Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Histopathological Changes of Male Mice Kidneys Treated with Fresh *Aloe vera* whole Leaf Extract

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Abstract

Background *Aloe vera* is an evergreen perennial plant widely used in modern herbal practice and is often available in proprietary herbal preparations. Evidence of efficacy is strongest for the laxative effects of *Aloe vera* latex; however the anthraquinones in the latex are associated with considerable risks.

Objective To investigate the histopathological changes of kidney tissues of male mice administered low dose of whole leaf fresh *Aloe vera* extract.

Methods Forty immature male Swiss Webster mice divided into two equal groups (experimental and control) (G and C respectively). The experimental group (G) was given 20 µl of *Aloe vera* extract orally for 21 days. While the control groups (C) were given by the same dose and route of administration with normal saline only. After six weeks (around puberty), the male were sacrificed to get their kidneys, then fixed with 10% formalin, and histological sections with a thickness of 5 microns were prepared.

Results Histological studies of mice kidneys from groups that consume low dose fresh *Aloe vera* whole leaf extracts showed disrupted entire structure of kidneys including degenerative changes in most parenchymatous elements in comparison with control.

Conclusions Using low doses of fresh *Aloe vera* whole leaf extract induce adverse effects on the histological features of mice kidneys and impaired their entire structure.

Key words *Aloe vera*, Kidney, anthraquinones, whole leaf extract.

List of Abbreviations: IASC = International *Aloe* Science Council, CIREP = cosmetic ingredient review expert panel, FDA = Food and Drug Agency, LOAEL = lowest-observed-adverse-effect level, GFR = glomerular filtration rate, PCTs = proximal convoluted tubules, DCTs = distal convoluted tubules, ROS = reactive oxygen species, LMWF: Low molecular weight fraction, PMN = polymorphonuclear.

Introduction

The kidney is one of the most vital bodily organs. It has a former role in the whole body homeostasis, controlling the electrolyte concentrations, acid-base balance, extracellular fluid volume, as well as playing a crucial role in the blood pressure regulation. It eliminates a wide variety of waste products. It secretes a group of hormones, such as erythropoietin. It also secretes the enzyme renin and calcitriol which is activated form of vitamin D [1,2].

Aloe plants have been used medicinally for centuries. Among them, *Aloe barbadensis*, commonly called *aloe vera*, is one of the most widely used healing plants in the history of mankind [3]. The molecular studies put *Aloe* in the order *Asparagales*. Within *Asparagales*, it is either in the family *Asphodelaceae* or the family *Xanthorrhoeaceae* [4]. The species is used widely in the traditional herbal medicine of China, Japan, Russia, South Africa, the United States, Jamaica, Latin America and India [5]. Briggs, 1995 [6] described the leaf of *Aloe* plants as consisting of two main parts. One part, the pericyclic cells, is found just below the plant's skin. The pericyclic cells produce a bitter, yellow latex known as *Aloe* juice, or latex. When this juice dries it forms a dark

brown solid material. The main active constituents of the latex are anthraquinones, which include aloins A, and B, barbaloin, isobarbaloin, and emodin. Also included are aloe-emodin, resins, aloesin and its aglycone, aloesone, and chromone derivatives [7]. *A*loe juice is approximately 99% water [8], and the remainder consists of minerals, vitamins, polysaccharides, lipids, phenolic compounds, and organic acids [9]. The second part, the inner central area of the leaf, contains the thin walled parenchymal cells that produce the clear slightly viscous (mucilaginous) fluid known as *Aloe* gel or inner gel. This gel contains the polysaccharides and three malic acid acylated carbohydrates [10]. Other potentially active constituents are lipids, amino acids, sterols [11] and high concentration of mannose 6-phosphate [12].

Many biological activities, including *Aloe* species have been used for centuries for their laxative, antiinflammatory, immunostimulant, antiseptic, wound and burn healing activities [13]. There have been reports, also, on the antidiabetic activity of *Aloe* extracts [14]. Additionally, numerous constituents within *Aloe vera* have demonstrated enhancement of immune system functioning within the body [15]. Leaf pulp extract also showed hypo-glycaemic activity in type I and II diabetic rats [16]. *Aloe vera* is a common ingredient in cosmetics and pharmaceutical industries [17].

In addition to the well-documented positive effects, there have been also reports of negative actions. There is, however, little scientific evidence of the effectiveness or safety of *Aloe vera* extracts for either cosmetic or medicinal purposes, and what positive evidence is available is frequently contradicted by other studies [18]. The International *Aloe* Science Council (IASC) distinguished between the anthraquinones found in the outer cell layer of the aloe leaf and the rest of the plant. According to this group, the maximum allowable aloin content in aloe-derived material for non medicinal use is 50 ppm or lowers [19]. The laxative effect of the anthraquinone glycosides found in *Aloe vera* latex is well established [20]. The Cosmetic Ingredient Review Expert Panel (CIREP) (2007) concluded that *Aloe* latex, but not the polysaccharide material derived from the inner gel, is cytotoxic [21].

Oral use may cause diarrhea or vomiting [22]. Many of these reactions appear to be associated with anthraquinone contaminants of the gel product. Moreover use of *Aloe vera* as a laxative during pregnancy may pose potential teratogenic and toxicological effects on the embryo and fetus [20]. Pregnant women are advised not to take *Aloe* latex because of its cathartic action, which may cause severe uterine contractions and increase the risk of miscarriage. It should also not be ingested by nursing mothers because of the possibility of causing severe cramps and diarrhoea in the infant [23].

In 1998, 27 adverse events due to *Aloe vera* were reported to the Food and Drug Agency (FDA) [24]. Adverse effects of *Aloe* whole-leaf powder have been reported at concentrations of 2 g/kg BW, and the lowest-observed-adverse-effect level (LOAEL) for aloin is estimated at 11.8 g/kg BW [25]. The distribution and/or accumulation of aloen in the stomach, liver, and kidneys indicated that aloin and its metabolites accumulated in the liver and the kidneys. In fact, liver and kidney were the only organs that had higher concentrations of aloe-emodin than plasma [26], leading to nephro-toxicity [27]. Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication on the kidneys decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glucosuria, decreased ammonium excretion and lowering of glomerular filtration rate (GFR) [28]. Until now, there are no published controlled *in vivo* toxicology studies of *Aloe vera* in humans; further research in humans is required to confirm these effects [29]. Due to vital function of kidneys, the present study was, therefore, designed to investigate the histopathological changes of kidney tissues of male mice

administered low dose of fresh *Aloe vera* whole leaf extract.

Methods

Forty immature Swiss Webster male mice (3) weeks old were divided into two equal groups: experimental (G) and control (C), twenty animals each.

Fresh leaves of plant having a length of approximately 25 to 50 cm were washed with fresh water then cut from the middle. The whole leaf extract was separated by scratching with a spoon. The obtained substance is then divided into 2 ml volume tubes, kept at 4 ^{o}C overnight, before being used. The aloe juice was prepared daily to get fresh extracts [30].

Immature male mice were obtained from animal house of the High Institute for Infertility Diagnosis and ART/ Al-Nahrain University randomly selected. The mice weighed 15-18 g and were about 3 weeks old. They were kept in metal cages at room temperature (27 ^{o}C - 30 ^{o}C) in the animal room and exposed to photo-periodicity 12:12. The mice, divided into 2 groups of twenty mice each, were fed on mice pellet and had access to water *ad libitum*. The experimental group (G) was given 20 µl of fresh *Aloe vera* whole leaf extract orally for 21 days. While the parallel control group was given normal saline by the same rout and dose as that used in the experimental group. After six weeks (around puberty) the mice were sacrificed to get their kidneys, cleared of adhering tissues, then fixed with 10% formalin, and histological sections with a thickness of 5 microns were prepared using the routine histological technique [31].

Results

Light microscopic study

Control mice

The histological section of control mice revealed normal renal parenchyma. Cortical portion of the kidney constitute of renal corpuscle and convoluted tubules. Acidophilic cells distinguish the proximal convoluted tubules (PCTs) from the distal convoluted tubules (DCTs), whose smaller, less intensely stained cells (Fig. 1).

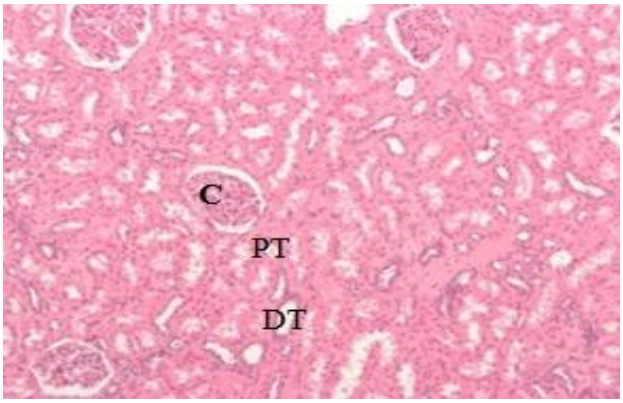

Fig. 1. Section in control mice kidney showing normal renal tissue. Normal renal corpuscle (C), the glomerular capillary loops are thin and delicate. The surrounding proximal (PT) and distal renal tubules (DT) are normal. The renal corpuscle is surrounded by Bowman's capsule. A urinary space (which appears as a clear space) is visible (20X, H&E).

The renal corpuscle exhibits the glomerular capillaries, parietal layer and visceral epithelium of the glomerular (Bowman's) capsule and the capsular space [32,33] (Fig. 2).

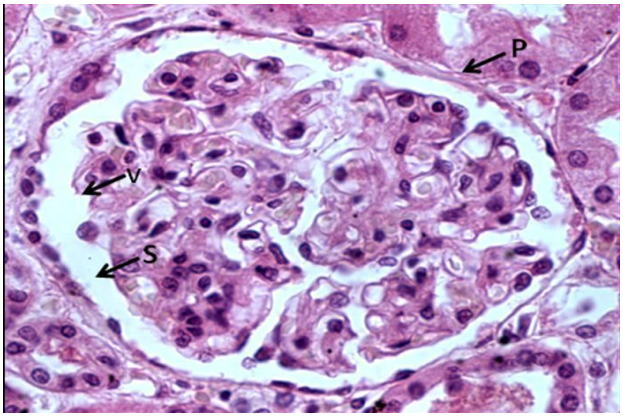

Fig. 2. Renal corpuscle of control adult male rat, P: parietal layer, V: visceral layer and S: urinary space (40 X, H & E) [34].

Histopathological changes

The kidney revealed degenerative changes in most of its entire structure in comparison with control. Atrophy of renal corpuscle with

shrinked glomeruli were represented by decrease in glomerular cellularity (Fig. 3).

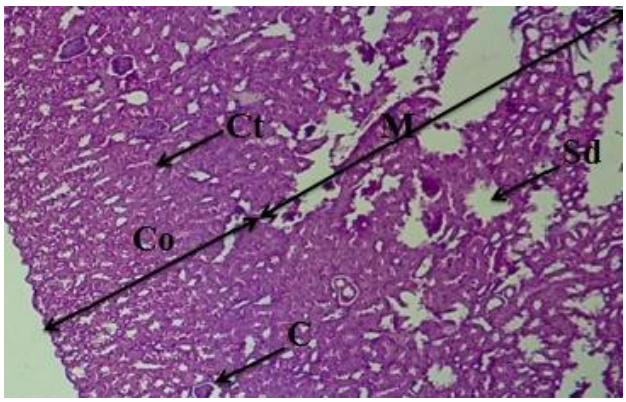

Fig. 3. Section of *Aloe vera* treated mice kidney showing atrophy of renal corpuscle (C) (arrow) with shrinked glomeruli in renal cortex (Co) (double head arrow) with degenerative cortical renal tubules (Ct). Sloughing of necrotic areas (Sd) (arrow) in most parenchymatous elements in renal medulla (M) (double head arrow) (4X, H&E).

Proximal tubules and distal renal tubules showed histological changes in the form of widening of tubular lumen with hyalinization. Glomeruli appears to be degenerative with necrosis (Fig. 4).

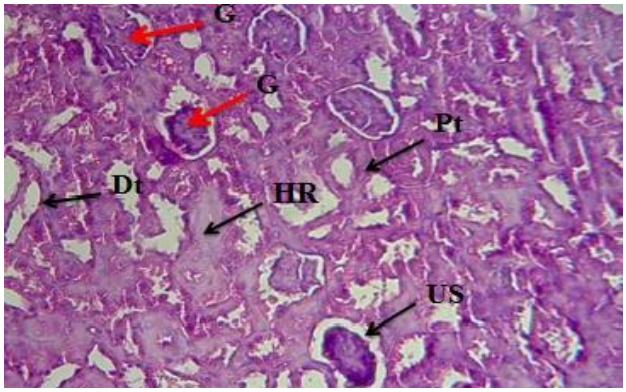

Fig. 4. Section of *Aloe vera* treated mice kidney showing widened deformed proximal renal tubules (Pt) and distal renal tubules (Dt) (arrows), along with hyalinization of renal tubules (HR). Glomeruli (G) reveal features of degenerative and necrosis (Red arrows). Widened urinary space (US) of the Bowman's capsule was observed (arrow) (20X, H&E).

Other observation demonstrates both proximal and distal tubules showing degenerated epithelial lining. The cells showed decrease in height, the cytoplasm was stained deeply acidophilic. The nuclei were small and deeply stained (pyknotic). The rest of the tubules showed dilated lumen. Massive regions of necrosis in renal parenchyma markedly observed (Fig. 5).

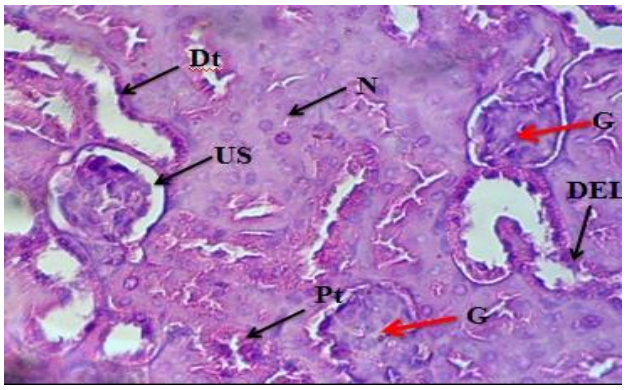

Fig. 5. Micrograph of mouse kidney treated with 20 µl *Aloe vera* showing degenerative and necrotic glomeruli (G) (Red arrows) with widened urinary space (US) of the Bowman's capsule (arrow). Both proximal (Pt) and distal tubules (Dt) showed degenerated epithelial lining (DEL). The cells showed decrease in height, the cytoplasm stained dark acidophilic, and the nuclei are small, pyknotic and dark stained. Distal tubules showed wide lumina. Massive regions of necrosis in renal parenchyma markedly observed (N) (40X, H&E).

Discussion

Estimation of the renal excretion of the waste metabolites and histological changes in the kidney has provided useful information on the health status of the kidneys [35]. Renal systems actively involved in drug elimination from the body through renal filtration process, proximal tubule secretion and distal tubule reabsorption. It is well known that most of drugs, including: antibiotics, nonsteroidal anti-inflammatory, radiographic contrast media and some of cancer remedies, may be the cause of renal failure. Although damage may be

reversible, it may cause chronic changes in kidney parenchyma [36].

The histological observations from this study illustrate atrophy of renal corpuscle with shrinked glomeruli represented by decrease in glomerular cellularity (Fig. 3 and 4). The decrease in glomerular cellularity may be explained by the fact that *aloe vera* leaves contain phytochemicals such as anthraquinone [37] and it was suggested that the presence of yellow sap (rich in anthraquinones) in *Aloe vera* gel reduces cell growth [38], while dilation of the urinary space of the renal corpuscles in *Aloe vera* treated mice may be due to basement membrane alterations and epithelial changes in the PCTs, decreasing the functional properties of PCTs and resulting in a decrease in the glomerular filtration rate (GFR) and the accumulation of urine in the urinary space. It is documented that normality of the basement membrane of glomerulus is essential for normal GFR and any change in this structure leads to proteinuria [39].

One finding of the present study was disorganization and necrosis of renal convoluted tubules, wide spacing of tubules, and atrophy of the lining epithelium along with hyalinization in most renal tissues (Fig. 3-5). This histopathological alterations could lead to nephrotoxicity which characterized by direct tubular necrosis [40,41]. The toxic effect of *Aloe vera* gel could be due to the generation of anthraquinones formed by oxidation of low molecular weight components such as aloin which are present in the plant leaves [42]. Aloin accumulates in proximal straight and distal convoluted tubules and promotes cellular damage, by multiple mechanisms including oxidative stress, DNA damage and apoptosis [43,44]. Furthermore the cytotoxic effects could be masked by the production of reactive oxygen species (ROS) by redox cycling induced by anthraquinones of the low molecular weight fraction (LMWF). Indeed, 'tHart *et al.* (1990) observed that the (LMWF) seems to have a stimulatory effect on the O_2 consumption by resting polymorphonuclear leukocytes (PMN), due perhaps to the mentioned mechanism of redox cycling. At longer times the toxic effect predominates and becomes evident. Likewise, it is also possible that the inhibitor is present in *Aloe vera* extracts, although in different amounts. It is apparent that LMWF obtained from *Aloe vera* gel has cytotoxic activities [45].

Another histopathological change is degeneration and necrosis of glomeruli (Fig. 4 and 5) and massive regions of necrosis with loss of renal parenchyma in some areas was clearly observed (Fig. 3 and 5). The degenerative alterations mentioned in the present study may be due to that anthraquinones, which are poorly absorbed from the GIT, are cleaved by gut bacteria to produce aloe-emodin, which is more readily absorbed and responsible for the purgative properties of these preparations [27]. The liver and kidney were the only organs that had higher concentrations of aloe-emodin than plasma [26]. In addition to that, oxidative stress has also been proposed to play a role in the pathogenesis of renal and hepatic tissue damage [46,47]. Several lines of evidence suggest the role of ROS in the pathogenesis of nephrotoxicity [48]. Moreover elevated generation of free radicals may lead to disruption of cellular functions and oxidative damage to membranes and may enhance susceptibility to lipid peroxidation [49]. Likewise abnormal production of ROS may damage some macromolecules to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage [50].

This study suggests that continuous consumption of *Aloe vera* whole leaf extract in the tested dosage range result in histopathological changes of the renal tissue in mice. It is important to note that the amount of *Aloe vera* used in many previously published studies were very high, in contrast to the present study which showed evidence of organ injury at relatively lower doses, suggesting that further research is warranted to examine the safety profile of this widely used food additive.

Moreover, herbs have a variety of complex chemical constituents that act on the body as a whole or on specific organs and systems. Some of the chemical constituents are mild and safe even in large doses while, some act more strongly or are toxic in large doses or when taken continuously [51].

In conclusion, using low doses of *Aloe vera* whole leaf extract induce adverse effects on the histological features of mice kidneys and impaired their entire structures.

Acknowledgment

I would like to express my deep gratitude to Allah for helping me to complete this study.

Conflict of interest

None.

Funds

None.

References

1. Stevens LA, Coresh J, Greene T, et al. Assessing kidney function measured and estimated glomerular filtration rate. New Engl J Med. 2006; 354: 2473-83.
2. Ruth R. A historical introduction to Gray's anatomy. In: Standring S (ed). Gray's Anatomy: The anatomical basis of clinical practice. 39th ed. Edinburgh: Elsevier Churchill Livingston; 2005. P. 4.
3. Moon EJ, Lee YM, Lee OH, et al. A novel angiogenic factor derived from *Aloe vera* gel: b-sitosterol, a plant sterol. Angiogenesis. 1999; 3: 117-23.
4. Dagne E, Bisrat D, Viljoen A, et al. Chemistry of *Aloe* species. Curr Org Chem. 2000; 4: 1055-78.
5. Liao Z, Chen M, Tan F, et al. Microprogagation of endangered Chinese aloe. Plant Cell Tiss Organ Culture. 2004; 76: 83-6.
6. Briggs C. Herbal medicine: *Aloe.* Can Pharmaceut J. 1995; 128: 48-50.
7. Wichtl M. Herbal drugs and phytopharmaceuticals. Practice on scientific basis. Stuttgart: Medpharm Scientific Publishers, 1994; 5: 463-9.
8. Hamman JH. Composition and applications of *Aloe vera* leaf gel. Molecules. 2008; 13: 1599-616.
9. International *Aloe* Science Council. Position paper of the international *Aloe* scientific council on the national toxicology program study of orally ingested *Aloe Vera*, 2011.
10. Esua MF, Rauwald JW. Novel bioactive maloylglucans from *Aloe vera* gel: isolation, structure elucidation and *in vitro* bioassays. Carbohydr Res. 2006; 341: 355-64.
11. Bruneton J. Pharmacognosy, Phytochemistry. Medicinal plants. 1987; 81(3): 434-6.
12. Davis RH, Donato JJ, Hartman GM, et al. Anti-inflammatory and wound healing activity of growth substance in *Aloe vera*. J Am Podiatr Med Assoc. 1994; 84: 77-81.
13. Fostel JM, Lartey PA. Immerging novel antifungal agents Drug Discov Today. 2000; 5: 25-32.
14. Rajasekaran SK, Sivagnanam K, Subramanian S. Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats. Pharmacol Rep. 2005; 57: 90-6.
15. Rajasekaran SK, Sivagnanam K, Subramanian S. Modulatory effects of *Aloe vera* leaf gel extract on oxidative stress in rats treated with streptozotocin. J Pharma Pharmacol. 2005; 57: 241-6.
16. Okyar A, Can A, Akev N, et al. Effect of *Aloe vera* leaves on blood glucose level in type I and type II diabetic rat models. Phytother Res. 2001; 15: 157-61.
17. Eshun K, He Q. *Aloe vera*: A valuable ingredient for the food, pharmaceutical and cosmetic industries a review. Crit Rev Food Sci Nutr. 2004; 44: 91-6.
18. Boudreau MD, Beland FA. An Evaluation of the Biological and Toxicological Properties of *Aloe Barbadensis* (*Miller*), *Aloe Vera*. J Environ Sci Health Part C. 2006; 24: 103-54.
19. International *Aloe* Science Council (IASC). IASC addresses aloe nomenclature for CTFA. Inside *Aloe.* 2003.www.iasc.org.
20. Ulbricht C, Armstrong J, Basch E, et al. An evidence-based systematic review of *Aloe vera* by the Natural Standard Research Collaboration. Herb Pharmacother. 2008; 7: 279-323.
21. Cosmetic Ingredient Review Expert Panel. Final report on the safety assessment of an *Aloe andongensis* extract, *Aloe Andongensis* leaf juice. Int J Toxicol. 2007; 26: 1-50.
22. Chinnusamy K, Nandagopal T, Nagaraj K, et al. *Aloe vera* induced oral mucositis: A case report. Internet J Pediatr Neonatol. 2009; 9: 35-49.
23. Brinker F. Herb contraindications and drug interactions. Oregon: Eclectic Medical Publishers; 1998. p. 28-30.
24. Food and Drug Administration (FDA). The special nutritionals/adverse event monitoring system web report for aloe. 1998.
25. Zhou Y, Feng Y, Wang H, et al. 90-day subchronic toxicity study. J Toxicol. 2013; 12: 67-78.
26. Lang W. Pharmacokinetic-metabolic studies with 14C-aloe-emodin after oral administration to male and female rats. Pharmacology. 1993; 47: 110-9.
27. Blumenthal M. The complete German commission E monographs. Boston: Mass Integrative Medicine Communications; 1998. p. 423.

28. Geevasinga N, Coleman PL, Webster AC, et al. Proton pumps inhibitors and acute interstitial nephritis. Clin Gastroenterol Hepatol. 2006; 4: 597-604.
29. Steenkamp V, Stewart MJ. Medicinal applications and toxicological activities of *Aloe* products. Pharm Biol. 2007; 45: 411-20.
30. Jasem E, Nasim J. Spermatogenic activity of *Aloe vera* in adult male rats. Pharmacology online. 2011; 2: 886-9.
31. Bancroft JD, Gamble M. Theory and practice of histological techniques. Nottingham, UK. Churchill Livingstone. 9th ed. 2007.
32. Dellmann HD, Eurell J. Textbook of veterinary histology. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 1998. p. 213-7.
33. Victor P. Atlas of histology with functional correlations. 11th ed. Philadephia: Walters Clawer Health. Lippincott William and Wilkins; 2008; p. 364.
34. Eleiwi SA, Continuous darkness induces changes in the urinary space of the rat's kidney. IJMS. 2014; 12: 60-5.
35. Panda NC, Talwar GP, Srivastava LM, et al, eds. Textbook of biochemistry and human biology. 2nd ed. India: Prentice-Hall; 1989. p. 276-92.
36. Murcia MA, Egea I, Romojaro F, et al. Antioxidant evaluation in dessert spices compared with common food additives. J Agric Food Chem. 2004; 52: 1872-81.
37. Liao Z, Chen M, Tan F, et al. Microprogagation of endangered Chinese aloe plant cell. Tiss Organ Culture. 2004; 76: 83-6.
38. Chapman M. Excessively high cell proliferation in sigmoid colon after an oral purge with anthraquinone glycosides (aloins). J Nat Cancer Institute. 1995; 87: 1086-7.
39. Nakamura S, Terashima M, Kikuchi N, et al. A new mouse model for renal lesions produced by intravenous injection of diphtheria toxin A-chain expression plasmid. BMC Nephrol. 2004; 5: 11-5.
40. Khandelwal V, Kumar M, Koneri R, et al. Biological activities of some Indian medicinal plants. J Adv Pharm Edu Res. 2011; 1: 12-44.
41. Laskshmi BV, Sudhakar M. Protective effect of zingiber officinal aleon gentamicin induced nephrotoxicity in rats. Int J Pharmacol. 2010; 6: 58-62.
42. Westendorf J, Marquardt H, Poginsky B, et al. Genotoxicity of naturally occurring hydroxy anthraquinones. Mutation Res. 1990; 240: 1-12.
43. PablaN, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int. 2008; 73: 994-1007.
44. Xiao T, Choudhary S, Zhang N, et al. Possible involvement of oxidative stress in cisplatin induced apoptosis in LLC-PK1 cells. J Toxicol Environ Health. 2003; 66: 469-79.
45. Horacio A, Joserivero, Flor H, German F. Cytotoxicity of a low molecular weight fraction from *Aloe vera* (*Aloe barbadensis miller*) gel. Toxicon. 1997; 35: 1423-1430.
46. Ha H, Lee HB. Oxidative stress in diabetic nephropathy: basic and clinical information. Curr Diab Rep. 2001; 1: 282-7.
47. Kashihara N, Haruna Y, Kondeti VK, et al. Oxidative stress in diabetic nephropathy. Curr Med Chem. 2010; 17: 4256-69.
48. Chilwant, Kalyan S, Muglikar AG. Effect of honey on gentamicin induced nephrotoxicity in albino rats. Int J Pharm Biosci. 2012; 3: 459-64.
49. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991; 40: 405-12.
50. Chaware VJ. Protective effect of the aqueous extract of phaseolus radiates seeds on gentamicin induced nephrotoxicity in rats. Int J Res Pharm Biomed Sci. 2012; 3: 73-5.
51. Chen XW, Plasmag ES, Sneed KB, Zhou SF. Herbal bioactivation molecular targets and the toxicity relevance. Chem Biol Interact. 2011; 15: 161-76.

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Received 8th Jan. 2015: Accepted 10th Jun. 2015