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EFFECT OF *CASSIA AURICULATA* (CAESALPINIACEAE) FLOWERS ON GENTAMICIN-INDUCED NEPHROTOXICITY IN RATS

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for attainment of any degree or qualification.

I hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

While gentamicin is effective against severe gram-negative infections, its nephrotoxicity restricts clinical applications. This study was aimed at investigating effect of *Cassia auriculata* aqueous and polyphenolic extracts against gentamicin-induced nephrotoxicity. Polyphenolic extract was prepared by extracting flower aqueous extract with ethyl acetate (1:1 v/v). Effect of *C. auriculata* aqueous or polyphenolic extracts on cellular viability and H_2O_2-induced cell death on Vero cells were measured by (MTS) assay. To examine nephroprotective effect, male Sprague-Dawley rats were pretreated with different doses of *C. auriculata* aqueous (100, 300, 500 mg/kg/day, p.o.) or polyphenolic extracts (50, 100, 300 mg/kg/day, p.o.) for 2 weeks, followed by 8 days treatment with gentamicin (100 mg/kg, i.p.). To examine therapeutic effect, rats were injected with gentamicin (100 mg/kg, i.p.) for 8 days, followed by treatment with different doses of *C. auriculata* aqueous (100, 300, 500 mg/kg/day, p.o.) or polyphenolic extracts (50, 100, 300 mg/kg/day, p.o.) for 2 weeks. On day 22, rats were placed individually in metabolic cages and 24 h urine samples were collected. On day 23, rats were killed and blood was collected. Renal function was assessed by serum urea, creatinine, albumin, total protein, urine creatinine, urinary protein excretion and creatinine clearance ratio. Kidney was subjected to histopathologic examination. Expression levels of antioxidant genes (catalase, superoxide dismutase, glutathione peroxidase) and proinflammatory cytokines (IL-1β, TNF-α) were measured by RT-PCR. *In vitro* results indicated that pretreatment of Vero cells with both aqueous and polyphenolic extracts for 24 h significantly ameliorated the cytotoxicity of H_2O_2 in a concentration-dependent manner. Aqueous extract produced a maximal protection afforded by 50 μg/mL at which, cell viability was 67%. Polyphenolic extract showed higher protective effect as compared to aqueous extract with the maximum protection at 50-200 μg/mL with about 73% cell viability. *In vivo* results indicated that *C. auriculata* aqueous (300 mg/kg) and polyphenolic (100 mg/kg) extracts significantly reduced gentamicine-induced elevations in serum creatinine, serum urea and urine total protein. Gentamicin-induced decrease in serum total protein, serum albumin, urine creatinine, and creatinine clearance were restored. Histopathological evaluation of renal cortex of rats treated with *C. auriculata* aqueous or polyphenolic extracts showed protective and therapeutic effect against tubular necrosis, infiltration of inflammatory cells and thickening of basement membrane. Antioxidant genes were significantly up-regulated by *C. auriculata* aqueous or polyphenolic extracts. Gentamicin-induced excessive expression of proinflammmatory cytokines were normalized by *C. auriculata* aqueous and polyphenolic extracts. The findings suggest that *C. auriculata* aqueous (300 mg/kg) and polyphenolic (100 mg/kg) extracts possess nephronprotective and nephrocurative activities by enhancing renal antioxidant enzymes and inhibiting proinflammmatory cytokines.

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CHAPTER ONE
INTRODUCTION

1.1 OVERVIEW

Aminoglycosides are wide spectrum antibiotics that have been used against severe gram negative bacterial infections for many years. However, their clinical applications are limited due to the fact that these antibiotics have high potential of inducing nephrotoxicity in humans and have been associated with serious nephrotoxicity in 10 to 30% of patients receiving this class of antibiotics (Lin, 2011).

Gentamicin is an important aminoglycoside antibiotic, which was discovered in 1963. It was the first aminoglycoside isolated from *Micromonospora,* a bacterial source different from *Streptomyces*, from which streptomycin was isolated (Lopez-Novoa *et al.,* 2011). Gentamicin presented a significant advance in the treatment of gram negative and some gram positive bacterial infections. In many cases, gentamicin was the only effective therapeutic tool against multidrug resistant bacterial strains (Lin, 2011). At the present time, gentamicin is the most widely used aminoglycoside against life-threatening gram-negative bacterial infections. This is primarily because of its chemical stability, availability and low cost (Lopez-Novoa *et al.,* 2011). However, nephrotoxicity remains a major side effect of gentamicin. Although changing dosage regimens from multiple-daily to once-daily dose and close monitoring of serum drug concentration has reduced the risk of kidney damage, its clinical applications is limited due to its contribution in increased morbidity and prolonged hospital stay (Buijk *et al.*, 2002). Clinical studies have shown more than 30% of patients who received gentamicin for more than 7 days had developed signs of nephrotoxicity (Rahman *et al.,* 2009).

The toxicity of gentamicin results from two important factors. Firstly, due to accumulation of the drug in proximal tubular cells and secondly, due to interaction of gentamicin with cellular membranes and organelles (Rahman *et al.,* 2009). Since the kidney is the excretory route of aminoglycosides, renal tubular brush borders are exposed to higher concentration of gentamicin than the concentration found in serum.