International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 6, Issue 4 -2019

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.04.024

Alleviative effects of marjoram and propolis against negative influences of adenine on renal and testicular efficiencies of male rats

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Abstract

Background: The present study evaluated the effect of marjoram (*Origanum majorana*), propolis, and their combination against adenine-induced dysfunctions in kidneys and testes of male albino rats. Thirty-five rats, weighing 200±10g, were divided into seven groups (n=5): the control group, the adenine-treated group (300 mg/kg body weight), the marjoram-treated group (100 mg/kg body weight), the propolis-treated group (70 mg/kg body weight), and other three groups received simultaneously adenine + marjoram, adenine+propolis, or adenine+ marjoram + propolis. All treatments were given orally (by gavage) for 30 consecutive days. **Results**: The results showed that adenine induced a significant decrease (P<0.05) in plasma testosterone concentration, but a significant increase (P<0.05) in the levels of total protein, albumin, and NAG in urine was observed. Also, adenine induced a disturbances in plasma and urine mineral levels as compared with control groups. On the other hand, all different treated groups showed a significant decrease in the NAG activity and a significant increase in plasma testosterone level as compared with the adenine-treated group. Histopathological results of the testes of the rats treated with adenine revealed that degeneration in seminiferous tubules and reduction of spermatogenic cells.
Conclusions: Treatment rats with marjoram, or propolis after adenine administration, exhibited marked recovery and prevent the testicular tissue damage**.** Administration of marjoram and propolis in combination restored the testicular architecture, as well as improving the male fertility in rats after adenine administration.

Keywords: Adenine; Marjoram; Propolis; Renal dysfunction; Testosterone

Background

Chronic kidney disease (CKD) is a worldwide public health problem. The prevalence of CKD is about 6.3 % in individuals with 30 years or older and over 20 % in individuals with60 years or older **(Collins et al., 2014)**. As CKD progresses there are numbers of co-morbidities related to the disease process including cardiovascular, respiratory and endocrine conditions **(Webster et al., 2017).** Sexual and gonadal dysfunction/infertility is quite common in patients with CKD **(Finkelstein and Finkelstein, 2002)**.

AD induced all the characteristics of CKD, which was confirmed by biochemical and histological findings. Feeding of an adenine-rich diet-induced renal failure and decreased bone mineral density, the plasma testosterone levels and proteinuria in male rats **(Diwan et al., 2013)**. Also, it reduced body weight and increased urine output as well as induced a significant increase in blood pressure **(Ali et al., 2013).** Gonadal abnormalities may develop in male patients with CKD that lead to impotence, reduced libido, reduced testicular size, impaired spermatogenesis, and gynecomastia **(Diwan et al., 2018)**. **Edey (2017)** reported that the high dose adenine reduced the testicular function of male rats, with reduced plasma concentration of testosterone.

Marjoram considered as antiseptic, antidiabetic, carminative, antispasmodic, stimulant, diaphoretic, and diuretic agent **(Rau et al., 2006)**. Administration of marjoram oil and sodium nitrite improved the plasma biochemical values compared to sodium nitrite rats group only**(Aita and Mohammed, 2014)**. Marjoram according to **Nirumand et al. (2018),** preventing loss of body weight, polyurea, crystalluria, oxaluria and increase plasma urea and creatinine levels**.** Marjoram volatile oil regulates and normalizes the testis weight, epididymis and accessory sex organs that are positively related to the significant increase in plasma testosterone level (**El-Ashmawy et al., 2007**).

Propolis is a honeybee product with extensive biological actions and strong therapeutic effects. The most vital constituents in propolis are flavonoids flavones, phenolics, and aromatics **(Huang et al., 2014)**. Treatment with propolis extract regularized the level of magnesium, creatinine, sodium, potassium, and chloride. Propolis extract has a defensive effect on delight and stops urinary calculus, crystalluria and proteinuria **(El Menyiy et al., 2016).**

It has tremendous protection against sterility by improving sperm production, motility, count and quality and augmented the process of steroidogenesis, in addition, elevated testosterone production. Propolis raised the levels of testosterone, the weight of testis and epididymis and deteriorated the negative effects of triphenyltin chloride **(Yousef and Salama, 2009)**. Moreover, Brazilian green propolis extract was found to reduce proteinuria significantly in patients with diabetic and non-diabetic CKD **(Silveira et al., 2019).**

From the above-mentioned, the aim of this study was to evaluate the effect of *Origanum majorana* and propolis separated or in combination on proteinuria, mineral levels and testis of chronic kidney disease rats-model induced by adenine.

Materials and Methods

Experimental animals

In this study, 35adult male rats (*Rattus norvigicus*) weighting (200+10 g) were used. They obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals housed in metal cages bedded with wood shavings and kept under standard laboratory conditions of aeration and room temperature at about 25^{o}C. The animals were allowed to free access to standard diet and water *ad libitum*. The animals accommodated to the laboratory conditions for two weeks before being experimented.

Extraction of the plant marjoram

Marjoram (*Origanum majorana* L.) from the family Lamiaceae is commonly known as sweet marjoram. Plant collected from the Faculty of Pharmacy field Zagazig University. Six hundred and fifty g of the dried plant extracted by using 70% alcohol, by maceration method at room temperature 3 times each time for 48 hrs. The total extract after concentration by Rotary Vaporator was 9.79 g (**Ramadan et al., 2013**).

Chemicals

In this study, propolis powder obtained from (Dosic IMP &EXP. Co, Ltd).China. Propolis dissolved in distilled water and administered orally for 30 successive days *via* gastric tube at dose 70 mg/ Kg (**Yousef and Salama, 2009**). The adenine was obtained from (Central Drug House (P), Ltd).New Delhi INDIA as adenine sulfate which dissolved in

distilled water and administered orally for 30 successive days *via* gastric tube at dose 300 mg/ Kg **(Wang et al., 2012).**

Experimental design

The study was performed on 35 mature male albino rats **(*Rattus norvegicus*)**, animals were divided into 7 main groups (5 rats each) as follows:

The 1st (control group): Animals received 1 mL of distilled water.

The 2nd (adenine group): Animals were daily received oral doses of adenine (300 mg/kg).

The 3rd(marjoram group): Animals were daily received oral doses of marjoram extract (50 mg/kg).

The 4th (propolis group): Animals were received orally propolis extract (70mg/kg).

The 5th (adenine+ marjoram group): Animals were administered adenine (300 mg/kg) and marjoram extract (100 mg/kg) simultaneously.

The 6th (adenine+propolis group): Animals were administered adenine (300 mg/kg) and propolis extract (70mg/kg) simultaneously.

The 7th(adenine + marjoram +propolis group): Animals were administered adenine (300 mg/kg), marjoram extract (100 mg/kg), and propolis extract (70mg/kg)simultaneously.

All treatments administered orally for 30 consecutive days.

Blood, urine and testis sampling:

At the end of the experimental period, all rats placed individually in metabolic cages to collect the urine voided in 24 hrs. Twenty-four hours after the end of the treated-rats were anesthetized by light inhalation of diethyl ether then sacrificed. Blood sample was collected from the retro-orbital vein, that allows bleeding of the same animal more than one time with minimal stress**(Van Herck et al., 2001)** and the blood directly transported to tubes containing ethylenediamine tetra-acetic acid (EDTA). The blood and urine were centrifuged at 900 rpm for 15 min. The plasma obtained, together with the urine specimens were stored at –20^{o}C for further biochemical analyses.

Immediately after collection blood, the abdomen was exposed, dissected by longitudinal incision then the testis is obtained, and fixed in 10% neutral formalin solution for histological examination.

Determination of plasma and urine total proteins and albumin levels

Protein forms a violet colored complex with cupric ions in an alkaline medium with maximum absorption at 546 nm proportional to total protein concentration in the sample **(Henry, 1974).** Albumin binds with bromocresol green at pH 4.2 results on a change in the dye color. The blue-green color formed is directly proportional to the amount of albumin present in the sample; the color change is measured at 620 nm **(Doumas and Biggs, 1976).**

Determination of urine N-acetyl-ß-glucosaminidase (NAG) activity

N-acetyl-ß-glucosaminidase (NAG) catalyzes the hydrolysis of p-nitrophenyl- N-acetyl-ß-D-glucosaminide to p-nitrophenol and N-acetylglucosamine. The liberated p-nitrophenol is proportional to the enzymatic activity and is colorimetrically defined in an alkaline medium **(Gressner and Roebruck, 1982).**

Determination of plasma and urine Na, K, Ca and P mineral concentrations:

Amount of potassium (K) is determined by using sodium tetraphenylboronin a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2 –7mmol/L (**Teeri and Sesin, 1958**).

The present method is based on a modification of those first described by **Maruna (1958)** in which sodium (Na) is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Calcium (Ca) reacts with cresolphthalein complex one to form a purple color complex in alkaline medium. The intensity of color measured photometrically between 540 and 600 nm with maximum absorbance at 575 nm is directly proportional to calcium concentration in the specimen **(Moorehead and Briggs, 1974).**

The measurement of inorganic phosphorus in plasma is regularly accomplished by forming a phosphomolybdate complex and in turn reducing it to a molybdenum blue color complex. Inorganic phosphorus reacts with ammonium molybdate in an acidic medium to form a phosphomolybdate complex. This complex is reduced by ferrous ammonium sulfate to produce a molybdenum blue complex **(Goldenberg and Fernandez, 1966).**

Measurement of testosterone hormone

Testosterone hormone concentration was determined by using a commercial ELISA kit (IMMULITE), according to the method of **Santners et al. (1981).**

Histopathological studies

Tissue sampling and histopathological examination part of testis tissue separated from either normal or treated groups. The fixed tissues were processed habitually, embedded in paraffin, sectioned of 5 µm thick, deparaffinized and rehydrated then stained with hematoxylin and eosin (H&E) using typical techniques of **Bancroft and Gamble (2002**) for histopathological examinations.

Statistical analysis

Results are presented as mean ± standard error. One-way analyses of variance ANOVA test was applied, and if significant differences between means were found, Duncan's multiple range test (significant level was defined as $P<0.05$) was used according to **Snedecor and Cochran (1989)** to estimate the effect of different treated groups.

Results

Results showed a significant decrease in the plasma total protein (TP) content in adenine-treated rats compared to the control group. The total protein in urine recorded a significant increase compared to the control group (Table 1). In adenine+marjoram, adenine+propolis, and adenine+ marjoram+propolis groups showed a significant decrease in plasma TP and showed a significant increase in urine TP when compared to the control animals. Comparing AD+marjoram, AD+propolis, AD+ marjoram + propolis groups with an adenine-treated group showed a slight increase in plasma TP and a significant decrease in urine TP.

The data presented in Table(1) showed that the plasma and urine albumin concentration of adenine-treated rats decreased significantly in plasma and increased significantly in urine in comparison with the control group. There is not any change in plasma albumin of adenine+marjoram, AD+propolis, AD+marjoram+ propolis treated-groups. However, a significant increase in urine TP was found in the previous groups when compared to the control animals. Comparing adenine+propolis, adenine+marjoram+propolis treated-groups with adenine-treated rats showed slightly increase in plasma albumin and a significant decrease of albumin in the urine. In plasma albumin, there is no significant change between the adenine-fed group and adenine-fed+ marjoram group.

Table (1): Effects of marjoram (Ma) and propolis (Pr) on the total protein and albumin concentrations in plasma and urine, as well as urinary N-acetyl glucosaminidase (NAG) activity of male rats treated with/without adenine (Ad).

	Total protein (mg/dL)		Albumin (mg/dL)		Urine NAG (U/L)
	Plasma	Urine	Plasma	Urine	
Control	7.60±0.12	0.91±0.02	4.18±0.09	0.46±0.01	0.29±0.01
Ma	6.88±0.32	1.23±0.04	3.94±0.13	0.43±0.02	0.26±0.01
Pr	6.32±0.13[a]	1.36±0.02	4.33±0.07	0.40±0.01	0.26±0.02
Ad	6.20±0.14[a]	6.80±0.22[a]	3.47±0.11[a]	3.95±0.09[a]	1.83±0.03[a]
Ad+Ma	6.40±0.12[a]	3.98±0.03[ab]	3.47±0.02[a]	2.67±0.05[ab]	0.87±0.03[ab]
Ad+Pr	6.54±0.09[a]	4.35±0.04[ab]	3.95±0.02	2.72±0.04[ab]	0.87±0.01[ab]
Ad+Ma+Pr	7.04±0.11[b]	4.61±0.23[ab]	4.14±0.20[b]	2.82±0.04[ab]	0.89±0.02[ab]

Data are presented as means ± standard error (n=5).
(a) Significant difference from the control group ($P<0.05$).
(b) Significant difference from the adenine-treated group ($P<0.05$).

NAG activity of adenine-treated rats showed a significant increase compared to the control value. In the meanwhile, adenine+ induction of rats with adenine followed by treatment with marjoram or propolis separately or in combination showed a significant decrease in the enzyme activity of NAG as compared to adenine-treated group (Table 1).

The data in the table (2) showed a significant decrease in plasma Na and Ca, in addition, a significant increase in plasma K and P levels in the adenine-treated group compared to the control group. Also, adenine + marjoram, adenine + propolis, and adenine + marjoram + propolis treated groups showed a significant increase compared to control group in plasma Na, the significant decrease in Ca concentration and slightly increase in plasma K and P levels except for AD+marjoram+propolis there was slightly decreasing. Compared to control group, adenine-treated group showed significant decrease in plasma Na, Ca levels, significant increase in plasma K and significant increase in plasma P. Compared to adenine-treated rats, AD+marjoram, adenine + propolis, adenine + marjoram + propolis treated groups showed a significant increase in plasma Na, Ca levels and significant decrease in plasma P, while showed a slight decrease in plasma K.

The data in the table (2) showed a significant decrease in urine K, P concentrations and a significant increase in urine Ca, Na levels in the adenine-treated group compared to the control group. Compared to adenine-treated rats, AD+marjoram, AD+propolis, AD+marjoram+propolis treated groups showed a significant decrease in urine Ca and a significant increase in urine Na, also a significant increase in urine K, and showed no significant in urine P level.

Table (2): Effects of marjoram (Ma) and propolis (Pr) on the mineral concentrations in plasma and urine of male rats treated with/without adenine (Ad).

	plasma				urine			
	Na (nmol/L)	K (nmol/L)	Ca(mg/dL)	P (mg/dL)	Na (nmol/day)	K (nmol/day)	Ca(mg/dL)	P (mg/dL)
Control	139.4± 1.6	5.08± 0.09	10.00± 0.04	5.04± 0.21	11.17± 0.28	3.28± 0.14	18.60± 0.26	1.84± 0.11
Ma	135.6± 1.7	5.57± 0.11	10.22± 0.19	4.30± 0.15	18.75± 0.32[a]	4.17± 0.19[a]	13.82± 0.27	2.06± 0.08
Pr	132.6± 2.0	5.02± 0.19	10.14± 0.11	4.38± 0.17	18.76± 0.29[a]	4.22± 0.22[a]	14.14± 0.34	1.98± 0.15
Ad	125.0± 2.2[a]	6.36± 0.23[a]	5.67± 0.09[a]	8.34± 0.15[a]	26.70± 0.75[a]	4.16± 0.17[a]	24.07± 0.45[a]	2.34± 0.15[a]
Ad+Ma	146.2± 1.6[b]	5.50± 0.09	7.11± 0.28[ab]	6.35± 0.15[ab]	21.64± 0.58[a]	4.50± 0.09[ab]	16.71± 0.39[b]	1.95± 0.12[b]
Ad+Pr	140.4± 1.0[b]	5.36± 0.21[b]	7.13± 0.20[ab]	5.32± 0.19[b]	23.51± 0.54[a]	3.76± 0.15[ab]	17.13± 0.20[b]	1.92± 0.13[b]
Ad+Ma+Pr	145.6± 1.2[b]	6.03± 0.19[a]	7.32± 0.25[ab]	4.42± 0.10[b]	21.98± 0.53[a]	4.03± 0.19[a]	17.32± 0.25[b]	1.82± 0.19[b]

Data are presented as means ± standard error (n=5).
(a) Significant difference from the control group ($P<0.05$).
(b) Significant difference from the adenine-treated group ($P<0$.

Compared to the control group, AD+marjoram, AD+propolis, AD+marjoram+propolis treated groups showeda significant increase in urine Na, slightly increase in urine Ca, while showed a significant decrease in urine K and P.

Figure (1) showed adiminish of testosterone level in the adenine-treated group compared to the control group. Compared to the control group, adenine+Marjoram, adenine+propolis, adenine+marjoram+propolis showed a decrease in testosterone level. In adenine+marjoram, adenine+propolis, adenine+Marjoram+propolis, there is an increase in plasma testosterone level compared to the adenine-treated group.

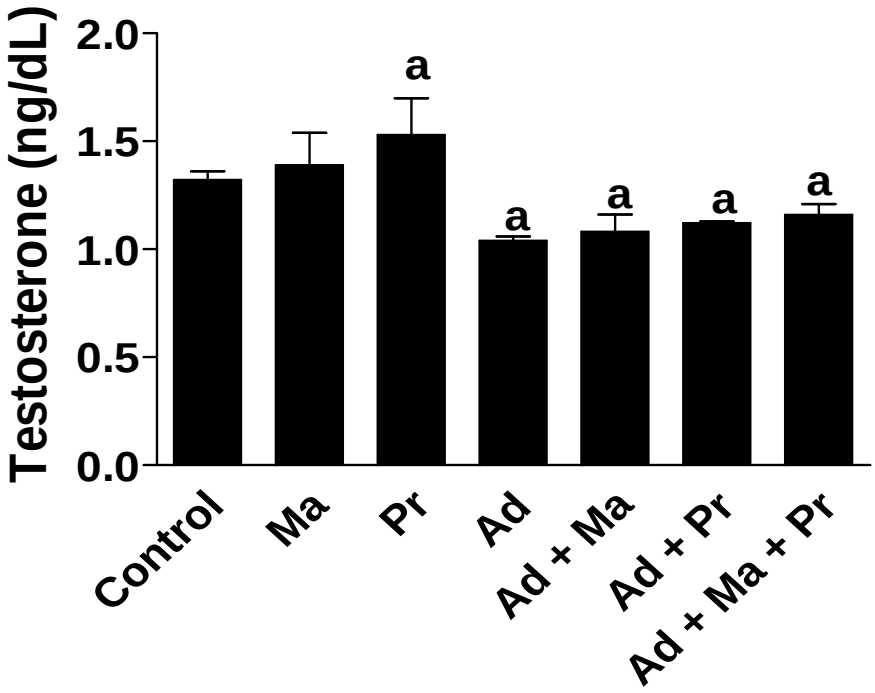

Figure 1 Effects of marjoram (Ma) and propolis (Pr) on the testosterone concentration in plasma of male rats treated with/without adenine (Ad).
Data are presented as means ± standard error (n=5).
(a) Significant difference from the control group (P<0.05).
(b) Significant difference from the adenine-treated group (P<0.05).

Histological observation in testis

Testis from the control group showed the normal structural organization of seminiferous tubules (ST) full of spermatogenic cells and normal sperm (SP) number was also observed **(Figs. 2a and 2b).** Marjoram and propolis groups have shown normal testicular architecture showing organized seminiferous tubules (ST) **(Figs. 3a, 3b) and (Fig. 4a, and 4b) respectively.**

Testis of rats treated with adenine revealed degenerative changes in seminiferous tubules with sloughing, vacuolization (V) and reduction of spermatogenic cells (SG) and a large number of sperms. **(Figs. 5a and 5b)**. Testis showed the good potential effect of marjoram and propolis on spermatogenesis in spite of Adenine Administration **(Figs. 6a, 6b) and (7a, and 7b) respectively.** Rats treated with adenine, marjoram, and propolis exhibited marked recovery, where the seminiferous tubules (ST) appeared with an almost regular distribution of spermatogenic cells and increased number of sperms (SP) **(Figs. 8a and 8b).**

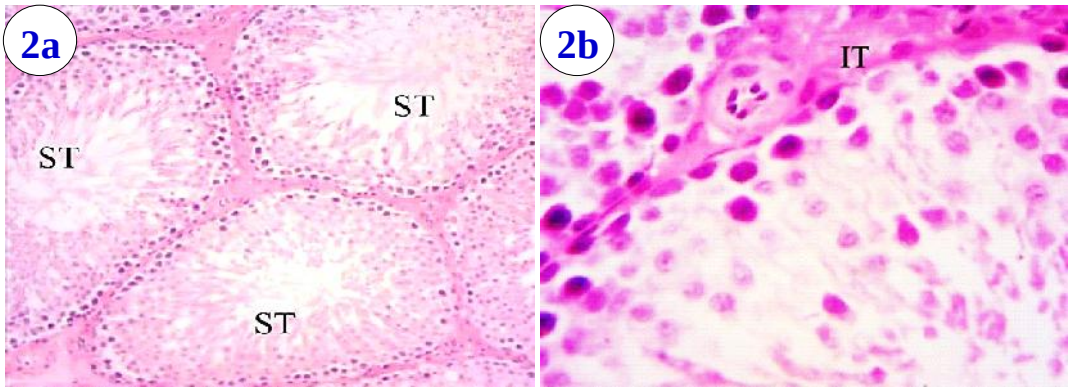

Figure 2: Photomicrograph of testis of a control rats showing (**a**) normal structural organization of seminiferous tubules (ST) full of spermatogenic cells and normal sperm shape (magnification power = ×400) and (**b**) normal histological pattern of the testis with interstitial tissue (IT, magnification power = ×1000).

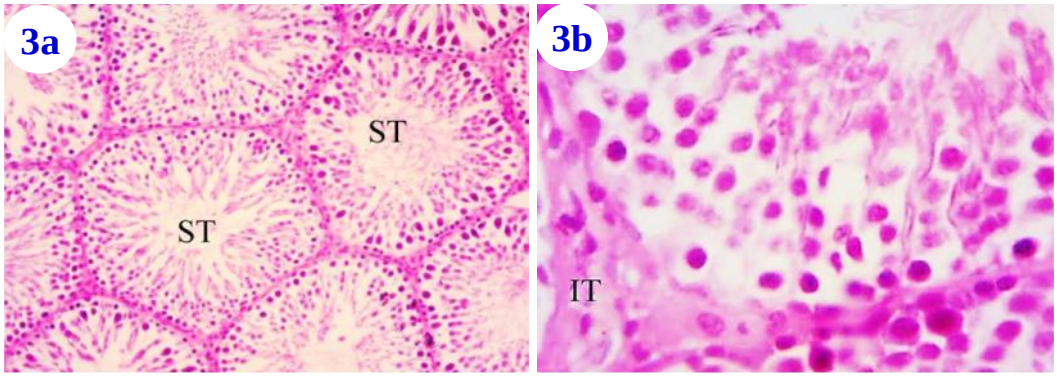

Figure 3: Photomicrograph of testis of a marjoram-treated rat showing (**a**) normal histological pattern of the testis with normal seminiferous tubules (ST, magnification power = ×400) and (**b**) interstitial tissue (IT, magnification power = ×1000).

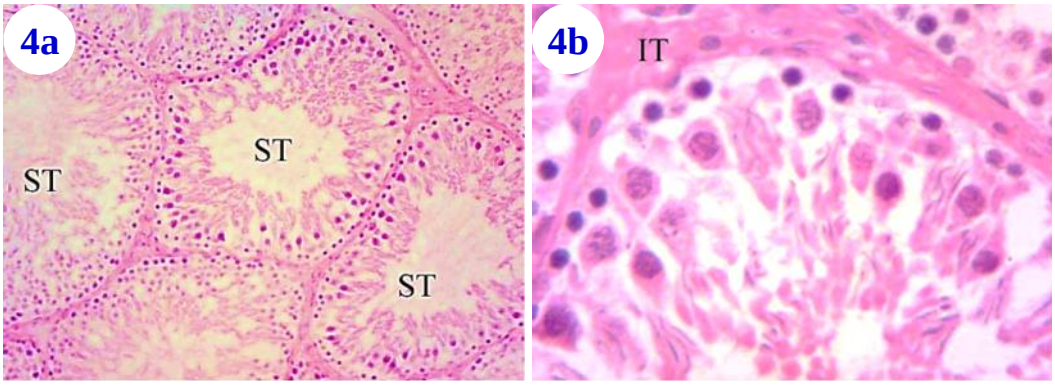

Figure 4: Photomicrograph of testis of a propolis-treated rat showing (**a**) normal testicular architecture with organized seminiferous tubules (ST, magnification power = ×400) and (**b**) interstitial tissue (IT, magnification power = ×1000).

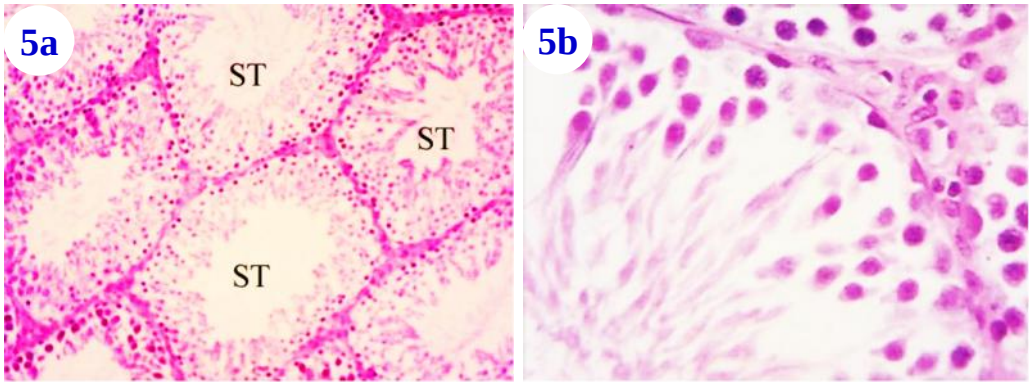

Figure 5: Photomicrograph of testis of an adenine-treated rat showing (**a**) degenerative changes in seminiferous tubules (ST) with sloughing and reduction of spermatogenic cells (magnification power = ×400) and (**b**) magnified ST exhibiting a vacuolization in the seminiferous tubules (magnification power = ×1000).

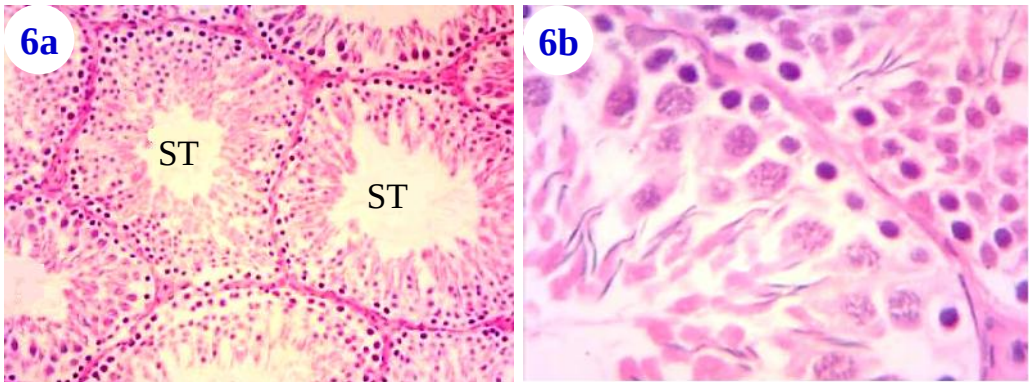

Figure 6: Photomicrograph of testis of rat received adenine + marjoram showing (**a**) active seminiferous tubules with spermatogenic cells (magnification power = ×400) and (**b**) magnified portions of seminiferous tubules illustrating increased incidence of sperms at the lumen (magnification power = ×1000).

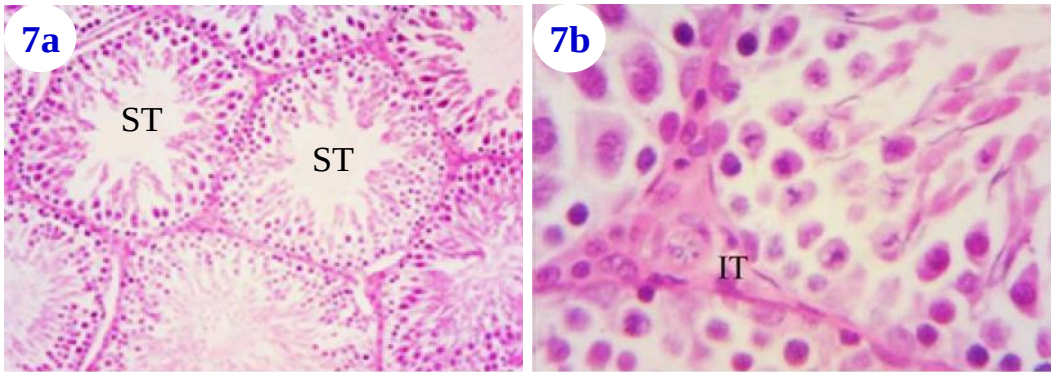

Figure 7: Photomicrograph of testis of rat received adenine + propolis showing the good potentiating effect of propolis on spermatogenesis in spite of adenine administration, (**a** and **b**) magnification power = ×400 and ×1000, respectively.

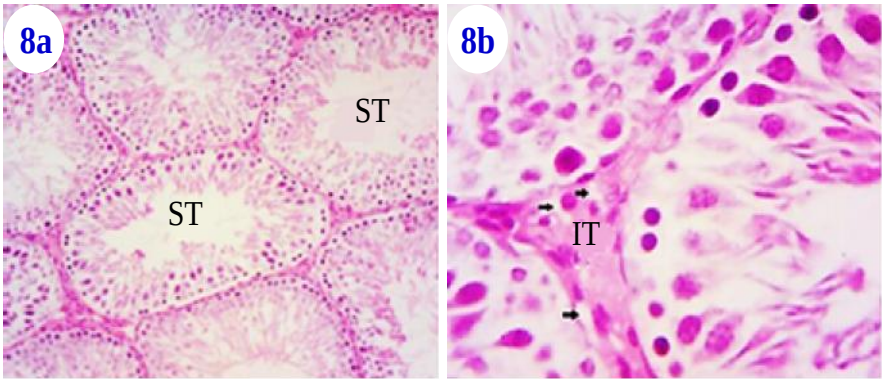

Figure 8: Photomicrograph of testis of rat received adenine + marjoram + propolis showing (**a**)the good potentiating effect of marjoram and propolis on spermatogenesis in spite of adenine administration (magnification power = ×400), **(b)**a restoration for the spermatogenic potential of testis, however some spermatogenic elements are still damaged and the interstitial tissue (IT) appeared vacuolated (arrow, magnification power = ×1000).

Discussion

In the present study, adenine has been used to induce chronic renal disease and the treatment with marjoram or propolis separately or in combination was evaluated. Moreover, the relationship between chronic kidney disease and the efficacy of testis was studied.

Chronic renal failure induced by adenine leads to decrease of total protein and albumin in plasma and increase their concentrations in the urine. It is interpreted, where adenine treatment caused renal disorders resembling CRF represented by increased the levels of total protein and albumin in urine and decreases their concentration in plasma **(El-Habibi et al., 2014**). **Yang et al. (2015)** reported that the administration of adenine to rats had a diminished tissue total protein, also albumin levels in pathological control group rats were significantly reduced. Adenine administration in rats produced a significant rise in urine protein excretion, suggesting high doses of adenine prompts proteinuria, which is a pathological sign in patients with CKD **(Rahman et al., 2018).** The reduction of plasma proteins may be due to decreased number of functional hepatocytes or due to nephrotoxicity which leads to leakage of albumin in urine with decreasing of plasma albumin and total protein concentration **(Sharma and Rathore, 2011).**From the present work, plasma total protein and albumin showed a significant increase in the groups treated with adenine with marjoram or adenine with propolis. These results are in parallel with **Aita and Mohammed (2014)** who mentioned that administration of marjoram oil and sodium nitrite improved the values of plasma biochemical compared to the values of sodium nitrite alone in the administered rats. There was a significant increase in the values of plasma total proteins and albumin. Also, **El Menyiy et al. (2016**) reported that propolis extract has a potent protective effect against ethylene glycol which causes hepatotoxicity and nephrotoxicity and has the potential to cure and prevent urinary calculus, crystalluria, and proteinuria. As ethylene glycol increased uric acid and protein excretion and decreased creatinine clearance, treatment with propolis alleviates urinary protein excretion and ameliorates the deterioration of liver and kidney function caused by ethylene glycol. **Abo-Salem et al. (2009)**, also stated that excretion of urinary albumin, as an indicator of early diabetic nephropathy, was found better after treatment with propolis compared to streptozotocin-induced diabetic group.

In this study, all results had assured that the urinary NAG activity was significantly increased in the rats which are treated with adenine. **Kern et al. (2010**) indicated that the increase in urinary NAG activity is a good indicator of renal tubular damage. **Ali et al. (2018)** reported that adenine treatment reduced body weight, raised water intake and urine output, as well as elevated NAG activity, and albumin in the urine. The uric intensity of NAG is trusted evidence for the kidney tubular function. Whenever a tubular cell is afflicted because of any disease process as (glomerular proteinuria, nephrolithiasis, hyperglycemia, interstitial, nephritis, transplant rejection), or even nephrotoxic factors such as antibiotics, or the factors of radio contrast, the levels of urine become on the rise and therefore it is used as a reflection of proximal tubular cell necrosis **(Kern et al., 2010).**On the other hand, co-administration of rats with adenine, marjoram, and propolis showed a significant decrease in the urinary

NAG activity compared to adenine treated rats indicating the protective effect of marjoram and propolis against the tubular damage induced by 2,8-dihydroxyadenine (DHA) that precipitated in tubules due to the adenine treatment.

Oral administration of adenine resulted in deregulation of mineral metabolism. Plasma (Ca and Na) and urine (P and K) were decreased, but that of plasma (P and K) and urine (Ca and Na) were increased. **Kim et al. (2013)** found that treatment with adenine significantly increased urine Ca level, meanwhile decreased urine P level and significantly increased plasma P level and reduced plasma Ca level. According to **Ikeda et al. (2010),** adenine-fed rats exhibited renal failure, ectopic calcification and altered plasma parameters, including elevated levels of plasma inorganic P, creatinine and parathyroid hormone (PTH). Plasma Ca levels were not obviously raised in rats that suffer from renal injury, however severe malformations of Ca dynamics were found in adenine-induced renal failure rats including a higher rate of urinary Ca excretion. In the present study, treatment with marjoram and propolis normalized dysregulation of mineral metabolism these results are in accordance with (**Saleh et al. 2018)**. It is reported that administration of male albino rats with paracetamol at a dose of 300 mg/kg/day resulted in a significant decrease in plasma values of Ca and Na and a significant increase in P and K. Treatment of paracetamol intoxicated rats with moringa and/or marjoram provided some improvement in plasma levels of minerals and electrolytes to be near the control levels particularly when both herbs were used together but the effect of marjoram was better than moringa in this regard. Patients with chronic renal disease have a tendency to retain phosphorus due to decreased kidney filtration and have diminished kidney hydroxylation of 25-hydroxyvitamin D to calcitriol (1, 25-dihydroxy vitamin D), resulting in hyperphosphatemia, calciferol deficiency, and ultimately hypocalcemia **(Gutierrez et al., 2005). Hsieh et al. (2011)** reported that the serum K level might increase along with deteriorating renal function. Subsequently, the elevated K level might by itself stimulate potassium excretion.

There was a lessening in body weight of adenine-treated group as compared with the control group. While an increment in body weight occurred in the other groups in comparing adenine-group at the end of the experiment. This finding in accordance with **Al Za'abi et al. (2017)** who found that rats fed with adenine alone lost about 5% of their body weight. Also, oral administration of propolis extract in doses of 100, 200 and 300 mg/kg significantly elevated the body weight recording 8%, 9%, and 13%, respectively compared to the streptozotocin-induced diabetic group **(Abo-Salem et al., 2009).**The results revealed, an increase of body weight in adenine-group with marjoram treatment separately or in combination with propolis as compared to adenine- group only at the end of the experimental period.

The present results revealed that the levels of testosterone are decreased in adenine treated rats and these result are in parallel with **Ogirima et al. (2006)**, who maintained that in male rats, the damage, which afflicted the kidney, was induced by 100 mg/mL adenine treatment and renal dysfunction was induced by 50 mg/mL adenine treatment. It was also clear that bone loss and the decreased level of testosterone were induced by the high rate of adenine. According to **Nakada and Adachi (1999**) in rats, renal insufficiency is responsible for gonadal impairment, the chronic renal deficiency was stimulated by giving an excess of adenine diet and the results showed that plasma testosterone level was decreased. It is clarified by **Carrero et al., (2009)** who suggested that Dysfunction of the hypothalamic-pituitary-testicular axis exists, and decreased synthesis and secretion of testosterone follows CKD and progressive renal failure. Whether persistent low-grade inflammation (as commonly observed in CKD) contributes to decreasing testosterone levels or vice versa is currently unknown, and it cannot be ruled out that it represents a vicious circle in which gonadal dysfunction causes or facilitates the increased inflammatory activity, which in turn further suppresses testosterone production. In the present study, treatment of marjoram and propolis with adenine induced CRF, ameliorate the level of testosterone and these results are in the same line with **El-Ashmawy et al. (2007)**, who reported that marjoram volatile oil regulate and normalize the testis weight, epididymis and accessory sex organs that are positively related to the significant increase in plasma testosterone level. Moreover, oral administration of marjoram extracts along with high-fat diet (HFD) increase testosterone level, decreased the accumulation of testicular lipid, increase androgens and sperm count and ameliorate the structure of testis **(El-Wakf et al., 2015).** Co-administration of local propolis extract to acrylamide treated group rats restored the sperm characteristics towards normal values which may be returned to restored in the plasma testosterone levels which reflect positively on the sperm characteristics **(Shalaby and Saleh, 2011)**, this give indicator that propolis extract hence ameliorate testosterone hormone level, sperm production and the process of

fertility**(Ghazi et al., 2013)**.The essential oil of marjoram was able to reduce the damaging effects of ethanol toxicity on male fertility, liver, and brain tissues **(El-Ashmawy et al., 2007)**. So marjoram showed both protective and curative effects on Cd-induced hepatotoxicity and nephrotoxicity **(Shati, 2011).**

Histopathological observations also revealed that rats treated with adenine showed degenerative changes in seminiferous tubules and reduction of spermatogenic cells. These results are explained by **Yu et al. (2014),** who reported that adenine treatment successfully induced the generation of spermatogenesis obstruction model, Adenine may reduce spermatogenesis and testosterone synthesis through a large number of free radicals generated by the xanthine oxidase reaction; it may also damage the gonads by affecting the testicular blood circulation therein-angiotensin-aldosterone system or by high blood pressure caused by renal failure.

Treatment with marjoram and propolis showed amelioration on spermatogenesis in spite of adenine administration. **El-Wakf et al. (2015)** described that rats fed high-fat diet (HFD) and received marjoram or sage oil exhibited marked recovery, where the seminiferous tubules (ST) appeared with an almost regular distribution of spermatogenic cells and increased number of sperms (SP) compared with that of HFD group, especially with the majoram HFD treated group. In addition, **Capucho et al. (2012)** informed that aqueous extract of Brazilian green propoliswas given to rats and the results showed higher sperm production and greater epithelium height of the epididymis initial segment.

Conclusions

The study suggests that marjoram and propolis are useful as renoprotection against chronic renal failure and playing an important role as anti-inflammatory agents and they have the ability to restore the testicular function and ameliorate testosterone level as well as improving the male fertility after adenine administration.

Abbreviations: CKD: chronic kidney disease**, TP:** Total protein**, ALB:** Albumin**, Ma:** Marjoram**, Pr:** Propolis**, Ad:** Adenine**.**

Declarations

Ethics approval and consent to participate: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and approved by the research scientific committee of the Zoology Department, Faculty of Science, Zagazig University.

Consent for publication: Not applicable

Availability of data and material: All data are available on Mendeley data repository**.**

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable

Authors' contributions: AD: made the planning and supervision overall steps of processing of the research**. HA:** carried out the physiological, biochemical and anatomical studies, participated in the sequence alignment and drafted the manuscript. **MZ:** participate in the physiological analysis and supervision of the research. **SE:** carried out the preparation of the chemicals and made the extraction of and propolis and supervision of the research. **BH:** carried out the practical part of this research.

Acknowledgments

To those who instilled in me the value of education and the rewards and opportunities it can generate to my parents, especially my Father, who supplied me with enthusiasm, support and creative insight. His critical reading of the manuscript that helped me refine the concept of this thesis, his deep interest in the topic and unfailing encouragement are highly appreciated.

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How to cite this article:

Abdelaziz A. Diab; Hani M. Abdelsalam; Mansour H. Zahra; Sameeh Eldehamy; Bassant A. Hendawy. (2019). Alleviative effects of marjoram and propolis against negative influences of adenine on renal and testicular efficiencies of male rats. Int. J. Adv. Res. Biol. Sci. 6(4): 194-207.

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.04.024