Protective effects of *Ficus racemosa* stem bark against doxorubucin-induced renal and testicular toxicity

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ABSTRACT

Background: *Ficus racemosa* Linn. (Moraceae) bark is a rich source of phenolic compounds known to possess potential antioxidant activity offering numerous health benefits. **Materials and Methods:** The present study evaluated the protective effects of sequential acetone extract of *Ficus racemosa* bark at two doses (FR_{250}; 250 mg kg^{-1} and FR_{500}; 500 mg kg^{-1} p.o.) against doxorubicin-induced renal and testicular toxicity in rats. **Results:** Doxorubicin administration resulted in significant decrease ($P \leq 0.05$) in total protein and glutathione concentrations, while increased ($P \leq 0.05$) serum urea, creatinine and thiobarbituric acid reactive substances (TBARS). Extract pretreatment restored biochemical parameters toward normalization. FR_{250} and FR_{500} decreased serum creatinine levels by 22.5% and 44%, while serum urea levels were decreased by 30.4% and 58.8%, respectively. Extract pretreatment (500 mg kg^{-1}) decreased TBARS and increased glutathione levels in the kidney and testis to control levels. These observations were substantiated by histopathological studies, wherein normal renal and testicular architecture was restored in FR500 group. **Conclusion:** Doxorubicin exposure results in pronounced oxidative stress, and administration of *F. racemosa* stem bark extract offers significant renal and testicular protection by inhibiting lipidperoxidation-mediated through scavenging free radicals.

Key words: Doxorubicin, glutathione, histopathology, oxidative stress, thiobarbituric acid reactive substances

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INTRODUCTION

Doxorubicin (Dox) is one of the most potent broad spectrum antitumor anthracycline antibiotic, widely used to treat variety of cancers, including severe leukemias, lymphomas, and solid tumors.[1] The clinical use of Dox is restricted because of its serious toxicity on various organs viz., heart, liver, lung, kidney, and testis. Doxorubicin administration is known to induce chronic progressive glomerular disease and also known to disturb spermatogenesis in a dose-dependent manner in animal studies.[2-4] Although, the precise mechanism is unclear,[5] production of free radicals as a byproduct of its metabolism is considered to be the primary mechanism of Dox toxicity, consequently warranting some new approaches, such as the potential use of natural antioxidants. The most commonly used and investigated antioxidant compounds against Dox toxicity are vitamins (E, C, A, carotenoids), coenzyme Q, flavonoids, polyphenols, herbal antioxidants, selenium, and virgin olive oil.[6]

Ficus racemosa Linn. (Moraceae) commonly known as 'Gular' is found throughout greater part of India in moist localities and widely used in the treatment of various diseases/disorders including jaundice, dysentery, diabetes, diarrhea and inflammatory conditions.[7] *F. racemosa* stem bark is a rich source of phenolic compounds and possess excellent antioxidant properties *in vitro*, *ex vivo*[8] and *in vivo* in streptozotocin-induced diabetic rats.[9] The bark has also shown to possess antidiabetic, antibacterial, anticholinesterase, acetylcholine enhancing and angiotensin converting enzyme inhibitory activities.[10-13] We have also reported *F. racemosa* bark extract to exhibit potential hepatoprotective activity against CCl_4-induced hepatotoxicity and cardioprotective effect against doxorubucin-induced cardiotoxicity in rats.[14,15] In view of this, the present study evaluated the protective effects of standardized extract of *F. racemosa* stem bark against doxorubicin-induced renal and testicular toxicity in albino rats.

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MATERIALS AND METHODS

Chemicals and reagents

Doxorubicin and 5,5-dithio (bis) nitro benzoic acid (DTNB) were purchased from Sigma Aldrich, Bangalore, India. All the other chemicals and reagents used in the study were of extra pure analytical grade.

Collection of plant material and preparation of the extracts

F. racemosa stem bark was identified by Dr. Shivprasad Hudeda and the voucher specimen (BOT-001/2008) was deposited at the herbarium of Botany Department, University of Mysore, Mysore, India. The bark was cut into small pieces, dried (50°C) and powdered, passed through 60 mesh sieve (BS) and stored in an air tight container at 4°C till further use.

The bark powder was extracted sequentially with solvents of increasing polarity (petroleum ether - chloroform - acetone - methanol - water) in a soxhlet apparatus for 8 h each. Among all these extracts acetone extract (FRSACE) containing highest amount of phenolic compounds[8] was selected for the *in vivo* study. Earlier, we have reported the extract to have LD_{50} value of >2 g kg^{-1} and contain bergenin and bergapten as major components.[15]

Animals

Healthy male Wistar rats between 8 and 9 weeks of age and weighing 140 and 160 g were divided into following 4 groups ($n = 6$).

- Group I: Control group, received distilled water (1 mL kg^{-1} BW, p.o.) for 9 days followed by sterile water for injection (1 mL kg^{-1} BW, i.v.) on 10^{th} day.
- Group II: Untreated group, received distilled water (1 mL kg^{-1} BW, p.o.) for 9 days followed by a single dose of Dox injection (10 mg kg^{-1} BW, i.v.) on 10^{th} day.
- Group III: FRSACE group (250 mg kg^{-1} BW, p.o.) for 9 days followed by a single dose of Dox injection (10 mg kg^{-1} BW, i.v.) on 10^{th} day.
- Group IV: FRSACE group (500 mg kg^{-1} BW, p.o.) for 9 days followed by a single dose of Dox injection (10 mg kg^{-1} BW, i.v.) on 10^{th} day.

The rats were housed in polyacrylic cages and maintained at 27 ± 2°C, 45-60% RH and 12 h photo period and provided with standard pellet diet (Amrut feeds, Pune, India) and water *ad libitum*. All animal procedures have been approved by the Animal Ethical Committee of University of Mysore in accordance with animal experimentation and care. After 48 hours of the injection of either Dox or vehicle, the animals were starved overnight (to minimize metabolic variations), euthanized, blood was collected by direct cardiac puncture and used for serum separation. Kidneys and testis were immediately excised. A portion of these organs were homogenized (1:5 w/v) in phosphate-buffered saline (pH 7.4) for estimation of TBARS and GSH while, the other portions were fixed in 10% formalin for histopathological studies.

Biochemical parameters

Total protein and albumin levels were determined in serum using diagnostic kits and urine proteins were detected using urine protein strips. Serum urea and creatinine were determined as markers of kidney function. The contents of glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) in kidneys and testis were determined as markers of oxidative stress according to the methods of Ellman[16] and Ohkawa *et al*.,[17] respectively.

Histopathological procedures

Various organs fixed in 10% formalin were dehydrated in graduated ethanol (50-100%), cleared in xylene and embedded in paraffin. The sections (4-5 µm) were then examined with a photomicroscope (Leica DM LS2, Switzerland) after staining with haematoxylin and eosin (H-E) dye. The morphological changes included cell necrosis, mononuclear infiltration, vacuolation, and degenerative changes.

Statistical analysis

All analyses were carried out in triplicates. Data were presented as mean ± standard deviation (SD). Statistical analyses were performed by one-way ANOVA followed by Tukey's multiple comparisons test for significant differences using SPSS 14.0 software. The values were considered significant at $P \leq 0.05$. The graphs were plotted using Origin 6.1 software.

RESULTS AND DISCUSSION

Doxorubicin is a powerful anthracycline antibiotic used to treat a multitude of human neoplasms whose use in clinical chemotherapy is limited due to diverse toxicities, including renal and testicular toxicity.[3,18-21] The major pathogenic mechanism for its toxicity appears to involve the generation of reactive oxygen species. A number of natural and synthetic compounds are known to alleviate Dox-induced toxicity,[22-26] of which polyphenols possessing significant free radical scavenging activity are considered important.[27] A number of animal models including mice, rats, dogs, swine, hamsters, and rabbits have been used for studying Dox-induced organotoxicity.[18]

In the present study, the protective effects of sequential acetone extract of *F. racemosa* bark (250 and 500 mg kg^{-1}) was studied against doxorubicin-induced renal and testicular toxicity in rats. Administration of Dox, significantly decreased ($P \leq 0.05$) total protein, albumin and A/G ratio which might

be ascribed to the hepatic damage caused by doxorubicin, as reports indicate that liver damage causes decreased amino acid uptake or hepatic protein synthesis.[28] However, extract pretreatment significantly restored serum proteins towards normalization. FR_{500} exhibited significantly higher ($P \leq 0.05$) protein restoration effect than FR_{250} [Figure 1].

Dox is a known nephrotoxic substance and produces chronic progressive glomerular disease manifested by increased plasma creatinine and urea levels associated with extensive glomerular lesions, tubular dilatation, vacuolization of renal glomeruli, protein deposits in tubular lumen and stromal fibrosis.[4,25] In the present study, serum urea and creatinine levels of control and FR_{500} pretreated groups were comparable and significantly lower than those of FR_{250} and Dox groups [Table 1]. Further, no proteinurea was observed in control and FR_{500} group. Moderate proteinurea was observed in FR_{250} group, while severe proteinurea was found in Dox group. These findings were further supported by the histopathological profiles of the kidneys. The sections from control group showed normal renal tubules associated with normal glomerulus. However, in Dox-treated group focal tubular and glomerular damage leading to shrinkage of the glomerulus was observed. The sections extract pretreated groups showed no tubular damage; however, slight shrinkage of glomerulus was observed [Figure 2]. These observations are in good agreement with earlier reports.[25,29,30]

Table 1: Effect of FRSACE on serum urea and creatinine levels

Group	Creatinine (mg dL^{-1})	Urea (mg dL^{-1})
Control	$0.56^{a} \pm 0.02$	$26.6^{a} \pm 5.31$
FR_{250}	$0.86^{b} \pm 0.17$	$42.1^{b} \pm 3.80$
FR_{500}	$0.62^{a} \pm 0.14$	$27.3^{a} \pm 4.01$
Dox	$1.11^{c} \pm 0.09$	$66.2^{c} \pm 3.09$

[a]Data expressed as mean ± SD of $n = 6$ rats ($P \leq 0.05$), [b]FR_{250} = FRSACE 250 mg kg^{-1}, FR_{250} = FRSACE 500 mg kg^{-1}, Dox = doxorubicin, [c]Values carrying different superscripts a, b, c¼. in columns differ significantly from each other ($P \leq 0.05$)

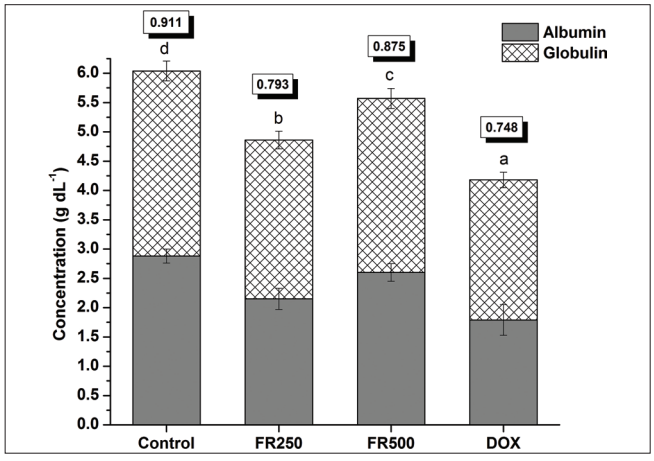

Figure 1: Effect of FRSACE on serum albumin, globulin and A/G ratio. Data expressed as mean ± SD of $n = 6$ rats ($P \leq 0.05$). Bars carrying different superscripts a, b, c¼. differ significantly from each other ($P \leq 0.05$). Values above each bar represents A/G ratio

Dox is also known to disturb spermatogenesis leading to low testicular sperm count and associated with increased ROS production.[3] The histopathological section of the control group showed normal seminiferous tubules with good number of sperms, while complete inhibition of spermatogenesis was seen in Dox-treated group, wherein severe damage to the seminiferous tubules with vacuolation and necrosis of the lining epithelial cells and damaged basement membrane resulting in the loss of architecture and spermatogenesis was observed. In FR_{500} pre-treated group the seminiferous tubules showed normal testicular architecture with sperms. In FR_{250} pretreated group, although, spermatogenesis was restored toward normalization, the number of sperms was significantly lower compared to FR_{500} group. These sections also showed vacuolation of the lining epithelium [Figure 3]. The observations are consistent with an earlier report, wherein green tea extract rich in polyphenolic compounds reversed Dox-induced pathological, biochemical changes, histology and lipid peroxidation in rats.[27] The testicular protective activity of FRSACE could be due to the presence of gallic acid and ellagic acid reported to exhibit significant protection against Dox-induced testicular toxicity.[27]

Oxidative stress was assessed by the levels of glutathione and thiobarbituric acid reactive substances (TBARS) in kidney and testis homogenates. FR_{250} and FR_{500} pretreatment significantly decreased ($P \leq 0.05$) lipid peroxidation induced by Dox as reflected by lower TBARS and higher GSH values [Figures 4 and 5]. It is noteworthy

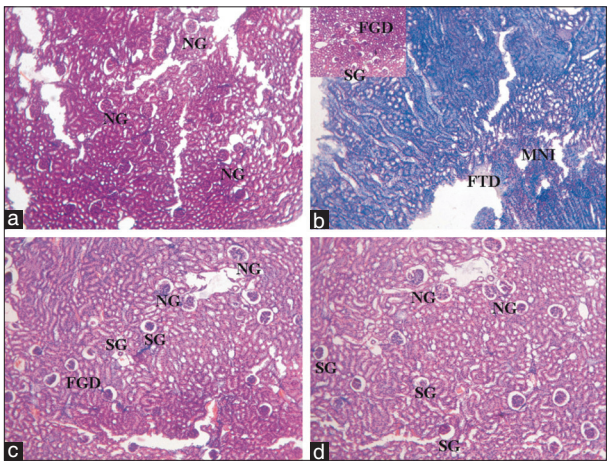

Figure 2: (a) Section of the kidney of control rats showing normal glomeruli. (b) Section of the kidney of untreated rats showing focal glomerular and tubular damage with mononuclear infiltrate. (c) Section of the kidney of FRSACE-treated rats (250 mg kg^{-1}) showing shrunken glomeruli. (d) Section of the kidney of FRSACE-treated rats (500 mg kg^{-1}) showing normal glomeruli *NG = normal glomerulus, SG = shrunken glomerulus, FGD = focal glomerular damage, FTD = focal tubular damage, MNI = mononuclear infiltrate

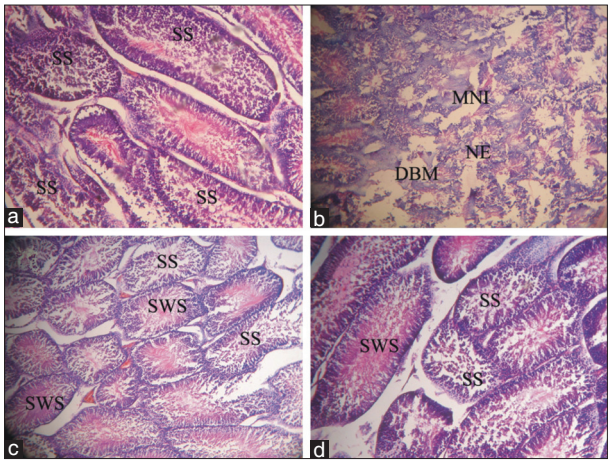

Figure 3: (a) Section of the testis of control rats showing normal seminiferous tubules containing sperms, (b) Section of the testis of untreated rats showing damage to the seminiferous tubules with damaged basement membrane and necrosed lining epithelium, (c) Section of the testis of FRSACE-treated rats (250 mg kg^{-1}) showing a few sperms, (d) Section of the testis of FRSACE-treated rats (500 mg kg^{-1}) showing more number of normal seminiferous tubules containing sperms *VC: vacuolation of the lining epithelium cells, SS: seminiferous tubules containing sperms, SWS: seminiferous tubules without sperms, DBM: damaged basement membrane, NE: necrosed epithelial cells, MNI: mononuclear infiltrate

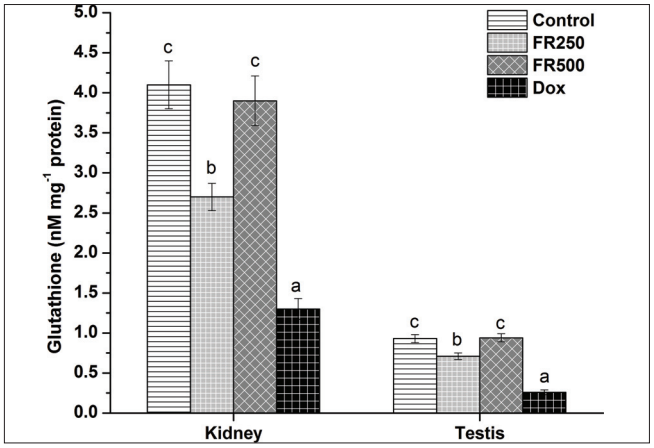

Figure 5: Glutathione levels in kidney and testis. Data expressed as mean ± SD of $n = 6$ rats ($P \leq 0.05$)

that, FR_{500} completely reversed oxidative stress to normal levels which could be attributed to the presence of various phenolic compounds and flavonoids such as quercetin, gallic acid, ellagic acid and trepenoidslupeol, lupeol acetate and a-amyrin that are reported to act as strong antioxidant and anti-inflammatory agents.[31]

CONCLUSIONS

From the findings of the present study, it is inferred that doxorubicin exposure results in pronounced oxidative stress, and administration of *F. racemosa* stem bark extract offers renal and testicular protection through its antioxidant properties. Further, there is a need to identify and isolate

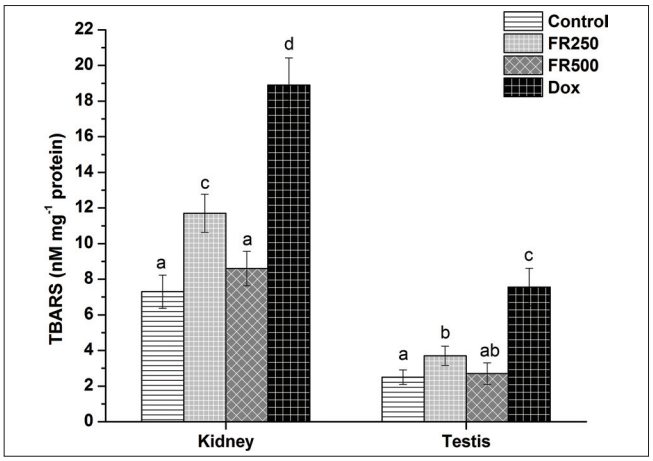

Figure 4: TBARS levels in kidney and testis. Data expressed as mean ± SD of $n = 6$ rats ($P \leq 0.05$)

the specific bioactive compound(s) from *F. racemosa* bark for its optimal utilization as a therapeutic agent to derive maximum benefits of doxorubicin as an anticancer drug by reducing its toxic effects considerably.

REFERENCES

1. Priestman T. Cancer chemotherapy in clinical practice. London: Springer-Verlag; 2008.
2. Ng R, Better N, Green MD. Anticancer agents and cardiotoxicity. Semin Oncol 2006;33:2-14.
3. Lui RC, Laregina MC, Herbold DR, Johnson FE. Testicular cytotoxicity of intravenous doxorubicin in rats. J Urol 1986;136: 940-3.
4. Injac R, Strukelj B. Recent advances in protection against doxorubicin-induced toxicity. Technol Cancer Res Treat 2008;7: 497-516.
5. Ratain MJ, Ewesuedo RB. Cancer chemotherapy. Berlin: Springer-Verlag; 1999. p. 76-83.
6. Quiles JL, Huertas JR, Battiono M, Mataix J, Ramírez-Tortosa MC. Antioxidant nutrients and adriamycin toxicity. Toxicology 2002;180:79-95.
7. Ananymous. The Wealth of India. Vol. 4. New Delhi: Council of Scientific and Industrial Research; 1952. p. 35-6.
8. Ahmed F, Urooj A. Antioxidant activity of various extracts of *Ficus racemosa* stem bark. National Journal of Life Sciences 2009;6:69-74.
9. Ahmed F, Urooj A. Glucose lowering, hepatoprotective and hypolipidemic activity of stem bark of *Ficus racemosa* in streptozotocin-induced diabetic rats. J Young Pharm 2009;1: 160-4.
10. Ahmed F, Hudeda S, Urooj A. Antihyperglycemic activity of *Ficusracemosa* bark extract in type II diabetic individuals. J Diabetes 2010;3:1-2.
11. Ahmed F, Sharanappa P, Urooj A. Antibacterial activities of various sequential extracts of *Ficusracemosa* stem bark. Pharmacognosy Journal 2010;2:203-6.
12. Ahmed F, Chandra JN, Manjunath S. Acetylcholine and memory enhancing activity of *Ficusracemosa* bark. Pharmacognosy Res 2011;3:246-9.
13. Ahmed F, Siddesha JM, Urooj A, Vishwanath BS. Radical scavenging and angiotensin converting enzyme inhibitory

activities of standardized extracts of *Ficusracemosa* stem bark. Phytother Res 2010;24:1839-43.
14. Ahmed F, Urooj A. Hepatoprotective effects of *Ficusracemosa* stem bark against carbon tetrachloride-induced hepatic damage in albino rats. Pharm Biol 2010;48:210-6.
15. Ahmed F, Urooj A. Cardioprotective activity of standardized extract of *Ficusracemosa* stem bark against doxorubicin-induced toxicity. Pharm Biol 2012;50:468-73.
16. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animals tissues by thiobarbituric reaction. Anal Biochem 1979;95:351-8.
18. Herman EH, Ferrans VJ. Animal models of anthracycline cardiotoxicity: Basic mechanisms and cardioprotective activity. Prog Pediatr Cardiol 1998;8:49-58.
19. Au WW, Hsu TC. The genotoxic effects of adriamycin in somatic and germinal cells of the mouse. Mutat Res 1980;79:351-61.
20. Sawada T, Tamada H, Mori J. Secretion of testosterone and epidermal growth factor in mice with oligozoospermia caused by doxorubicin hydrochloride. Andrologia 1994;26:151-3.
21. Imahie H, Adachi T, Nakagawa Y, Nagasaki T, Yamamura T, Hori M. Effects of adriamycin, an anticancer drug showing testicular toxicity, on fertility in male rats. J Toxicol Sci 1995;20: 183-93.
22. Goodman J, Hochstein P. Generation of free radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. Biochem Biophys Res Commun 1977;77:797-803.
23. Pritsos CA, Sokoloff M, Gustafson DL. PZ-51 (Ebselen) *in vivo* protection against adriamycin-induced mouse cardiac and hepatic lipid peroxidation and toxicity. Biochem Pharmacol 1992;44:839-41.
24. Shinozawa S, Gomita Y, Araki Y. Protective effects of various drugs on adriamycin (doxorubicin)-induced toxicity and microsomal lipid peroxidation in mice and rats. Biol Pharm Bull 1993;16:1114-7.
25. Mansour MA, El-Kashef HA, Al-Shabanah OA. Effect of captopril on doxorubicin-induced nephrotoxicity in normal rats. Pharmacol Res 1999;39:233-7.
26. Manabe F, Takeshima H, Akaza H. Protecting spermatogenesis from damage induced by doxorubicin using the luteinizing hormone-releasing hormone agonist leuprorelin: An image analysis study of a rat experimental model. Cancer 1997;79: 1014-21.
27. Patil LJ, Balaraman R. Green tea extract protects doxorubicin induced testicular damage in rats. Pharmacologyonline 2008;3: 913-25.
28. El-Shenawy NS, Abdel-Nabi IM. Hypoglycemic effect of *Cleome droserifolia* ethanolic leaf extract in experimental diabetes, and on non-enzymatic antioxidant, glycogen, thyroid hormone and insulin levels. Diabetologia Croatica 2006;35:15-22.
29. El-Shitany NA, El-Haggar S, El-desoky K. Silymarin prevents adriamycin-induced cardiotoxicity and nephrotoxicity in rats. Food Chem Toxicol 2008;46:2422-8.
30. Saad SY, Najjar TA, Al-Rikabi AC. The prevention role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. Pharmacol Res 2001;43:211-8.
31. Khan N, Sultana S. Chemomodulatory effect of *Ficusracemosa* extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats. Life Sci 2005;29: 1194-210.

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