity (Ki-67-ir) in liver and kidney sections in the different groups under study were revealed in Figures (3 & 4). Liver and kidney sections in control groups showed a mild or negative expression for Ki-67-ir (grade 0 & 1 respectively) were observed, also; faint to negative expressions for Ki- 67 in liver and kidney (grade 0 & 1 respectively) in propolis group. Strong positive expression for Ki-67-ir (grade 4 & 4 respectively) were detected in both liver and kidney sections in boldenone group. Moderate to mild positive expression for Ki-67-ir in liver and kidney sections in co-administration of propolis with boldenone (grade 3 & 2 respectively). The intensity of Ki-67-ir in liver and kidney sections in co-administration of propolis with boldenone showed significantly decreased when compared with boldenone group.

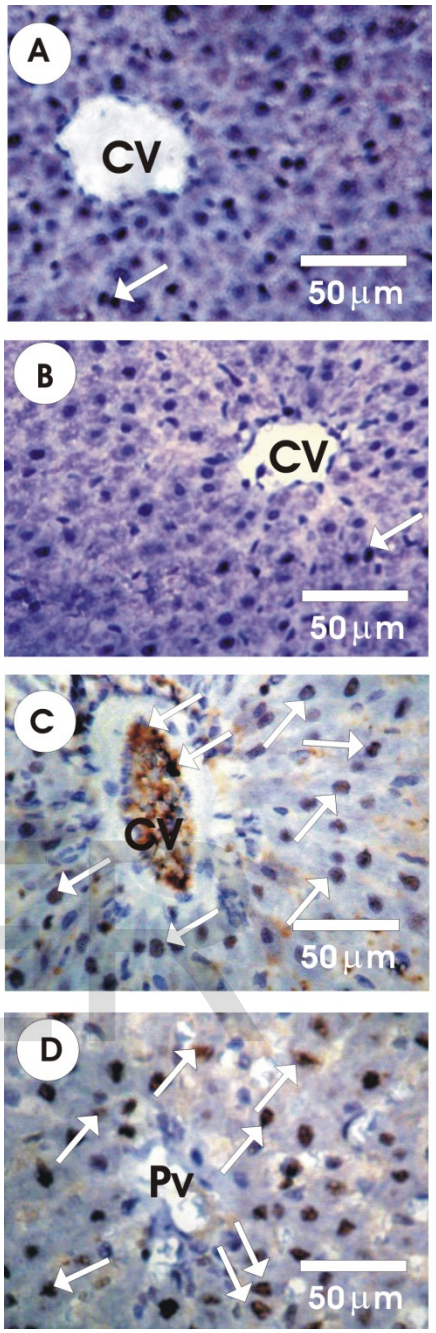


Figure 3: Ki-67 expressions in rat liver sections in different groups under study. A: faint expression in control group. B: negative expression in propolis group. C: strong positive expression boldenone group. D: moderate expression in co-treated boldenone group with propolis. White arrows indicate strong positive expression of Ki-67 hepatocytes.

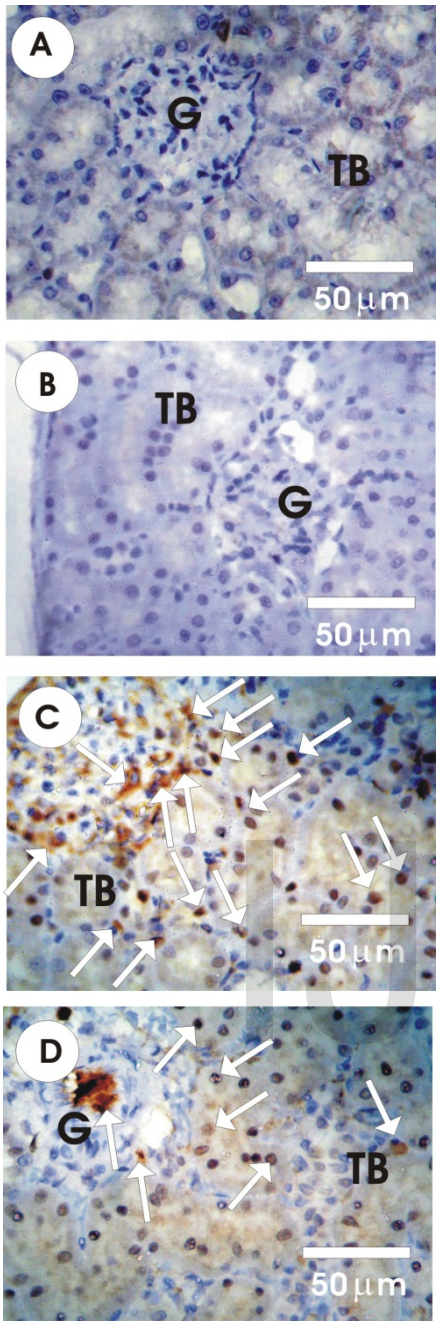


Figure 4: Ki-67 expressions in rat kidney sections in different groups under study. A&B: Faint or negative expression in control and propolis groups. C: strong positive expression boldenone group. D: moderate expression in co-treated boldenone group with propolis. White arrows indicate strong positive expression of Ki-67 renal tissues.

IV. DISCUSSION

Recently, boldenone used by bodybuilders in both off-season and pre-contest, where it is well known for increasing vascularity while preparing for a body building contest. It has a very long half-life and can show up on a steroid test for up to 1.5 years. Trace amounts of the drug can be easily detected for months after discontinued use [21]. Boldenone caused some adverse effects on many other adverse effects associated with anabolic androgenic steroids were recorded to be happened such as disturbance of the endocrine and immune function, alterations of sebaceous system and skin, changes of hemostatic system and urogenital tract [22]. The antioxidant activities of propolis are related to its ability to scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxynitrite [23]. Our data showed that rats that receive boldenone revealed gradual disturbances of the hepatocytes radially arranged cords with sinusoidal congestion in liver and a multifocal glomerular injury with markedly congested sinusoidal and dilated blood vessels in kidneys. Our results agree with El-Moghazy et al. [21] who reported that changes in the hepatic and renal structure and function after a growth promoter boldenone injection in rabbits. Propolis supplementation enhancement of liver and kidney damage induced by boldenone. Our results agree with Türkez et al. [23] who reported that propolis prevents aluminum-induced genetic and hepatic damages in rat liver. The expression of the Ki-67 protein is strictly associated with cell proliferation. The monoclonal antibody of Ki-67 has been developed and used in evaluating cellular proliferation rates of malignant tumor [20]. In the present study; the detection and distribution of Ki-67 in liver and kidney sections were observed. Our results indicated that; the intensity of Ki-67 after boldenone injection was significantly increased on liver and kidney sections when compared with control rat. Also, co-treatment with propolis significantly decreases the intensity of Ki-67 in liver and kidney sections when compared with boldenone group. Our results were coincided with that of Pižem et al. [24] who stated that PCNA and Ki-67-ir were useful for proliferative activity assessment of hepatocytes and their expressions were higher in HCC than in non-neoplastic liver. Also the study of Guzman et al. [25] showed that immunostaining of HCC lesions for Ki-67-ir was associated with higher mitotic activity.

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