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Original Article

# BROWN SEAWEEDS AMELIORATE RENAL ALTERATIONS IN MICE TREATED WITH THE CARCINOGEN AZOXYMETHANE

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**ABSTRACT**

**Objectives:** The current study was conducted to evaluate the possible protective effects of the brown seaweeds, *Turbinaria ornata* and *Padina pavonia*, against azoxymethane (AOM)-induced nephrotoxicity in mice.

**Methods:** The experimental mice were allocated randomly into 4 groups as follows; Group I (Normal): mice received two consecutive doses of saline, Group II (AOM): mice received intraperitoneal injections of 10 mg/kg AOM once a week for two consecutive weeks, Group III (AOM + *T. ornata*): mice received AOM once a week for two consecutive weeks + 100 mg/kg *T. ornata* extract, and Group IV (AOM + *P. pavonia*): mice received AOM once a week for two consecutive weeks + 100 mg/kg *P. pavonia* extract. Both extracts were supplemented orally for 3 weeks starting at the end of 10$^{th}$ week.

**Results:** AOM administration to mice induced renal damage evidenced by the histological alterations, including blood vessel dilatation, degenerated tubules, necrosis, inflammatory cell infiltrations, mildly congested blood vessels, degenerated glomeruli, thickened blood vessel wall and other histologic manifestations. In addition, AOM-administered mice showed significantly increased serum creatinine, urea and uric acid as well as elevated renal lipid peroxidation and nitric oxide levels. Consequently, renal glutathione content and superoxide dismutase and glutathione peroxidase activities were significantly declined. Supplementation of either *T. ornata* or *P. pavonia* markedly alleviated the AOM-induced alterations.

**Conclusion:** The current findings provide the first evidence that *T. ornata* and *P. pavonia* could protect mice against AOM-induced renal damage via abolishment of oxidative stress and potentiation of the antioxidant defense system.

**Keywords:** Azoxymethane, Nephrotoxicity, Seaweeds, Oxidative stress.

## INTRODUCTION

Seaweeds are a group of marine algae with various health benefits and of great potential as a supplement in functional food [1]. Seaweeds have been widely used by coastal populations for thousands of years due to their high nutritional values [2]. Mayer et al. [3] reported that seaweeds produce a wide range of new secondary metabolites with various biological activities. In addition, medical and pharmaceutical industries are currently interested in marine algae since they have proven to be rich sources of diverse bioactive compounds with valuable pharmaceutical and biomedical potential [4]. Further, several bioactive compounds from marine organisms have been experimentally tested for their biomedical efficacy [5]. In this regard, multiple studies demonstrated that marine algae are rich in proteins, dietary fiber, minerals, lipids, essential amino acids, omega-3 fatty acids, polysaccharides, and vitamins A, B, C, and E [2,6,7].

Azoxymethane (AOM) is one of the most used compounds for studying colon carcinogenesis in experimental animals [8]. It is an intermediary metabolite of dimethylhydrazine that gives rise to methyl diazonium and methyl carbonium which are known to damage a number of biomolecules and may induce colon cancer [8]. Also, AOM has been utilized to investigate the preventive potential of numerous natural products on colon cancer [9]. Chan et al. [10] stated that liver and lungs are the most affected organs during colon cancer induction using AOM. However, previous studies reported the secondary effects induced by AOM in the kidney of experimental animals [11].

Recently, we reported that the brown seaweeds *Turbinaria ornata* and *Padina pavonia* possess anti-inflammatory, antioxidant and antidiabetic effects in experimental animals [12,13]. To date, there is nothing yet reported on the use of the extracts of *T. ornata* and *P. pavonia* against AOM-induced renal biochemical and histological alterations. Therefore, the current study was conducted to evaluate the protective effects of *T. ornata* and *P. pavonia* against induced kidney damage in mice.

## MATERIALS AND METHODS

### Chemicals

Chemicals was purchased from Sigma (USA). All other chemicals were of analytical grade and obtained from standard commercial supplies.

### Collection of algae and extract preparation

*P. pavonia* and *T. ornata* were collected from the Red Sea (Egypt) and extracts were prepared as we formerly reported [12,13]. Briefly, collected seaweeds were washed, air-dried and pulverized to a fine powder then extracted by 80% aqueous ethanol. Following filtration, the filtrates were concentrated under reduced pressure in a rotary evaporator and lyophilisation. Extracts were then stored refrigerated until used.

### Experimental animals

Twenty four male mice weighing 20-25 g were obtained from the animal house of the National Research Center (NRC), El-Giza, Egypt. The animals were housed in plastic well-aerated cages at normal atmospheric temperature (25±5 °C) and 12-hour light/dark cycle. Mice had free access to water and were supplied daily with laboratory standard diet of known composition.

All animal procedures were approved by the Institutional Ethics Committee of Beni-Suef University, which confronted to the recommendations of the Canadian Committee for Care and Use of Animals [14].

**Experimental groups**

Mice were allocated randomly into 4 groups, each consisting of six animals as follows

Group 1 (Normal): Mice received two intraperitoneal (i.p.) Injections of saline.

Group 2 (AOM): Mice received i. p. injections of 10 mg/kg AOM once a week for two consecutive weeks.

Group 3 (AOM + *T. ornata*): Mice received AOM once a week for two consecutive weeks + 100 mg/kg body weight *T. ornata* extract suspended in 1% carboxymethylcellulose (CMC).

Group 4 (AOM + *P. pavonia*): mice Received AOM once a week for two consecutive weeks + 100 mg/kg body weight *P. pavonia* extract suspended in 1% CMC.

Extracts of both *T. ornata* and *P. pavonia* were supplemented orally for 3 weeks starting at the end of 10th week. The used doses of the extracts were selected based on our recent studies [12,13]. The doses were balanced weekly as indicated by any change in body weight to keep up comparable dosage for every kg body weight.

By the end of 13 weeks, mice were sacrificed under anesthesia after the last treatment and blood was collected. Serum samples were separated from the collected blood by centrifugation at 3000 rpm for 15 minutes. Kidney samples were excised and immediately perfused with ice-cold saline then homogenized in chilled saline (10% w/v). The homogenates were centrifuged to separate the nuclear debris. The clear homogenates were separated and used for subsequent biochemical assays.

**Assay of serum urea, uric acid and creatinine**

Serum creatinine, urea and uric acid levels were assayed using reagent kits purchased from spinreact (Spain), following the methods of Young[15], Kaplan[16] and Fossati et al. [17], respectively.

**Assay of oxidative stress and antioxidant system parameters**

Lipid peroxidation (assayed as malondialdehyde {MDA}) and nitric oxide (NO) levels were determined in kidney homogenates according to the methods of Preuss et al. [18] and Montgomery and Dymock [19], respectively. Reduced glutathione (GSH) content was determined according to the method of Beutler et al. [20]. Activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured according to the methods of Marklund and Marklund [21] and Matkovics et al.[22], respectively.

**Histopathology**

The kidney samples were flushed with saline and then fixed in 10% buffered formalin for at least 24 h. The specimens were then dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax. Blocks were prepared and 4µm thick sections were cut by a sledge microtome. The paraffin embedded sections were deparaffinized using xylene and ethanol. The slides were washed with phosphate buffered saline (PBS) and stained with hematoxylin and eosin (H&E). The stained slides were examined under light microscope.

**Statistical analysis**

Statistical analysis was performed using SPSS v.20. Results were expressed as mean ± standard error (SE) and all statistical comparisons were made by means of the one-way ANOVA test followed by Turkey's test post doc analysis. A P value <0.05 was considered significant.

**RESULTS**

AOM administration to mice significantly (P<0.001) increased serum urea levels when compared to the normal control mice (Table 1). Oral supplementation of *T. ornata* extract markedly (P<0.001) decreased the elevated serum urea when compared to the AOM control mice. Similarly, treatment of AOM-induced mice with *P. pavonia* significantly (P<0.01) ameliorated serum urea level.

Serum uric acid showed a significant (P<0.05) increase following AOM administration as represented in Table 1. Treatment of AOM-administered mice with *P. pavonia* extract significantly (P<0.01) improved serum uric acid levels when compared to AOM control mice. *T. ornata* supplementation ameliorated serum uric acid levels, however, the decrease is non-significant (P>0.05) compared to AOM control mice. Mice administered AOM showed a significant (P<0.05), increase in serum creatinine levels and both tested extracts produced a non-significant (P>0.05) effect compared to the AOM group of mice (Table 1).

Histopathological examination of the kidney sections of normal mice revealed normal histological structure (Fig. 1). AOM-administration induced kidney damage confirmed by blood vessel dilatation, degenerated tubules, inflammatory cell infiltrations, mildly congested blood vessels, degenerated glomeruli, tubular epithelial necrosis, thickened blood vessel wall, pyknosis, lobulated glomeruli and other histologic manifestations (Fig. 2). Oral supplementation of either *T. ornata* (Fig. 3) or *P. pavonia* (Fig. 4) extract potentially ameliorated the kidney architecture and protected against the AOM-induced histopathological alterations.

**Table 1: Serum urea, uric acid and creatinine of normal, AOM and *AOM mice treated with T. ornata and P. pavonia***

| | Urea (mg/dl) | Uric acid (mg/dl) | Creatinine (mg/dl) | |
|---|---|---|---|---|
| Normal | 23.40 ± 1.81 | 3.66 ± 0.32 | 0.42 ± 0.01 | |
| AOM | 47.15 ± 6.44*** | 5.70 ± 0.57* | | 0.52 ± 0.04* |
| AOM + *T. ornata* | 22.75 ± 1.52### | 4.16 ± 0.10 | 0.51 ± 0.01 | |
| AOM + *P. pavonia* | 26.88 ± 1.62## | 2.83 ± 0.51## | 0.47 ± 0.02 | |
| F-Prob. | P<0.001 | P<0.01 | P<0.05 | |

Data are expressed as M ± SE, *P<0.05, ***P<0.001 vs normal and ##P<0.01###P<0.001 vs AOM.

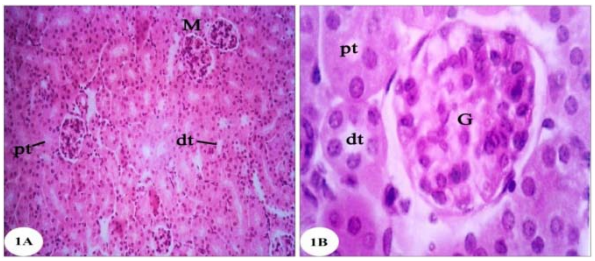


**Fig. 1: Photomicrographs of H&E stained kidney sections of normal mice showing glomerulus (G), Malpighian corpuscle (M), proximal tubules (pt) and distal tubules (dt). (1 A) (X200) and (1B) (X1000)**

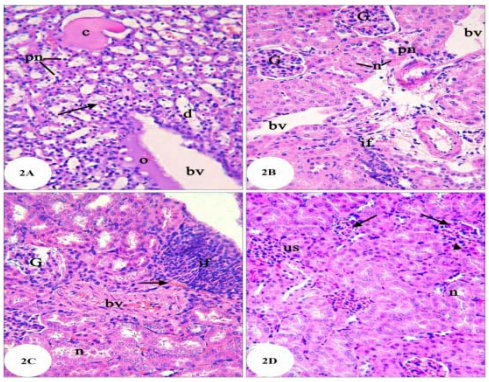


**Fig. 2: Photomicrographs of H&E stained kidney sections of AOM-administered mice showing dilatation in the blood vessel (bv), prevascular edema (o), tubular degeneration and dilatation (d), hyaline cast (c), necrosis (n), pyknosis (pn) and leucocyte infiltrations (if). (X400)**

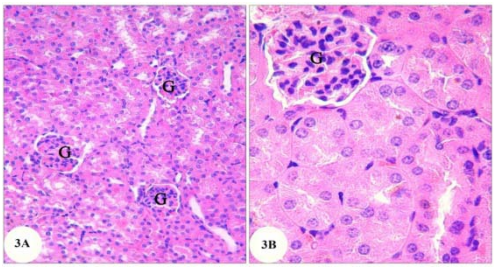


**Fig. 3: Photomicrographs of H&E stained kidney sections of AOM-administered mice treated with *T. ornata* showing nearly normal renal tubules and renal corpuscles. (3A X400, 3B X1000)**

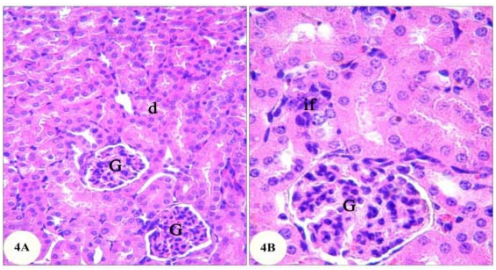


**Fig. 4: Photomicrographs of H&E stained kidney sections of AOM-administered mice treated with *P. pavonia* showing more or less normal renal tubules and corpuscles with slight infiltration and degeneration. (4A X400, 4B X1000)**

Mice administered AOM exhibited a significant (P<0.01) increase in kidney lipid peroxidation when compared with their respective normal mice, as represented in Figure 5. Oral supplementation with either *T. ornata* or *P. pavonia* to AOM-administered mice significantly (P<0.05) decreased the elevated kidney MDA.

Nitric oxide exhibited the same pattern, it was significantly (P<0.001) elevated in the kidney of AOM-administered mice and significantly (P<0.05) declined following supplementation with either *T. ornate* or *P. pavonia* (Fig. 6).

Conversely, GSH content showed a significant (P<0.05) decrease in the kidney of AOM-intoxicated mice as depicted in Figure 7. On the other hand, both *T. ornata* and *P. pavonia* were able to significantly (P<0.05) rejuvenate renal GSH content. More or less similar, AOM administration induced a significant decline in the activities of SOD (P<0.01) and GPx (P<0.05) as represented in Figures 8 and 9, respectively. Supplementation of AOM-administered mice with *T. ornata* and *P. pavonia* significantly alleviated the activities of SOD and GPx.

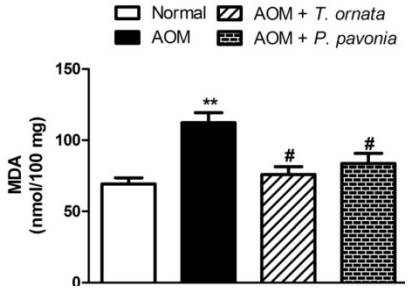


**Fig. 5: Lipid peroxidation in kidneys of normal, AOM and AOM mice treated with *T. ornata* and *P. pavonia*. Data are expressed as M ± SE, P<0.01. **P<0.01 vs normal and #P<0.05 vs AOM**

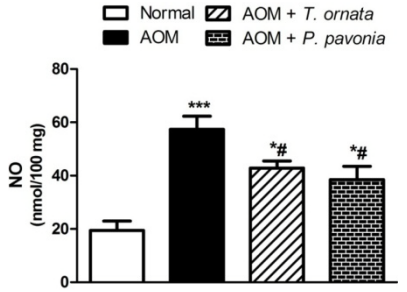


**Fig. 6: Nitric oxide levels in kidneys of normal, AOM and AOM mice treated with *T. ornata* and *P. pavonia*. Data are expressed as M ± SE, P<0.01. * P<0.05, ***P<0.001 vs normal and #P<0.05 vs AOM**

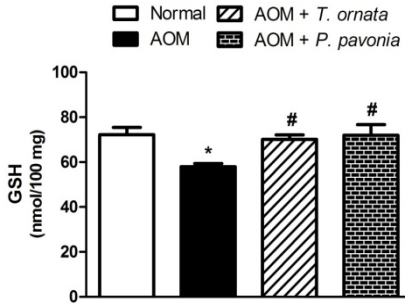


**Fig. 7: Reduced glutathione content in kidneys of normal, AOM and AOM mice treated with *T. ornata* and *P. pavonia*. Data are expressed as M ± SE, P<0.01. *P<0.05 vs normal and #P<0.05 vs AOM**

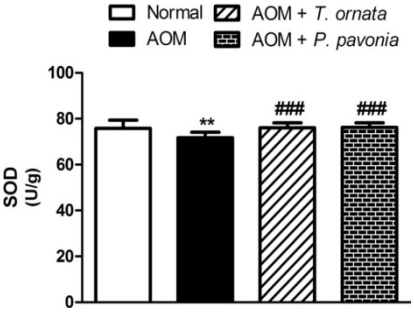


**Fig. 8: Superoxide dismutase activity in kidneys of normal, AOM and AOM mice treated with *T. ornata* and *P. pavonia*. Data are expressed as M ± SE, P<0.001. **P<0.01 vs normal and ###P<0.001 vs AOM**

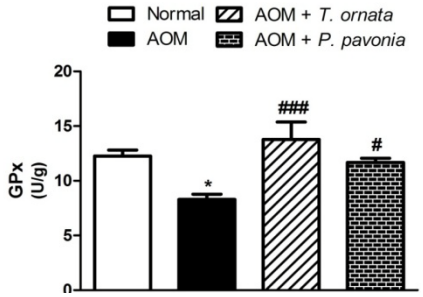


**Fig. 9: Glutathione peroxidase activity in kidneys of normal, AOM and AOM mice treated with *T. ornata* and *P. pavonia*. Data are expressed as M ± SE, P<0.01. *P<0.05 vs normal and #P<0.05 ###P<0.001 vs AOM**

## DISCUSSION

In this study, we described the renal alterations caused by administration of the colon carcinogen AOM in mice. Subsequent to its first identification in cycad palms, AOM was frequently used as a selective intestinal carcinogen [23-25]. Although exposure to AOM is uncommon, human exposures to structurally related hydrazines found in tobacco, rocket fuels, herbicides and drugs are frequent [26]. To the best of our knowledge, no studies have examined the protective effects of the brown seaweeds, *T. ornata* and *P. pavonia*, against AOM-induced nephrotoxicity in mice. Therefore, we conducted the current study to evaluate the possible beneficial effects of *T. ornata* and *P. pavonia* hydro-ethanolic extracts on AOM-induced renal alterations in mice.

AOM administration produced noticeable renal impairment evidenced by the significantly elevated circulatory creatinine, urea and uric acid levels. These finding are in contrary to Hajrezaie et al. [27] who reported a non-significant change in renal function markers in AOM-administered rats after 10 weeks experimental period. Therefore, the 10 weeks period may be insufficient for observing AOM-induced renal function impairment in experimental animals. In the same regard, Hue et al. [28] demonstrated a non-significant elevation of serum urea after 8 weeks following AOM administration in male F344 rats. On the other hand, Pence and Buddingh [29] revealed that administration of the AOM precursor, dimethyl hydrazine, to rats significantly increased serum urea levels. In agreement with our findings, Ward [30] demonstrated that rats given high doses of AOM developed renal tumors. Therefore, the effect of AOM on renal function is seemed to be dose and duration dependent. In addition, renal injury induced by AOM in mice is further confirmed by the observed histological alterations, including dilatation in the blood vessel, perivascular edema, tubular degeneration and dilatation, tubular necrosis, pyknosis, leucocyte infiltrations and other manifestations. On the other hand, supplementation of either *T. ornata* or *P. pavonia* ameliorated the altered renal function markers in serum of AOM-administered mice. Also, both treatments potentially alleviated the kidney architecture. In this regard, the renoprotective effects of brown seaweeds have been demonstrated in few studies. A recent study conducted by Karthikeyan et al. [31] reported that *Padina boergessenii* ameliorates carbon tetrachloride ($CCL_4$)- induced nephrotoxicity in rats. However, the renoprotective effect of *T. ornata or P. pavonia* against AOM-induced toxicity has not been previously reported. Hence, this study shows for the first time that *T. ornata or P. pavonia* could protect mice against AOM-induced nephrotoxicity.

Mice administered with AOM exhibited a significant increase in renal lipid peroxidation. This observation might be attributed to increased reactive oxygen species (ROS) generated through the metabolism of AOM. Multiple studies reported that the metabolism of AOM produces extremely reactive hydroxyl radicals that induce oxidative stress [25,32]. The produced ROS induce lipid peroxidation, protein damage and DNA fragmentation [33]. Due to the abundance of long chain polyunsaturated fatty acids in the composition of renal lipids, the kidney is highly vulnerable to damage caused by ROS [34]. In addition, Lopez-Novoa et al. [35] postulated that ROS are crucial in the mechanisms that lead to tubular necrosis and decrease of glomerular filtration rate. Similarly, mice received AOM showed significantly elevated renal NO levels. Under conditions of oxidative stress, NO reacts with superoxide anions to form the potent oxidant peroxinitrite [36,37]. In addition, NO activates nuclear factor-kappa B and thereby stimulates the production of pro-inflammatory cytokines [38]. Treatment of AOM-administered mice with either *T. ornata* or *P. pavonia* potentially decreased lipid peroxidation and NO levels in the kidney. These findings are in agreement with our recent study [12] where we reported that *T. ornata* significantly decreased cyclophosphamide-induced lipid peroxidation and inflammatory cytokines in rats. In addition, we demonstrated that both *T. ornata* and *P. pavonia* have anti-inflammatory effect in diabetic rats [13]. Another study conducted by Karthikeyan et al. [31] reported that *Padina boergessenii* significantly decreased lipid peroxidation and alleviated the nephrotoxicity induced by $CCl_4$ in rats.

Renal tissue GSH content was significantly depleted in the AOM treated group, with concomitantly declined renal activities of the antioxidant enzymes SOD and GPx. GSH is an antioxidant thiol compound [39,40] that protect cells against lipid peroxidation through the formation of S-conjugates with products of lipid peroxidation [41] and by acting as a co-factor of GPx [42,43]. In addition, GSH has been reported to be involved in detoxification of many xenobiotics through the formation of S-conjugates with toxic metabolites in the second phase of biotransformation [44]. The observed inactivation of the antioxidant enzymes may be attributed directly to the increased production of ROS in AOM-induced mice. Supplementation with either *T. ornata* or *P. pavonia* extracts potentially restored renal GSH content as well as activities of the antioxidant enzymes when compared with AOM control group. Recently, we reported that *T. ornata* extract significantly ameliorated GSH and activities of the antioxidant enzymes in cyclophosphamide- induced hepatotoxicity in rats [12]. In addition, Germoush [45] demonstrated the antioxidant effects of *T. ornata* and *P. pavonia* extracts, provided as a gift from our lab, in diabetic rats. Therefore, we assume that supplementation of both tested extracts protected mice against AOM-induced nephrotoxicity by preventing depletion of GSH and enhancing the renal antioxidant defense enzymes.

In conclusion, the present study conveys for the first time new information on the protective effects of *T. ornata* and *P. pavonia* against AOM-induced nephrotoxicity. Our findings suggest that the renoprotective effects of the brown seaweeds might be attributed to their ability to hamper oxidative damage induced by the chemical carcinogen AOM and to potentiate the renal antioxidant defense system.

## CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

## REFERENCES

1. Senthilkumar K, Manivasagan P, Venkatesan J, Kim S-K. Brown seaweed fucoidan: biological activity and apoptosis, growth signaling mechanism in cancer. Int J Biol Macromol 2013;60:366-74.
2. Mac Artain P, Gill CI, Brooks M, Campbell R, Rowland IR. Nutritional value of edible seaweeds. Nutr Rev 2007;65:535-43.
3. Mayer AMS, Rodriguez AD, Berlinck RGS, Hamann MT. Marine pharmacology in 2003-4. Comp Biochem Physiol 2007;145:553-81.
4. Madhusudan C, Manoj S, Rahul K, Rishi CM. Seaweeds: a diet with nutritional, medicinal and industrial value. Res J Med Plant 2011;5:153-7.
5. Arif JM, Al-Hazzani AA, Kunhi M, Al-Khodairy F. Novel marine compounds: Anticancer or genotoxic? J Biomed Biotechnol 2004;2004(2):93-8.
6. Cerna M. Seaweed proteins and amino acids as nutraceuticals. Adv Food Nutr Res 2011;64:297-312.
7. Tabarsa M, Rezaei M, Ramezanpour Z, Waaland JR. Chemical compositions of the marine algae *Gracilaria salicornia* (*Rhodophyta*) and *Ulva lactuca* (*Chlorophyta*) as a potential food source. J Sci Food Agric 2012;92(12):2500-6.

8. Bird RP. Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. Cancer Lett 1995;93(1):55-71.
9. De Robertis M, Massi E, Poeta ML, Carotti S, Morini S, Cecchetelli L, *et al*. The AOM/DSS murine model for the study of colon carcinogenesis: from pathways to diagnosis and therapy studies. J Carcinog 2011;24:1477-3163.
10. Chan CH, Cook D, Stanners CP. Increased colon tumor susceptibility in azoxymethane treated CEABAC transgenic mice. Carcinogenesis 2006;27(9):1909-16.
11. Kobæk-Larsen M, Fenger C, Ritskes-HoitingA J. Secondary effects induced by the colon carcinogen azoxymethane in BDIX rats. APMIS 2004;112:319-29.
12. Mahmoud AM, Hussein OE, Ramadan SA. Amelioration of cyclophosphamide-induced hepatotoxicity by the brown seaweed *Turbenariaornata*. Int J Clin Toxicol 2013;1:9-17.
13. Mahmoud AM, Germoush MO, Elgebaly HA, Elsayed KNM, Hassan S, Mousa NM, *et al*. Antidiabetic and insulin sensitizing effects of *Padina pavonia* and *Turbenaria ornata* in streptozotocin/nicotinamide diabetic rats. Asian J Pharm Clin Res 2014;7(4):74-8.
14. Canadian Council on Animal Care. Guide to the care and use of experimental animals, Vol. 2. Ottawa, Ontario, Canada: CCAC; 1993.
15. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press; 1995.
16. Kaplan A. Urea. Kaplan A. Clin Chem The CV Mosby Co St Louis. Toronto. Princeton; 1984. p. 1257-60 and 437 and 418.
17. Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 1980;26(2):227-31.
18. Preuss HG, Jarrell ST, Scheckenbach R, Lieberman S, Anderson RA. Comparative effect of *chromium vanadium* and *Gymnemasylvestre* on sugar-induced blood pressure elevation in SHR. J Am Coll Nutr 1998;17:116-23.
19. Montgomery HAC, Dymock JF. The determination of nitrite in water. Anal 1961;86:414-6.
20. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Mid 1963;61:882-8.
21. Marklund SL, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
22. Matkovics B, Szabo L, Varga IS. Determination of enzyme activities in lipid peroxidation and glutathione pathways (in Hungarian). Laboratoriumi Diagnosztika 1998;15:248-9.
23. Laqueur GL, Mickelsen O, Whiting MG, Kurland LT. Carcinogenic properties of nuts from *Cycas circinalis* L. indigenous to Guam. J Natl Cancer Inst 1963;31:919-51.
24. Hirono I. Natural carcinogenic products of plant origin. Crit Rev Toxicol 1981;8:235-77.
25. Waly MI, Al-Rawahi AS, Al Riyami M, Al-Kindi MA, Al-Issaei HK, Farooq SA, *et al*. Amelioration of azoxymethane induced-carcinogenesis by reducing oxidative stress in rat colon by natural extracts. BMC Complement Altern Med 2014;14:60.
26. Gamberini M, Cidade MR, Valotta LA, Armelin M, Leite L. Contribution of hydrazines-derived alkyl radicals to cytotoxicity and transformation induced in normal c-myc-overexpressing mouse fibroblasts. Carcinogenesis 1998;19:147-55.
27. Hajrezaie M, Hassandarvish P, Moghadamtousi SZ, Gwaram NS, Golbabapour S, Najihussien A, *et al*. Chemopreventive evaluation of a Schiff base derived copper (II) complex against azoxymethane-induced colorectal cancer in rats. PLoS One 2014;9(3):e91246.
28. Hue J-J, Lee YE, Lee K-N, Nam SY, Ahn B, Yun YW, *et al*. Phytic acid protects the formation of colonic aberrant crypt foci induced by azoxymethane in male f344 rats. J FdHyg Safety 2008;23(3):264-70.
29. Pence BC, Buddingh F. Effect of dietary selenium deficiency on incidence and size of 1,2-dimethylhydrazine-lnduced colon tumors in rats. J Nutr 1985;115:1196-202.
30. Ward JM. Dose response to a single injection of azoxymethane in rats: induction of tumors in the gastrointestinal tract, auditory sebaceous glands, kidney, liver and preputial gland. Vet Pathol 1975;12:165-77.
31. Karthikeyan R, Anantharaman P, Chidambaram N, Balasubramanian T, Somasundaram ST. *Padina boergessenii* ameliorates carbon tetrachloride induced nephrotoxicity in Wistar rats. J King Saud Univ-Sci 2012;24(3):227-32.
32. Khanum F, Anilakumar KR, Sudarshana Krishna KR, Viswanathan KR. Effects of feeding fresh garlic and garlic oil on detoxifying enzymes and micronuclei formation in rats treated with azoxymethane. Int J Vitamin Nutr Res 1998;68(3):208-13.
33. Nencini C, Giorgi G, Micheli L. Protective effect of silymarin on oxidative stress in rat brain. Phytomed 2007;14(2-3):129-35.
34. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol 2012;2012:465-897.
35. Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int 2011;79:33-45.
36. McKim SE1, Gäbele E, Isayama F, Lambert JC, Tucker LM, Wheeler MD, *et al*. Inducible nitric oxide synthase is required in alcohol-induced liver injury: studies with knockout mice. Gastroenterol 2003;125:1834-44.
37. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Intl J Biochem Cell Biol 2007;39:44-84.
38. Matata BM, Galinanes M. Peroxynitrite is an essential component of cytokines production mechanism in human monocytes through modulation of nuclear factor-kappa B DNA binding activity. J Biol Chem 2002;277:2330-35.
39. Parke DV, Piotrowski J. Glutathione: Its role in detoxication of reactive oxygen and environmental chemicals. Acta Pol Toxicol 1996;4:1-14.
40. Deneke S. Thiol-based antioxidants. Curr Top Cell Reg 2000;36:151-80.
41. Laurent A, Perdu-Durand E, Alary J, DE Brauwer L, Cravedi JP. Metabolism of 4-hydroxynonenal, a cytotoxic product of lipid peroxidation, in rat precision-cut liver slices. Toxicol Lett 2000;114:203-14.
42. Arai M, Imai H, Koumura T, Yoshida M, Emoto K, Umeda M, *et al*. Mitochondrial phospholipid hydroperoxide glutathione peroxidase plays a major role in preventing oxidative injury to cells. J Biol Chem 1999;274:4924-33.
43. Dringer A. Metabolism and function of glutathione in brain. Prog Neurobiol 2000;62:649-71.
44. Strange RC, Jones PW, Fryer AA. Glutathione S-transferase: genetics and role in toxicology. Toxicol Lett 2000;112-113:357-63.
45. Germoush MO. Antioxidant and anti-inflammatory effects of *Padina pavonia* and *Turbenaria ornata* in streptozotocin/nicotinamide diabetic rats. Life Sci J 2013;10(3):1265-71.