EFFECT OF CORN SILK EXTRACT ON ACETAMINOPHEN INDUCED RENAL DAMAGE IN MICE

Faiza Mehboob, Mohammad Tahir
University of Health Sciences Lahore

ABSTRACT

Objective: To evaluate the protective role of Corn Silk extract on Acetaminophen induced nephrotoxicity in albino mice.

Study Design: Laboratory based randomized controlled trials.

Place and Duration of Study: The study was carried out in experimental research laboratory University of Health Sciences and Anatomy department, Lahore. The study duration was one year from February 2012 to February 2013.

Material and Methods: Twenty seven male albino mice, 6-8 weeks old weighing 30 ± 5 gm, were used; these animals were randomly divided into three groups having nine mice in each group. Group A served as control and was given 16.6ml/kg normal saline intraperitoneally on first day of experiment and was sacrificed on 10th day of the experiment. Group B was treated with acetaminophen 600 mg/kg dissolved in 16.6 ml of normal saline intraperitoneally on 1st day of experiment and was sacrificed after 48 hours. Group C was given acetaminophen at a dose of 600 mg/kg intraperitoneally on first day of experiment and then corn silk extract was given by oral route at a dose of 400 mg/kg for next 8 days. The animals were sacrificed on 10th day of the experiment, the kidneys were removed; 3mm three tissue pieces were fixed in 10% formaline; processed and stained with H&E for histological study.

Results: It was observed on microscopic examination that Corn silk extract reduced deleterious effects of acetaminophen on tubules of kidney as evidenced by reduction of tubular vacuolation and necrosis, absence of protein casts, vascular congestion and inflammation.

Conclusion: It is concluded from current results that corn silk extract protects acetaminophen induced nephrotoxicity.

Keywords: Acetaminophen, Corn silk extract, Mice, Nephrotoxicity.

INTRODUCTION

Acetaminophen, commonly known as Paracetamol, is one of the widely used over the counter drug with potent antipyretic and analgesic effects. It is the drug of choice for management of pain, fever and osteoarthritis[1].

Acetaminophen inhibits prostaglandin synthesis by inhibiting cycloxygenases and exerts its antipyretic and analgesic effects[2]. It passes through first phase of metabolism in liver. A small fraction is oxidized by cytochrome P- 450 system resulting in production of highly reactive toxic metabolite, N- acetyl-p-benzo-quinine imine (NAPQI), which normally reacts with sulfhydryl groups in glutathione and is thereby rendered harmless[3].

Different products are used to treat acetaminophen induced nephrotoxicity such as Garlic oil[4], Propylthiouracil[5], Harungana madagascariensis[6] and Phyllantusamarus[7], these works were mainly focused on the antioxidant properties of the remedies mentioned to combat the toxic effects of acetaminophen.

Corn silk comprises the long silky threads which are the stigma and styles of the maize plant that cover the corn[8].The major constituents of corn silk are amines, fixed oils, saponins, pigments, resin, flavonoids, alkaloids, tannins, chlorogenic acid, phytosterols, allantoin, vitamin C, E and K[9]. Corn silk is traditionally used as herbal medicine which has been used in different parts of the world for the management of odema, gout, inflammatory conditions of bladder, renal stones, nephritis, diabetes mallitus and prostatitis[10]. It is a mild stimulant, diuretic and demulcent. It is useful in acute and chronic cystitis, in bladder irritation of uric acid and in gonorrhea. Recent studies have indicated that corn silk can reduce

Correspondence: Dr Faiza Mehboob, University of Health Sciences, Lahore, Pakistan
Email: hi2faiza@hotmail.com
Received: 19 Sep 2013; Accepted: 03 Sep 2014

clotting time and blood pressure[11].

Flavonoids from corn silk (FCS) have been investigated and confirmed to possess various pharmacological activities such as antihypertensive, anti-inflammatory, anti-oxidative and anti- diabetic[12]. Nephroprotective effect of corn silk extract alongside Gentamicin induced nephrotoxicity has also been reported[13].

Damage to the kidney by acetaminophen is reported to be due to oxidative stress produced by free oxygen radicals and on promise that corn silk extract possesses strong anti oxidant properties, it is presumed that it could serve as a protective agent for acetaminophen induced nephrotoxicity. The present study were therefore designed accordingly.

MATERIAL AND METHODS

These laboratory based randomized controlled trials were conducted in experimental research laboratory, anatomy department of University of Health Sciences Lahore from Feb 2012 to Feb 2013.

Dose of acetaminophen was based on previous studies[14] i.e., single dose of 600mg/kg intraperitoneally. Acetaminophen was obtained from Merck Pharmaceuticals in a powdered form. Solution of acetaminophen was prepared by dissolving 600 mg of acetaminophen in 16.6ml of 0.9% normal saline; thus each ml of solution would contain 36 mg of acetaminophen.

Corn silk extract was prepared at PCSIR Laboratory, Lahore. 100 gm of corn silk powder was swamped in 1000 ml of 80% methanol for 72 hours and the extract was made by a rotating evaporator. Prepared extract was collected, weighed and stored in refrigerator till use[13].

Dose of corn silk extract was calculated 400 mg/kg/day according to formula for dose translation[13].

Stock solution of the extract was prepared by dissolving 2000 mg of extract in 50 ml of distilled water; thus 40 mg of extract was present in one ml of solution.

Twenty seven male albino mice, 6-8 weeks old, weighing 30 ± 5gm were procured from Veterinary Research Institute, Lahore. Only healthy animals were included, weighed and housed in steel cages of appropriate size. Animals were kept under controlled environment having room temperature of 22 ± 3˚C, humidity (50% ± 10) and light and dark cycles of 12 hours each. Animals were fed on standard rodent diet and tap water ad libitum. They were allowed to acclimatize for a period of one week before starting the experiment. The animals were divided into three groups using random number table, each groups having nine mice.

The mice of group A were given 16.6 ml/kg of 0.9% normal saline solution intraperitoneally on first day of experiment and were sacrificed under anesthesia on the 10th day. The mice of groups B & C were given Acetaminophen at a dose of 600 mg/kg dissolved in 16.6 ml normal saline intraperitoneally, on first day of experiment; Group B mice were sacrificed after 48 hours while the Group C mice were given 400 mg/kg of corn silk extract orally which was dissolved in 10ml of distilled water, for next 8 days and were sacrificed on the 10th day.

All the animals were dissected and sacrificed under chloroform anesthesia. The kidneys were removed and sliced into small pieces (3mm), fixed in 10% neutral formaline for 48 hours, dehydrated in rising grades of alcohol, washed in xylene and embedded in molten paraffin wax of melting point 56^{0}C-58^{0}C. Paraffin blocks were prepared, solidified, refrigerated and fixed in the chuk of rotator microtome. 5µm thick sections were obtained and stained with haematoxylin and eosin (H&E) in a usual way and examined under light microscope.

The tubules were recorded as normal (N), mild tubular necrosis (+), moderate (++) and severe (+++). The damage was considered as mild (+), when the tubules were sparsely damaged, involving less the quarter of the total number of proximal tubules (< 25%); moderate (++), when involvement of the tubules was from 26-50%, and severe (+++) when more than half of the tubules showed necrosis and

desqumation (>50%). Intraluminal protein casts were also similarly graded[15].

In group B, Acetaminophen exposure for 48 hours showed degenerative changes

Table-1: Shows comparison of histological parameters between the groups.

		Group A n = 9	Group B n = 9	Group C n = 9	*p*-value
Degree of Tubular Necrosis	Absent	9 (100%)	0 (0.0%)	6 (66.7%)	
	Mild <25%	0 (0.0%)	2 (22.2%)	3(33.3%)	
	Moderate 26-50%	0 (0.0%)	7 (77.8%)	0 (0.0%)	<0.001
Degree of Tubular Vacuolation	Absent	9 (100%)	0 (0.0%)	2 (22.2%)	
	Mild <25%	0 (0.0%)	6 (66.7%)	7 (77.8%)	
	Moderate 26-50%	0 (0.0%)	3 (33.3%)	0 (0.0%)	<0.001
Degree of Tubular Casts	Absent	9 (100%)	0 (0.0%)	9 (100%)	
	Mild <25%	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Moderate 26-50%	0 (0.0%)	7 (77.8%)	0 (0.0%)	
	Severe >50%	0 (0.0%)	2 (22.2%)	0 (0.0%)	<0.001
Degree of Interstitial Inflammation	Absent	9 (100%)	1(11.1%)	9 (55.6%)	
	Mild <25%	0 (0.0%)	1(11.1%)	0 (0.0%)	
	Moderate 26-50%	0 (0.0%)	5 (55.6%)	0 (0.0%)	
	Severe >50%	0 (0.0%)	2 (22.2%)	0 (0.0%)	<0.001
Percentage of Vascular congestion	Absent	8 (88.9%)	0 (0.0%)	9 (100%)	
	Mild<25%	1(11.1%)	2 (22.2%)	0 (0.0%)	
	Moderate 26-50%	0 (0.0%)	5 (55.6%)	0 (0.0%)	
	Severe >50%	0 (0.0%)	2 (22.2%)	0 (0.0%)	<0.001

Data had been analyzed by SPSS (statistical package for social Sciences) version 18.0. Descriptive statistics were used to describe the results. To compare the morphological features between the groups, Chi-square/Fisher Exact test was applied. A *p*-value of <0.05 was considered as significant.

RESULTS

Examination of the cortex of the kidneys from group A showed renal corpuscles which looked like rounded structures containing glomeruli, surrounded by double layered Bowman's capsule lined by squamous epithelial cells with flattened nuclei. The cortical tubules mainly consisted of proximal and distal convoluted tubules together with collecting tubules. Proximal convoluted tubules were lined by simple cuboidal epithelium with prominent brush border and distal convoluted tubules were lined by simple cuboidal epithelium with relatively larger lumen.

mutually in proximal convoluted tubules

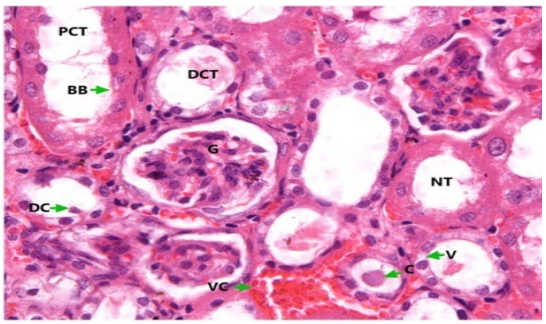

Figure-I: Photomicrograph of group B showing glomerulus (G) and degenerative necrotic tubules (NT). Proximal convulated tubule (PCT) having loss of the brush border (BB). Distal convoluted tubules (DCT) showing desquamating cell (DC), vacuolation (V) and cast (C). Vascular congestion (VC) is very prominent. H&E stain x400.

(PCTs) and distal convoluted tubules (DCTs); tubular necrosis with fractional loss of brush

border of proximal convoluted tubules was observed. Vacuoles were seen in the cells of both proximal and distal convoluted tubules with presence of cellular debris in their lumens. Basement membrane of the tubules was intact in all the animals of group B. Vascular congestion and inflammation were evident in all animals and so were protein casts in the lumen of DCT, ascending thick limb of loop of Henle and collecting ducts (Fig-1).

In group C there was no evidence of vascular congestion, inflammation and protein casts but some of the tubules exhibited desquamation of epithelial cells. Some tubules also showed areas of patchy necrosis with loss of brush border and vacuolation implying thereby that tubular injury was not in fact fully reversed (Fig-2).

Significant difference was observed between groups in percentages of tubular necrosis (p<0.001), tubular vacuolation (p <0.001), tubular casts (p<0.001), interstitial inflammation (p<0.001) and vascular congestion (p <0.001) between the groups (Table-1).

DISCUSSION

In the current study it was observed that there was statistically significant tubular necrosis and vacuolation of epithelial cell in the animals of group B when compared with the animals of groups A and C. This showed that toxic dose of acetaminophen was responsible for tubular damage especially in proximal convoluted tubules after 48 hours. Roberts[16] in 2008 stated that cytoplasmic granules are the first to show the evidence of degeneration process which is followed by the vacuolation. Results of current study is in accord with those reported earlier by Refaat and Mady[17] who observed proximal tubular distortion and vacuolization after single intraperitoneal dose of 1000mg/kg of acetaminophen in rats.

In present study, loss of brush border was observed in animals of group B. This loss of brush border was statistically significant among the groups. This result is in accord with the Khorsandi and Orazizadeh[18] who observed loss of brush border in proximal convoluted tubules after single oral dose of 500 mg/kg of acetaminophen in mouse. Brush border did not completely recover in group C as four animals of this group showed mild brush border loss. This result is comparable to that reported by Sepehri et al[13] who stated that brush border did not completely repair by methanolic extract of corn silk when it was given along with gentamicin intraperitoneal injections.

Current study showed that luminal cast was present in distal convoluted tubules of group B. Gulnaz et al4 also reported many protein casts in distal convoluted tubules and in thick ascending limb of loop of Henle after single intraperitoneal dose of 1000 mg/kg acetaminophen. There was no protein cast in animals of group C of current study which showed that treatment with corn silk extract has improved the deleterious effects of acetaminophen on renal tubules. Present study also showed that there was marked interstitial vascular congestion and inflammation (lymphocytic infiltration) in the group treated with acetaminophen. Li et al[14] also reported widespread renal interstitial congestion after single intraperitoneal dose of 600 mg/kg of acetaminophen to mouse. In current study, interstitial congestion and inflammation was not seen in mice of groups A and C.

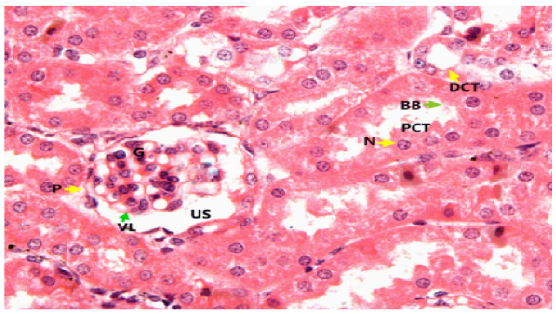

Figure-2: Photomicrograph of group C showing normal glomerulus (G) and Bowman's capsule comprising of parietal (P) and visceral (VL) layers separated by a urinary space (US).Proximal convoluted tubule (PCT) with partial loss of brush border (BB) but with normal nucleus (N). Distal convoluted tubules (DCT) with no protein casts. No vascular congestion and inflammation is observed in this group. H&E stain x400.

Extracts of corn silk and corn silk have antioxidant activities. This antioxidant activity is stated to provide protection from the injury produced by free radicals[19]. Corn silk extract has recovered the interstitial congestion and inflammation in group C. The exact mechanism is unknown but it is probably because antioxidant causes vasodilatation hence it may reduce the toxicant induced congestion and renal injury[20].

Antioxidant, antinflammatory and free-radical scavenger activity of corn silk explains its nephroprotective role[12]. Antioxidants present in corn silk extract are volatile oxygen containing chemicals, such as R-terpineol, eugenol and citronellol[21]. The free radical scavenging ability of these extracts is due to the existence of phenolic compounds for example eugenol, carvacrol and thymol, along with some polyphenols and flavonoids[22].

Present study revealed that corn silk extract administration ameliorated histopathological lesions induced by acetaminophen, vascular congestion and inflammation to almost normal, reduced deleterious effects of acetaminophen on tubules and caused noticeably decreased vacuolation. This indicates that considerable protection is provided by corn silk extract to acetaminophen nephrotoxicity.

CONCLUSION

On basis of our observations that administration of a single dose of 600mg/kg body weight of acetaminophen to mice resulted in tubular necrosis, cast formation, inflammation and congestion which disappeared upon treating the mice with corn silk extract with a dose of 400 mg/kg body weight for next 8 days, clearly indicating that corn silk protects renal injury induced by acetaminophen. Further investigations are required to explore the mechanism of action of corn silk extract and may possibly have a significant impact on future clinical treatments of patients with renal malfunction.

Acknowledgment

I would like to thank the Vice Chancellor of University of health Sciences Lahore for his moral help and providing funds for the research. I am also grateful to the Laboratory staff for their unhesitating help to support my work.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Stockler M, Vardy J, Pillai A, Warr D. Acetaminophen (Paracetamol) Improves Pain and Well-Being in People With Advanced Cancer Already Receiving a Strong Opioid Regimen. J Clin Oncol 2004; 22(16): 3389-3394.
2. Hinz B, Cheremina O, Brune K. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. The FASEB J 2008; 22 (2): 383–390.
3. Katzung BG. Non Steroidal anti-inflammatory Drugs. In: Wagner W, Khanna P, Furst DE, eds. Basic and clinical pharmacology. 10th ed. USA: Mc Graw Hill; 2007. p. 591.
4. Gulnaz H, Tahir M, Munir B, Sami W. Protective effects of garlic oil on acetaminophen induced nephrotoxicity in male albino rats. Biomedica 2010; 26: 9-15.
5. Abraham P, Kanakasabapathy I, Dian BJ. Propylthiouracil attenuates acetaminophen-induced renal damage in the rat. Nephrology 2005; 10: 588–593.
6. Adeneye AA, Olagunju JA, Benebo AS, Elias SO, Adisa AO, Idowu BO, et al. Nephroprotective effects of the aqueous root extract of Harungana madagascariensis (L.) in acute and repeated dose acetaminophen renal injured rats. IJARNP 2008; 1(1): 6-14.
7. Adeneye AA, Benebo AS. Protective effect of the aqueous leaf and seed extract of Phyllanthus amarus on gentamicin and acetaminophen-induced nephrotoxic rats. J Ethnopharmacol 2008; 118: 318–323.
8. BioWeb Home. Zea Mays-sweet Corn. [Internet]. [Cited 2012 oct 05]. Available from: bioweb.uwlax.edu/bio203/s2008/Knudsen_ashl/index.htm.
9. Bushman BS, Snook ME, Gerke JP, Szalma SJ, Berhow MA, Houchins KE, et al. The genetic basis of chlorogenic acid synthesis in maize. Crop Sci 2002; 42: 1669–1678.
10. Hu Q, Deng Z. Protective effects of flavonoids from corn silk on oxidative stress induced by exhaustive exercise in mice. Afr J Biotechnol 2011; 10(16): 3163-3167.
11. Rosli W, Nurhanan WI, Mohsin AR, Farid SSJ, Ghazali C. Aqueous, Alcoholic Treated and Proximate Analysis of Maydis stigma (Zea mays Hairs). Annals Of Microscopy, 2008; 8: 66-72.
12. Liu J, Wang CN, Wang ZZ, Zhang C, Lu S, Liu JB. The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (Zea mays L.) and related flavone glycosides. Food Chem 2011; 126: 261-269.
13. Sepehri G, Amen D, Farnaz YZ. Protective effects of corn silk extract administration on Gentamicin induced nephrotoxicity in rats. Comp Clin Pathol 2011; 20: 89-94.
14. Li C, Liu J, Saavedra JE, Keefer KL, Waalkes MP. The nitric oxide donor, V-PYRRO/NO, protects against acetaminophen-induced nephrotoxicity in mice. Toxicology 2003; 189: 173-180.
15. Erdem A, Gundogan NU, Usubutun A, Kilinc K, Erdem SR. Kara A, et al. The protective effect of taurine against gentamicin induced acute tubular necrosis in rats. Nephron Dial Transplant 2000; 15: 1175-1182.
16. Roberts, G. Gentamicin (WHO food additive series 34). [Internet] 2008. [Cited on 2012 December 30] Available from: URL: //www.inchem.org docouments/jecfa/jwcmono/v34jeo6.htm.
17. Refaat SH, Mady AA. Vitamin A against the acetaminophen- induced toxicity in the renal cortex of albino rats. Egypt J Histol 2008; 31: 321 - 331.
18. Khorsandi L, Orazizadeh M. Protective effect of Curcuma longa extract on acetaminophen induced nephrotoxicity in mice. Daru 2008; 16: 155-159.
19. Eman, Alam A. Evaluation of antioxidant and antibacterial activities of Egyptian Maydis stigma (Zea mays hairs) rich in some bioactive constituents. Am J Sci 2011; 7: 726-729.

20. Cameron, N.E, Cotter M.A. Effect of antioxidant on nerve and vascular dysfunction in experimental diabetes. Diabetes Research and Clinical Practice 1999; 45: 137-146.
21. El-Ghorab, El-Massry KF, Shibamoto T. Chemical composition of the volatile extract and antioxidant activities of the volatile and nonvolatile extracts of Egyptian corn silk (Zea mays L.). J Agric Food Chem 2007; 55: 9124 – 9127.
22. Maksimovic Z, Malencic D, Kovacevic N. Polyphenol contents and antioxidant activity of maydis stigma extracts. Bioresource Technology 2005; 96: 873-877.

.....