Journal of Applied Pharmaceutical Science Vol. 4 (07), pp. 064-068, July, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40711
ISSN 2231-3354

Ameliorative effect of citrus peel extract on castration-induced oxidative stress in liver and kidney of rats

Nada A. E. Mohamed[1], Amany A. Tohamy[1], Basma Elgamal[2] and Ahmed E. Abdel moneim[1]*

[1]Zoology and Entomology Department, Faculty of Science, Helwan University, Cairo, Egypt.
[2]Clinical pathology Department, National Cancer Institute, Cairo University, Cairo, Egypt.

ARTICLE INFO

Article history:
Received on: 15/01/2014
Revised on: 02/02/2014
Accepted on: 16/03/2014
Available online: 28/07/2014

Key words:
Castration; Citrus peel; Oxidative stress; Liver; Kidney.

ABSTRACT

The present work aimed to investigate the effect of methanolic extract of citrus peel in the redox status of liver and kidney in castrated rats. Twenty four Wistar albino rats were used. They were divided into 4 groups (n = 6). Group I was used as control. Group II was castration group, Group III was normal rats treated with citrus peel and Group IV was citrus peel castration group. Liver and kidney function and oxidative stress markers were measured. In addition, histopathological changes of liver and kidney were examined. Castration enhanced lipid peroxidation and nitric oxide production in both liver and kidney with concomitant reduction in glutathione. In addition, castration caused liver and kidney injuries as indicated by histopathological changed of the liver and kidney with a disturbance in the functions of liver and kidney. Citrus peel protected liver and kidney through decreasing the oxidative stress stimulating the antioxidant defense system. From the present results, it can be concluded that the decrease in liver and kidney damages during citrus peel treatment may be due to the inhibition of oxidative stress overproduction and maintenance of antioxidant defense mechanisms of this extract.

INTRODUCTION

Testosterone concentrations change as a function of age. In the course of aging, circulating testosterone concentrations in healthy men decline an average of 1–2% per year, starting at the third decade of life. With aging, the decrease in bio-available testosterone appears to be greater than the decline in total testosterone due to an age-related increase in sex hormone-binding globulin (SHBG). Testosterone affects almost every organ in the body (Matsumoto, 2002; Liu *et al.*, 2007).

Testosterone deficiency leads to a higher proportion of body fat and to the accumulation of predominantly visceral fat (Christoffersen *et al.*, 2006). Castration has been shown to induce oxidative stress in the acinar epithelium of the rat ventral prostrate, as evidenced from marked increase in 8-hydroxy-2'-deoxy-guanosine and 4-hydroxynonenal protein adducts in the regressing epithelium. Quantification of steady-state mRNA levels of 14 genes involved in the anabolism and catabolism of reactive oxygen species (ROS), showed that castration resulted in dramatic increases of three ROS-generating NAD(P)H oxidases (NOXS) genes; significant reductions of key ROS-detoxifying enzymes (superoxide dismutase 2, glutathione peroxidase 1, thioredoxin and peroxiredoxin 5); and unchanged levels of catalase and glutathione reductase (Afolabi *et al.*, 2013).

One could hypothesize that animals with hypo-androgenism would display the same or similar features and thus be useful as a model for the metabolic syndrome related to testosterone deficiency. Castration is a way of studying the consequences of extreme testosterone deficiency in animal models (Christoffersen *et al.*, 2006).

Oxidative stress is the state of a cell, which is characterized by excess production of reactive oxygen species (ROS) and or a reduction in antioxidant defense responsible for metabolism. ROS are formed as a natural by-product of the normal metabolism of oxygen. Under normal circumstances, the cell is able to maintain an adequate homeostasis between the formation of ROS and its removal through enzymatic pathways or via antioxidants (Abdel Moneim, 2014).

* *Corresponding Author*
Ahmed Esmat Abdel Moneim , Department of Zoology and Entomology Faculty of Science, Helwan University, 11795 Helwan, Cairo, Egypt
Tel. number: (+20) 1090579791. E-mail: aest1977@helwan.edu.eg

Citrus fruits contain various bioflavonoids. Naringin and hesperidin, glycosylated citrus flavonoids, are two major bioflavonoids identified in tangerine-peel extract (Bok *et al.*, 1999). Citrus industry by-products, if utilized optimally could be major sources of phenolic compounds as the peels, in particular, have been found to contain higher amounts of total phenolics compared to the edible portions. Citrus peels are waste materials, obtained after extraction of juice from citrus fruit. Methanolic extract of citrus peel is known to have different antioxidative compounds (Ziaur, 2006; Xu *et al.*, 2008; Green *et al.*, 2013).

Therefore, the present work aimed to investigate the effect of methanolic extract of citrus peel in the redox status of liver and kidney in castrated rats.

MATERIALS AND METHODS

Plant Material Extract

Citrus sinensis (*C. sinensis*) fruits were collected from market of East Cairo, Egypt in the months of February-March, 2012. The plant material was authenticated in Botany Department, Faculty of Science, Helwan University, Cairo-Egypt on the basis of taxonomic characters and by direct comparison with the herbarium specimens that available at the herbarium of the Botany Department.

Extraction

Fresh fruit peels of *C. sinensis* were taken and grounded, and about 500 g of the plant material was consecutively macerated for one day in petroleum ether, ethyl acetate, chloroform, and methanol, respectively. On basis of the preliminary phytochemicals tests conducted, the methanol extract was found to be rich in terms of chemical constituents and therefore was selected for the experiment. The methanol was removed under reduced pressure to obtain a semisolid mass of methanolic extract of citrus peel (MECP). The MECP was then stored in -20ºC until used.

Animals

Adult male Wister albino rats weighing 180–200 g were obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). After an acclimatization period of one week, the animals were divided into four groups (7 rats per group) and housed in wire bottomed cages in a room under standard condition of illumination with a 12 hours light-dark cycle at 25±1ºC. They were provided with water and balanced diet ad *libitum*. All animals received care in compliance with the Egyptian rules for animal protection.

Surgical Procedure and Experimental Design

Under anesthesia, the scrotum was incised at the midline and the testes were exposed. In the castration groups (Group II), the testes were removed after en bloc ligature of the spermatic cord, and in the control groups (Group I), the testes were exposed, manipulated and reinserted into the scrotum. Group citrus peel castration (Group IV) administered with citrus peel 200 mg/kg bwt after 30 days of castration. Group III was administered with citrus peel at a dose of 200 mg/kg bwt for 30 days.

After 30 days of daily administration, overnight fasting animals were euthanized under mild ether anesthesia. Blood was collected from abdominal aorta using syringe puncture. Liver and kidney were promptly excised, washed in chilled saline, blotted and processed for biochemical and histological studies.

Biochemical Estimations

Liver and kidney functions tests

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in serum (Reitman and Frankel, 1957). Serum alkaline phosphatase, uric acid, urea and creatinine levels were determined by commercially available diagnostic kits (Biodiagonstic-Egypt) as per manufacturer's instructions.

Oxidative stress and the antioxidant enzymes

Homogenates of liver and kidney were prepared in 50 mM Tris-HCl, pH 7.4, to determine lipid peroxidation (LPO) by reaction of thiobarbituric acid (TBA) (Ohkawa *et al.*, 1979). Similarly, those homogenates were used to determine nitrite/nitrate (nitric oxide; NO) (Green *et al.*, 1982) and glutathione (Ellman, 1959).

Estimation of serum testosterone

Quantitative measurement of serum testosterone was carried out adopting ELISA technique using kits specific for rats purchased from BioVendor (Gunma, Japan) according to the protocol provided with each kit.

Photomicroscopic Observations

The liver and kidney tissues were collected and immediately fixed with 10% buffered formalin, and embedded in paraffin. Sections (5-7 µm) were prepared and then stained with hematoxylin and eosin dye for photomicroscopic observations.

Statistical Analysis

Results were expressed as the mean ± standard error of the mean (SEM). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a *post hoc* test according to the statistical package program (SPSS version 17.0) and figures were drawn with Origin (version 8). All p values are two-tailed and $p< 0.05$ was considered as significant for all statistical analysis in this study.

RESULTS

Biochemical Results

The activities of ALT, AST and ALP in the control and castrated rats are given in the Table 1. Castrated rats had increased activities of ALT, AST and decreased the activity of ALP in the

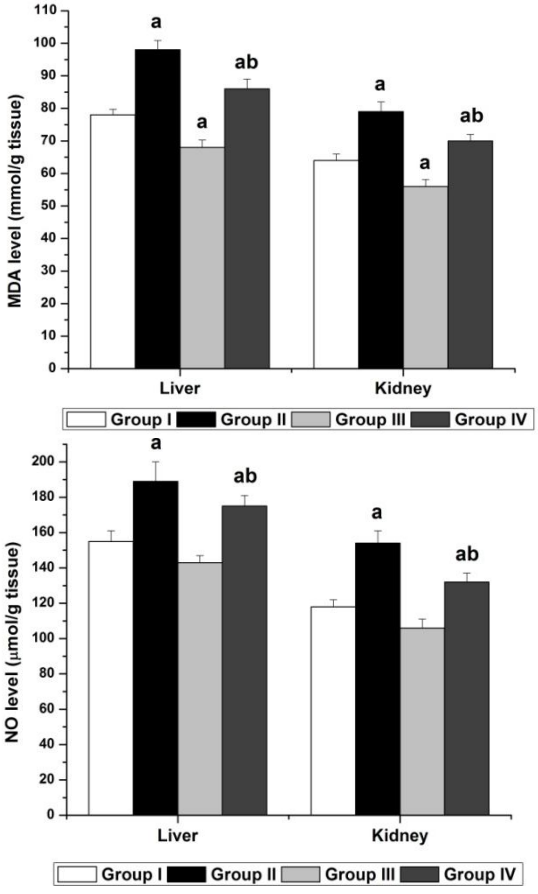
serum and treatment with MECP reversed these changes to near the normal. However, MECP was failed to reverse the change in ALP. Table 1 shows the levels of urea, uric acid and creatinine in the serum of control and castrated rats. Castrated rats show decreased levels of urea and uric acid and increased the level of creatinine. Treatment with MECP has reversed these parameters to near control values.

Table. 1: Serum levels of liver and kidney parameters in different studied groups.

Parameters	Group I	Group II	Group III	Group IV
ALT (U/ml)	65.52±2.53	153.91±1.56[a]	69.57±3.87	79.10±5.12[b]
AST (U/ml)	55.86±1.96	123.75±7.21[a]	61.54±3.43	107.08±1.45[b]
ALP (IU/L)	147.73±4.07	67.49±5.41[a]	159.05±8.36	64.66±4.22[a]
Uric acid (mg/dl)	55.79±2.52	132.56±10.85[a]	59.74±3.97	56.08±5.31[b]
Urea (mg/dL)	1.97±0.08	2.89±0.18[a]	2.13±0.08	1.87±0.24[b]
Creatinine (mg/dL)	0.85±0.01	1.71±0.02[a]	0.94±0.02	1.03±0.01[b]

Values are means ± SEM (n=7)
[a]p<0.05, significant change with respect to group I; [b]p<0.05, significant change with respect to group II.

The levels of MDA, NO and GSH in the liver and kidney of castrated and control rats are presented in Figure 1. Castrated rats had elevated levels of MDA and NO in the liver and kidney when compared with normal rats. Treatment with MECP showed reversal of these parameters to near normalcy.



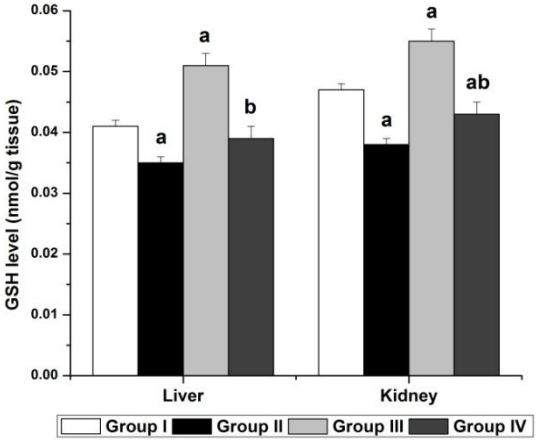


Fig. 1: Effect of MECP on MDA, NO and GSH levels in the liver and kidney of control and castrated rats. Values are given as means ± SEM from seven rats in each group. [a,b] Values not sharing a common superscript differ significantly at $p < 0.05$.

Castration of rats was caused overproduction of cellular oxidants and modulation of antioxidant defense system. As observed during the study, castration led to a modulation of several parameters of oxidative stress relative to control animals. After 30 days of castration, GSH contents in the hepatic and renal homogenates were significantly decreased ($p < 0.05$) compared to the controls (Figure 1). On the other hand; MECP treatment elevated the content of GSH significantly compared to castrated group. Moreover, MECP treatment elevated GSH on liver and kidney homogenates up to the control values.

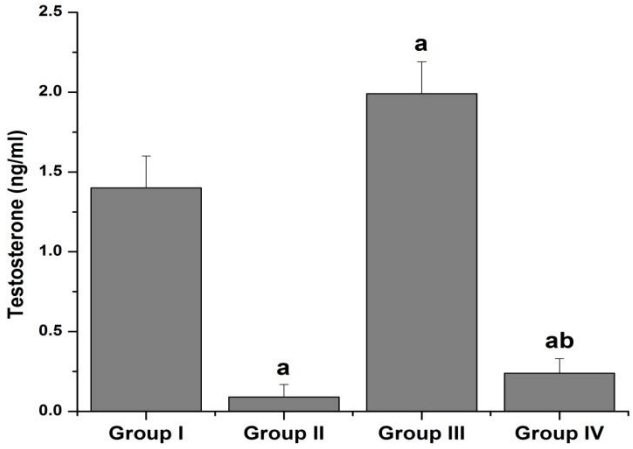


Fig. 2: Effect of MECP on testosterone level in control and castrated rats. Values are given as means ± SEM from seven rats in each group. [a,b] Values not sharing a common superscript differ significantly at $p < 0.05$.

The mean values of the serum hormone, testosterone, are shown in Figure 2. After castration of rats, the mean value of testosterone was decreased as compared to the control group (-93.6 %,). In the MECP treatment in castrated group, testosterone level was increased significantly as compared with the castrated group (P <0.05), but it still decreased significantly compared to the control group (-82.4%). Statistically significant increase in the level of testosterone was observed in rats treated with citrus peel extract on its own as compared to the control group.

Histological Results

Histopathological changes in liver and kidney of rats were shown in Figure 2. Castrated rats contained a hepatic vein congestion and inflammatory cells invasion, variability in the nuclear size, pyknosis and karyolysis in the hepatic nuclei (Figure 3). Kidney sections appeared with shrunken glomeruli, intratubuler blood congestion, loss of glomerular lobulation tubular cytoplasmic vaculation and some pyknotic nucleus. The treatment with MECP reversed these changes to near normalcy.

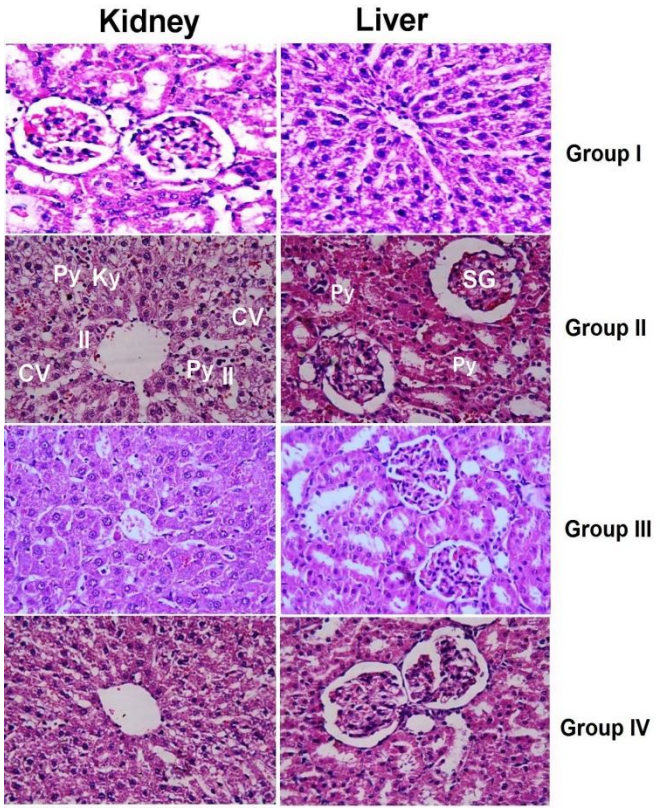


Fig. 3: Photomicrographs of hematoxylin–eosin staining of hepatic and renal tissues of control and experimental rats. Histological photograph of (control) Group I, (castrated) Group II, (MECP) Group III and (castrated-MECP) Group IV rats. Hepatic and renal tissue sections were showed at 40× magnification. Cytoplasmic vacuolation (CV), pyknosis (Py), karyolysis (Ky), inflammatory infiltration (II) and shrunken glomeruli (SG).

DISCUSSION

Testosterone is the major male androgen and deficiencies in circulating levels are a prominent feature observed in aging males. Testosterone exerts a variety of anabolic and androgenic effects on many organs, most of which are mediated by the nuclear androgen receptor (AR). The antioxidant potential of various steroid hormones (estriol, estradiol, estrone, progesterone, testosterone, androstenedione, cortisol and others) have been evaluated and it was shown that estrogens, especially estriol and estradiol, are natural antioxidants (Barp *et al.,* 2002).We found that testosterone concentrations in castrated rats were 93.6% lower than in control rats. Castration clearly induced an elevated prooxidant state in the liver and kidney, as proved by a significant decrease in the activities of antioxidant enzymes and a tendency toward lower concentrations of GSH. The underlying mechanism suggested by Tam et al. (2003) was the activation of ROS generating NAD(P)H oxidases in response to castration. There is much evidence that reactive oxygen species (ROS) are implicated in the pathology of cardiovascular disease and cardiac remodeling, though it is difficult to find out the specific regulatory mechanism while taking into consideration that ROS may be provided from multiple sources, such as excessive generation in mitochondria, xanthine oxidase reaction, infiltration of inflammatory cells, or uncoupled nitric oxide synthases (NOSs) (Klapcinska *et al.*, 2008).

Membrane lipids, which are rich in polyunsaturated fatty acids, are vulnerable to peroxidation by oxidants. The level of MDA, which occurs due to lipid peroxidation, is an indicator of oxidative damage. We observed that hepatic and renal MDA levels significantly increased in castrated rats compared to control mice. This increase demonstrates that oxidative damage occurs in liver and kidney as a result of lipid peroxidation attributed to testosterone deficiency. In agreement with our findings, castration leads to higher MDA content (Klapcinska *et al.,* 2008) and an increase in lipid peroxidation (Sreelatha Kumari *et al.,* 1993) in heart. Androgens have been shown to effectively prevent the formation of lipid peroxidation (Bilinska *et al.,* 2006). It has been reported that testosterone administration leads to a decrease in MDA levels in rat liver (Huh *et al.,* 1994) and brain (Meydan *et al.,* 2010). In the study by Sreelatha Kumari *et al*., (1993) administration of testosterone in castrated rats partially reversed the increase in lipid peroxidation in the hearts. Our study indicates that MECP therapy caused a decrease in the MDA levels in liver and kidney, which are increased by testosterone deficiency.

The extract of citrus peel was able to partially prevent castration-induced decay of antioxidant molecule and elevation of oxidant markers; this preventive effect was also be observed at the histological level (Figure 3). A similar scavenger role of citrus peel in rats after exposure to oxidative stress had been determined (Abdel Moneim, 2014).

Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Flavonoids act as anti-oxidants by neutralizing oxidizing free radicals, including the superoxide and hydroxyl radicals. The redox properties of flavonoids also allow them to act as reducing agents and presence of flavonoids and phenolic compounds have been recognized as excellent scavengers of superoxide, hydroxyl ion and peroxyl radicals there by inhibiting lipid peroxidation (Abdel Moneim, 2014).

Treatment of rats with citrus peel ameliorated the effects of castration and the level of testosterone was increased slightly. Orange contains phenols and flavonoids which can directly or indirectly reduce oxidative damage by preventing the excessive generation of free radicals. In addition, treatment of rats with citrus peel alone, slightly increases the serum level of testosterone. The increase in sex hormones in the present study due to the citrus peel can be in part due to the ability of orange to reduce stress hormones, such as cortisol, as seen by Hong *et al.,* (2008). Our

results clearly show that the absence of testosterone involves morphological and biochemical changes in the liver and kidney. All morphological variations, observed at a microscopic level after castration, can be summarized in terms of cell distress.

Taken together, the results suggest that a gonadectomy significantly and negatively affected the antioxidant status of the rat liver and kidney. Reduced concentrations of testosterone in the elderly and in some pathological conditions may contribute to the development of different diseases through the induction of oxidative stress, and the administration of citrus peel in castrated animals resulted in a tendency toward a decrease in the oxidative stress status in the liver and kidney tissues.

REFERENCES

Abdel Moneim AE. Citrus peel extract attenuates acute cyanide poisoning-induced seizures and oxidative stress in rats. CNS Neurol Disord Drug Targets, 2014;13(4):638-646.

Afolabi AO, Osho OO, Alagbonsi IA. Tomato supplement ameliorates castration-induced oxidative stress in the rat. Afr J Biochem Res, 2013; 7(3):33-38.

Barp J, Araújo ASR, Fernandes TRG, Rigatto KV, Llesuy S, Belló-Klein A, Singal P. Myocardial antioxidant and oxidative stress changes due to sex hormones. Braz J Med Biol Res, 2002; 35:1075-1081.

Bilinska B, Wiszniewska B, Kosiniak-Kamysz K, Kotula-Balak M, Gancarczyk M, Hejmej A, Sadowska J, Marchlewicz M, Kolasa A, Wenda-Rozewicka L. Hormonal status of male reproductive system: androgens and estrogens in the testis and epididymis. In vivo and in vitro approaches. Reprod Biol, 2006; 6 Suppl 1:43-58.

Bok SH, Lee SH, Park YB, Bae KH, Son KH, Jeong TS, Choi MS. Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. J Nutr, 1999; 129:1182-1185.

Christoffersen B, Raun K, Svendsen O, Fledelius C, Golozoubova V. Evalution of the castrated male Sprague-Dawley rat as a model of the metabolic syndrome and type 2 diabetes. Int J Obes (Lond) 2006; 30:1288-1297.

Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys, 1959; 82:70-77.

Green CO, Wheatley AO, McGrowder DA, Dilworth LL, Asemota HN. Citrus peel polymethoxylated flavones extract modulates liver and heart function parameters in diet induced hypercholesterolemic rats. Food Chem Toxicol, 2013; 51:306-309.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem, 1982; 126:131-138.

Hong MY, Seeram NP, Heber D. Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor. J Nutr Biochem, 2008, 19(12):848-855.

Huh K, Shin US, Choi JW, Lee SI. Effect of sex hormones on lipid peroxidation in rat liver. Arch Pharm Res, 1994; 17:109-114.

Klapcinska B, Jagsz S, Sadowska-Krepa E, Gorski J, Kempa K, Langfort J. Effects of castration and testosterone replacement on the antioxidant defense system in rat left ventricle. J Physiol Sci, 2008; 58:173-177.

Liu PY, Beilin J, Meier C, Nguyen TV, Center JR, Leedman PJ, Seibel MJ, Eisman JA, Handelsman DJ. Age-related changes in serum testosterone and sex hormone binding globulin in Australian men: longitudinal analyses of two geographically separate regional cohorts. J Clin Endocrinol Metab, 2007; 92:3599-3603.

Matsumoto AM. Andropause: clinical implications of the decline in serum testosterone levels with aging in men. J Gerontol A Biol Sci Med Sci, 2002; 57:M76-99.

Meydan S, Kus I, Tas U, Ogeturk M, Sancakdar E, Dabak DO, Zararsiz I, Sarsilmaz M. Effects of testosterone on orchiectomy-induced oxidative damage in the rat hippocampus. J Chem Neuroanat, 2010; 40:281-285.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979; 95:351-358.

Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol, 1957; 28:56-63.

Sreelatha Kumari KT, Menon VP, Leelamma S. Testosterone and lipid peroxide metabolism in orchidectomised rats. Indian J Exp Biol, 1993; 31:323-326.

Tam NN, Gao Y, Leung YK, Ho SM. Androgenic regulation of oxidative stress in the rat prostate: involvement of NAD(P)H oxidases and antioxidant defense machinery during prostatic involution and regrowth. Am J Pathol, 2003; 163:2513-2522.

Xu GH, Chen JC, Liu DH, Zhang YH, Jiang P, Ye XQ. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J Food Sci, 2008; 73:C11-18.

Ziaur R. Citrus peel extract – A natural source of antioxidant. Food Chemistry, 2006; 99:450-454.

How to cite this article:

Nada A.E. Mohamed, Amany A. Tohamy, Basma Elgamal and Ahmed E. Abdel moneim. Ameliorative effect of citrus peel extract on castration-induced oxidative stress in liver and kidney of rats. J App Pharm Sci, 2014; 4 (07): 064-068.