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Impact of Grape Seed Extract (Proanthocyanidins) on Pathophysiological Changes and Antioxidant Capacity in Rabbit Does Expose to Heat Stress

Emad A. Abd allah, Doaa S. Abdel-Hady, Ali E. Ghodaia, Hany M. A. Lethy, Entesar Z. Eliraqy, Zeinab M. Farouk and Sameh M. Shedeed

Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Abstract

THE MAIN aim of this study is to assess the effects of grape seed extract (GSE) on immune performance and, antioxidant capacity in rabbit does exposed to heat stress in Sakha Animal Research Station, Kafr El-Sheikh governorate, Egypt. Twenty-four healthy mature females of APRI bred were randomly allocated into 3 groups (eight rabbit does each). The first group (GI), received a regular rabbit diet and was maintained as a control group. Rabbits from the second group (G2) were treated with GSE by oral gavage at the dose of 125 mg/kg body weight. The third group (G3) of rabbits was treated with GSE by oral gavage at a dose of 250 mg/kg body weight. Rabbits were kept in cages under a constant temperature of 28-30Cº and 12:12 hour "light: dark cycle". Food and water were provided adlibitum. The lipid parameters, Cholesterol, Triglycerides, and High-Density Lipoprotein and Low-Density Lipoprotein were determined. For biochemical and histopathological examinations specimens from liver, kidney, and ovary tissue were collected. The obtained results from in vitro data demonstrated that GSE (125-250 mg/kg) treatments increased immunosystem and decreased oxidative stress, and also suggested that GSE plays a beneficial role in the enhancement of liver and kidney function and improves the ovarian activity.

Keywords: Grape seed extract, pathophysiological changes, antioxidant capacity, heat stress, Rabbits.

Introduction

According to [13], oxygen is necessary for every life on Earth to survive. About 5% of oxygen is univalently reduced during the course of oxygen use in typical physiological and metabolic processes, producing oxygen-derived free radicals such as superoxide, hydrogen peroxide, hydroxyl, and nitric oxide radicals [35]. The cell is subjected to around 10,000 oxidative hits each second by all of these radicals, also referred to as reactive oxygen species (ROS) [24]. Free radicals begin damaging cell proteins, lipids, and carbohydrates when a formation of ROS overwhelms the antioxidant defense of the cells. Several physiological disorders were the result of this [13].

According to [6], free radicals play a role in the development of degenerative illnesses. Additionally, according to [4], they have been linked to the etiology of cancer, neurological diseases, alcoholism, diabetes, nephrotoxicity, inflammation, atherosclerosis, cancer, and the aging process. According to [22], a variety of plants can be used as a scavenger for excess free radicals since they contain significant levels of antioxidants such as carotenoids, flavonoids, vitamins C and E, and tannins.

When rabbits experience heat stress, their bodies are unable to regulate the equilibrium between heat generation and dissipation. Raising rabbits in the summertime can be detrimental to their health due to heat stress, which is easily caused by high ambient temperatures [30]. A number of variables, including high temperatures, humidity, radiant heat, and airspeed, can combine to cause heat stress. As a result, heat stress is mostly caused by high ambient temperatures [27].

The average body temperature of a rabbit ranges from 38.5 to 39.5◦C, whereas each rabbit has individual variances that range from 0.4 to 1.1◦C. When the outside temperature goes beyond 30°C, heat stress occurs. If the temperature rises above 35°C, rabbits cannot control their body temperature and will eventually have heat failure [33, 29]. For rabbits, 15 to 25°C is the appropriate temperature range, and 55–65% is the ideal humidity level.

*Corresponding author: Emad A. Abd allah, E-mail: dremad2019@gmail.com, Tel.: 00201004901993
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Heat stress has been shown to have a number of detrimental effects on rabbit health and productivity. These include a 20–25% reduction in daily weight gain, an 8–15% decrease in feed conversion ratio, a 9–12% increase in mortality rate, a 6–10% decrease in reproductive performance, and more.

Nutritional intervention is one of the possible ways for mitigating heat stress, and it has been shown to be an effective mitigation method [41].

Polyphenols, lipids, proteins, and carbs can all be found in grape seed, a naturally occurring plant component. The most common phenolic compounds in grape seeds are called proanthocyanidins, and they are higher molecular weight polymers made up of dimers or trimers of (+) -catechin and (−) -epicatechin. Proanthocyanidins are more potent antioxidants than other types of phenolic compounds [1]. Moreover, antibacterial, antiviral, anti-inflammatory, anti-allergic, and vasodilatory properties are possessed by vitamin C, E, and gallic acid [5] [9]. According to [16], GSE shields the cellular membrane from oxidative damage, preventing liver lipid peroxidation. The ovarian activities are controlled by various hormonal substances which act as themselves oxidative damage [17]. Reactive oxygen species (ROS) are one of the many physiological regulators that control the complex antioxidant defense system present in all non-pathological cells [29].

ROS are produced in the ovarian follicle and corpus luteum, and they play crucial roles in follicular development and survival [45]. The effects of oxidative stress on female reproduction have been widely described [3]. It is also commonly known that, when preserved at physiological cellular concentrations, ROS play a significant role in the control of cell signaling. For instance, cell metabolism produces superoxide, which is produced by membrane-bond enzyme complex oxidases [28]. On the other hand, oxidants' effects can be offset by an excess of ROS generation [12]. Moreover, proanthocyanidins have been demonstrated to prevent platelet aggregation, lipid peroxidation, capillary permeability, and fragility [49]. Proanthocyanidins have also been shown to potently block xanthine oxidase, an enzyme that is crucial in the synthesis of free radicals. Also, they have the ability to bind free iron molecules and prevent lipid peroxidation brought on by iron [18]. Proanthocyanidins have a monomer structure made up of C4 to C8 or C4 to C6 links, which link (epi) gallocatechin or (epi) catechin [42]. Condensed tannin, as defined by [7], is a term used to describe flavan-3-ol that is typically condensed into oligomeric and polymeric molecules with a degree of polymerization ranging from 2 to 11.

Proanthocyanidin has been shown to have a variety of pharmacological and nutraceutical benefits over the past few decades, including the prevention of atherosclerosis, the amelioration of ischemic cardiovascular disease, anticancer effects, and antibacterial, antiviral, and antifungal activities [48, 14]. These benefits have been demonstrated by both experimental and clinical studies. Due to their 20-fold greater capacity to scavenge free radicals than other well-known antioxidants (e.g., vitamin C, vitamin E, or β-carotene), proanthocyanidins were thought to have positive benefits.

The purpose of the present study was to evaluate the possible protective effect of grape seed extract on the heat stressed rabbits under hot environments.

Material and Methods

Grape seed collection

According to [2], grape seeds (Vitis vinifera) were acquired from El-Ahram Heineken for drinks (Gianaclis Company), which is situated in the Gianaclis area, Abu El-Matamir, Beheira Governorate, Egypt.

Preparation of alcoholic extract

After being cleaned with tap water, the grape seeds were ground into a powder using a knife mill and dried. 30 g of the powder was combined with 300 ml of 96% ethanol and allowed it to remain at room temperature in a shaking water bath for a full day. With filter paper (Whatman No. 1), the extract was filtered out of the solid concentration. After twice extracting the leftover residue, the extracts were combined. A rotary vacuum evaporator was used to extract the solvent under vacuum at 40 °C [31].

Animals and Experimental Procedures

The current study was carried out at the Animal Production Research Institute in Sakha Station, Kafr El-Sheikh, Egypt. The biochemical analysis was done in APRI laboratories during June 2022.

A total of 24 healthy mature rabbit females of the APRI line were used in this study, weighing 3.0-3.5 kg, were used throughout this study. The Rabbits from Sakha Research Station were kept in cages. These cages were cleaned once a week. The feed (pellets) and water (tap water) were given freely and housed in a constant 28-33Cº temperature environment with12:12-hour light: dark cycle where the maximum temperature used to reach heat stress point is 30.5 Cº.

The rabbits were split up into three equal groups of eight each at random. The initial group (GI) was kept as a control group and was fed a regular diet of rabbits. Proanthocyanidins dissolved in water, or GSE, was administered orally to rabbits in the second group (G2) at a dose of 125 mg/kg body weight. GSE was administered orally to the third group (G3) of

rabbits at a dose of 250 mg/kg body weight [25]. The experiment was run up to three weeks.

At the end of experiment the blood samples were obtained via jugular veins punctured using sterile syringe needle for biochemical assessments. Blood samples were centrifuged at 700 g for 10 minutes at room temperature after clotting for an hour. The sera were carefully gathered and stored at -20°C until they were needed for the biochemical assessments. After that, each was euthanized under a light ether anesthetic. Kidney and liver were taken for histological and biochemical analyses.

Biochemical Analysis

Using a conventional enzymatic assay (Fortress/UK kit), the lipid parameters—cholesterol, triglycerides, and high- and low-density lipoprotein—were measured [11]. To estimate MDA using the thiobarbituric acid test, one gram of liver and heart tissue was taken, as previously mentioned by [47]. The liver's GSH content was determined using [40]. Both creatinine and urea levels in the serum were determined using the techniques outlined by [36] and [20]. Using an auto-analyzer (Olympus AU 600, Japan), the activities of serum alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase (AST) were determined.

Histopathological Examination

For histological study liver and kidney tissue samples were preserved in 10% buffered neutral formalin. Histo-section processing was performed on paraffin-embedded blocks, and 5 µm thick sections were stained with hematoxylin-eosin and then viewed using an Olympus BX51 optical microscope with a 200 X magnification applied to 10 microscopic fields.

Statistical Analysis

Results were expressed as mean ± SD, statistical evaluation was done using one-way analysis of variance (ANOVA) which is followed by the Duncan test. The level of statistical significance was set at $P<0.05$ [44].

Results

Serum biochemical enzymes

Aspartate aminotransferase (AST) and alanine transaminase (ALT) activities were significantly ($p < 0.05$) decreased in group 3 rabbit does (30.8±2.8 and 37.3±2.0) when compared to other treatment groups G2 (41.4±2.8, 57.6±1.4) and G3 (54.5±2.4, 71.6±1.6) respectively table (1).

Creatinine and Urea

The effects of GSE on serum creatinine and urea concentrations are shown in Table (2). Comparing G1 to other treatment groups, there was a substantial ($P<0.05$) rise in urea and creatinine concentrations. Compared to groups 2 and 3, which had creatinine levels of 1.16±0.28 and 0.8±0.09 mg/dL, G1 did had substantially higher creatinine levels (1.31±0.24 mg/dL). In contrast, G3 had urea concentrations that were considerably ($P<0.05$) less than those in the other groups receiving therapy. At 37.6±1.45 mg/dL, urea concentrations in G1 were significantly ($P<0.05$) lower than those in the other groups.

Serum lipid profile

Lipid profiles expressed by cholesterol (C), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) of rabbit doe groups treated with Grape seed extracts are shown in Table (3).

Table 3 indicates that, compared to the other groups, G1 had a considerably higher ($P<0.05$) concentration of total cholesterol (13.1±0.24 mg/dL) than the other groups, but there was no significant difference in the concentration of total triglycerides. Group 3 had a considerably greater concentration of high-density lipoproteins (HDL) (4.3±0.78 mg/dL) than the other two groups ($P<0.05$). Low-density lipoprotein (LDL) concentrations in groups 2 and 3 (6.5±0.48 and 2.2 ± 0.31 mg/dL, respectively) were considerably lower ($P<0.05$) in G1 (7.1±0.15 mg/dL).

Tissue GSH and MDA concentrations

After receiving GSE therapy for 21 days, the animals' tissue thiobarbituric acid reactive chemicals were considerably higher than those of the control group (G1). The animals' livers that received 250 mg/kg of MDA showed the biggest reduction in MDA content. In contrast to the control value, GSE also demonstrated a decrease in the lipid peroxidation product. However, there was a noteworthy decrease in the animals when compared to the control group, and the animals treated with 250 mg/kg GSE showed the largest rise in GSH content in their livers (Table 4).

Histopathology

Histological Examination of the Liver

A microscopic examination of liver tissues in animals treated with GSE (G2 and G3) showed improvement in the histological picture somewhat normal appearance hepatocytes, vacuolated and normal hepatocytes central vein (Fig 1, b and c). It is worth mentioning that histological (Structural) changes observed in the liver, supported the observed biochemical (Functional) changes in all experimental groups.

Histological examination of the kidney

Representative samples of kidneys from groups 2 and 3 showed normal glomerulus and tubules, group 1 showed disruption and loss of glomerular histo-architecture. FIG. 2c

Histological examination of ovary

An ovarian sample was indicative of group 1 (Fig 3a). The ovarian cortex is primarily made up of primordial follicles, it does not contain mature graffian follicles or primordial follicles (H&E X200). Group 2's sample (Fig. 3b) revealed mature graffian follicles with antral fluid, while a primary follicle consisting of an oocyte and one layer of granullosa cells was also visible (H&E x200). In a similar manner, group 3's sample (Fig. 3c) showed primary follicles in the ovarian cortex beneath the ovarian capsule in addition to numerous primordial follicles (H&E X200).

Discussion

The purpose of this study was to examine the effects of grape seed extract (proanthocyanidins). Grape seeds are important because they have flavonoids, which act as free radical scavengers to protect LDL-c from oxidation during the early stages of lipid peroxidation, hence explaining their protective effect as an antioxidant [19]. It has been demonstrated that quercetin and flavonoids bind to the surface of LDL particles by forming an ether bond [23].

In addition to lowering the levels of Ch, TGs, and LDL-c and decreasing the concentration of MDA in the liver tissues, alcoholic extracts from grape seeds have a significant positive impact on lipid peroxidation and antioxidant status. They also increase the levels of HDL-c in serum and GSH in the liver tissues. According to our research, grape seeds rich in proanthocyanidins and polyphenolic compounds are crucial for preventing endothelial cell-mediated LDL-c lipid peroxidation [37]. Nonetheless, grape seed extract increased the activity of antioxidant enzymes in cells and decreased the propensity of LDL-c to oxidize [21]. This study showed that the alcoholic extract from grape seeds had a protective effect at the histopathological levels at a concentration of 250 mg/kg.

Because of its anti-proliferating qualities, ability to inhibit a redox-sensitive signal transduction pathway linked to cell growth on vascular smooth muscle cells, and changes in antioxidant enzyme chain breaking antioxidant activity, GSE may therefore have a protective effect against oxidative stress [26]. In this investigation, the treatment with grape seed extracts prevents the rabbits' increased lipid peroxidation in comparison to the control group.

The observed outcome may be attributed to either oxidative inactivation or feedback inhibition of the enzyme protein as a result of excessive ROS production. These enzymes may become inactive as a result of producing α-hydroxyethyl radical [37]. Following treatment with extracts from grape seeds, these enzymes exhibited a marked increase in activity. By scavenging reactive oxygen species (ROS), boosting the activity of cellular antioxidant enzymes, and raising glutathione levels in cells, these extracts may improve antioxidant action.

Because it can neutralize the peroxyl radical, uric acid is a metabolite that can act as an antioxidant in the plasma. Uric acid is a good indicator of stress, according to [43] and Superoxide anions are produced by ubiquinone from the electron transport chain and catalyze the conversion of hypoxanthine into xanthine and uric acid, which is carried out by xanthine oxidase [15, 39]. [34] found that very short exposure to high temperatures (15 min at 42ºC) results in a reduction in N retention, an increase in plasma uric acid levels (which may indicate active protein catabolism), a decrease in protein synthesis, and a depletion of both essential and non-essential plasma-free amino acids.

On the other hand, prolonged exposure to heat stress lowers the levels of most plasma-free amino acids, particularly sulfur and branched-chain amino acids, and suppresses protein synthesis in a variety of muscles as well as protein breakdown. Concentrations of uric acid are kept at values similar to those observed in control pigeons [46]. Thus, when heat stress persists, protein breakdown may first rise quickly before declining. During these processes, there appears to be a depression in both protein synthesis and N deposition.

Vegetables, fruits, different flowers, and their seeds all contain proanthocyanidins, which are polyphenolic anti-oxidants [8]. Polyphenols are abundant in grape, and 60–70% of these polyphenols are found in According to [32], GSPE is derived from grape seeds and is a physiologically active mixture of polyphenolic flavonoids that includes oligomeric proanthocyanidin [8]. Various experimental studies have established the antiapoptotic, anticarcinogenic, antiallergic, anti-inflammatory, and immunomodulator effects of GSPE, in addition to its antioxidant capabilities [8,10] Studies, however, indicate that its anti-apoptotic hepatoprotective action is achieved by downregulating the expression of bcl-XL, a bcl2 family member that creates resistance to apoptotic cell death. [38].

Conclusion

The results of the in vitro research showed that GSE (125–250 mg/kg) treatments boost the immune system and reduce oxidative stress. They also suggest that GSE may be useful in improving kidney and liver function as well as ovarian activity and fertility rate. To ascertain the molecular mechanism of GSE and confirm the potential in vivo application

of these natural phytoproducts, more research is required.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Animal Production Research Institute, Agriculture Research Center, Egypt (ethics approval number, 14/4/2019).

TABLE 1. Levels (Mean ± SEM) of serum aspartate aminotransferase (AST) and alanine transaminase (ALT) of the rabbit doe groups treated with Grape seed extracts.

Group	AST (U/ml)	ALT (U/ml)
G1 (Control)	54.5±2.4^{a}	71.6±1.6^{a}
G2 (125 mg/kg)	41.4±2.8^{b}	57.6±1.4^{b}
G3 (250 mg/kg)	30.8±2.8^{c}	37.3±2.0^{c}

SEM Standard error of the mean, a-c Means within a column with different superscript letters were significantly different (P< 0.05).

TABLE 2. Levels of serum creatinine and urea (mean ± SEM) of the rabbit doe groups treated with Grape seed extracts.

Group	creatinine (mg /dl)	Urea (mg/ dl)
G1 (Control)	1.31±0.24^{a}	57.6±5.3^{a}
G2 (125 mg/kg)	1.16±0.28^{ab}	42.6±1.45^{ab}
G3 (250 mg/kg)	0.8±0.09^{b}	37.6±1.45^{b}

SEM: Standard error of the mean, a-b Means within a column with different superscript letters were significantly different (P< 0.05).

TABLE 3. Lipid profile* in serum rabbit does treated with Grape seed extracts.

Group	Cholest. (mmol/L)	TG (mmol/L)	HDL (mmol/L	LDL (mmol/L
G1 (Control)	13.1 ± 0.24^{a}	6.5 ± 0.28^{a}	2.2 ± 0.14^{b}	7.1 ± 0.15^{a}
G2 (125 mg/kg)	10.8 ± 0.91^{b}	8.8 ± 0.16^{a}	2.5 ± 0.12^{ab}	5.7 ± 0.58^{ab}
G3 (250 mg/kg)	6.5 ± 0.28^{c}	8.8 ± 0.16^{a}	4.3 ± 0.78^{a}	2.2 ± 0.31^{b}

*Lipid profile expressed by cholesterol (C), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), SEM: Standard error of the mean, a-c Means within a column with different superscript letters were significantly different (P< 0.05).

TABLE 4. Level of Glutathione (GSH) and malondialdehyde (MDA) in liver tissues of rabbit (mean± SD) treated with Grape seed extracts.

Group	GSH.(mmol/L)	MDA (mmol/L)
G1 (Control)	1.90 ± 0.14^{b}	6.3 ± 0.18^{a}
G2 (125 mg/kg)	2.05 ± 0.05^{b}	4.3 ± 0.08^{b}
G3 (250 mg/kg)	6.0 ± 0.08^{a}	4.0 ± 0.06^{b}

a-b Means within a column with different superscript letters were significantly different (P< 0.05)

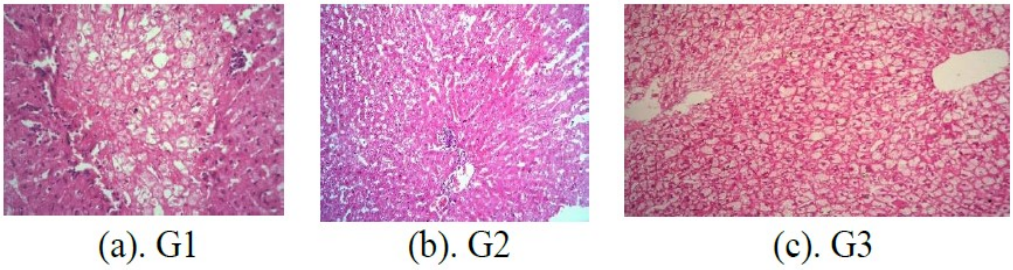

Fig. 1. Photomicrograph of liver tissue from rabbit does. H & E stain 200X.

(a) Representative sample of liver from G1 showing loss of the normal architecture, degenerated hepatocytes (arrow), vacuolated hepatocytes (circle),
(b) Representative sample of liver from group G2 showing of normal architecture, and less degenerated hepatocyte (arrow) and vacuolated hepatocytes (circle).
(c) Group 3 showed improvement of histological picture somewhat normal appearance hepatocytes, vacuolated and normal hepatocytes central vein.

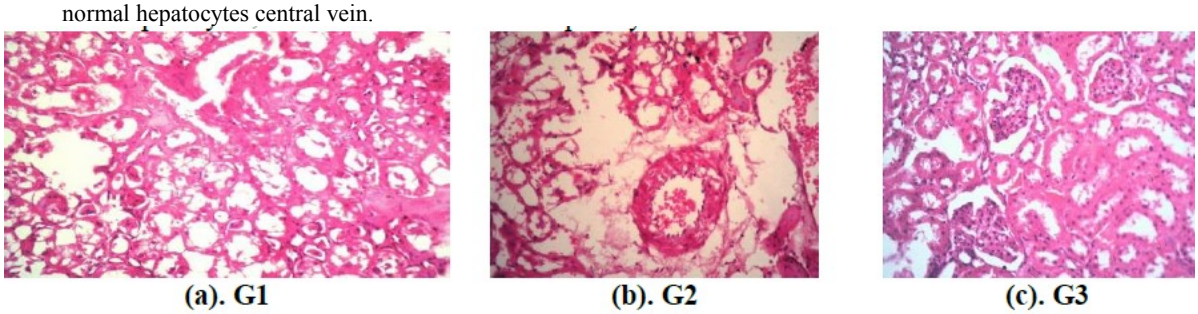

Fig. 2a. kidney G1 Section in the kidney not treated rabbit received grape seed showed change of the renal tubules epithelium eosinophilic hyaline renal casts in the lumen. (H & E X400).

Fig.2. Showing one of the renal blood vessels surrounded by pink homogeneous epithelium. (H& E X 400).

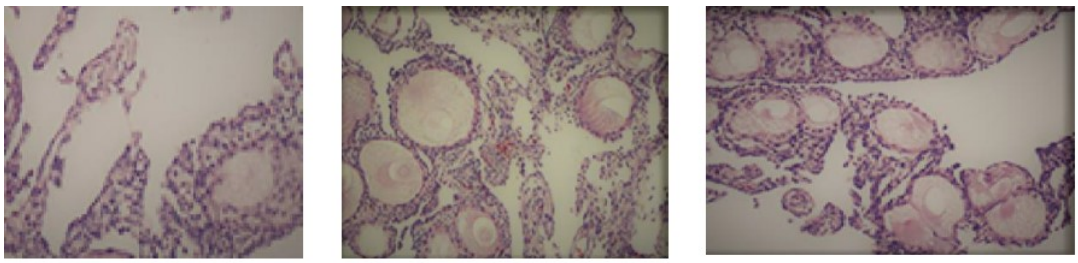

Fig. 3.Effects of GSPE on morphological changes of ovarian tissues. Representative morphology of ovarian tissues where, a represents G1 while b and c represent G2 and G3, respectively.

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تأثير مستخلص بذور العنب (البروانثاسياندين) على الأداء المناعي وقوته كمضاد اكسدة على اناث الارانب عند تعرضها للإجهاد الحراري

عماد عبد العزيز عبد الله ، دعاء سيد عبد الهادي، على السعيد غدية ، هاني محمد امين ،انتصار زكريا العراقي، زينب محمد فاروق و سامح محمد شديد

معهد بحوث الإنتاج الحيواني - مركز البحوث الزراعية – الدقي – الجيزة – مصر.

الملخص

أجريت هذه التجربة لتقييم تأثير مستخلص بذور العنب (البروانثاسياندين) على الأداء المناعي ومقدرته كمضاد اكسدة لإناث الارانب عند تعرضها للإجهاد الحرارى تم تقسيم الارانب الى ثلاث مجموعات المجموعة الأولى (المجموعة القياسية) وفيها لم تعامل الارانب بالمستخلص و المجموعة الثانية و فيها تم تجريع الارانب ب 125 ملج من المستخلص / كجم حى و تم تجريع المجموعة الثالثة ب 250 ملجم من المستخلص / كجم حي. في نهاية التجربة تم اخذ عينات دم من المجموعات الثلاثة لتقدير الكوليسترول- الجليسريدات الثلاثية - GSH- MDA– Urea- Creatinine – GPT-GOT – LDL-HDL واخذت عينات هستولوجى من الكبد و الكلى و المبايض. أوضحت النتائج ان تجريع مستخلص بذور العنب لعب دورا هاما كمضاد اكسدة قوى في تحسين الحالة المناعية ووظائف الكبد و الكلى بالإضافة الى تحسن النشاط المبيضى في اناث الارانب .

الكلمات الدالة: مستخلص بذور العنب ، التغيرات الباثوفسيولوجية ، قوة مضادات الاكسدة ، الاجهاد الحرارى ، الارانب.