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Analysis The Nephro Protective Activity of The Oleanolic Acid Isolated from The Medicinal Plant - *Cassia Auriculata (Linn.)*

KEYWORDS: Cassia auriculata, Methanol extract, oleanolic acid, nephro protective activity.

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ABSTRACT *The nephro protective activity was studied using male albino rats of Wistar strain by treatment with oleanolic acid. The treatment with oleanolic acid at 50 mg/kg was found to reduce the level of urea for (23.73 mg/dL) as well as creatinine (0.64 mg/dL). The results showed that the administration of oleanolic acid significantly decreased the levels of MDA, urea and creatinine in Gentamycin-induced rats.*

INTRODUCTION

Plants have been an essential part of human society since the start of civilization. Around 400 plants having therapeutic values were mentioned by Rig-Veda, Ayurveda, and Atharvana Veda (Rajasekara Pandiyan 2007). Nowadays, the development of resistance by a pathogen to many of the commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents (Ali-Shtayeh et al, 1998, Primo et al, 2001).

The leaves of Cassia auriculata family of Fabaceae, are used for ulcer, leprosy, jaundice, liver disease and skin diseases (Rajagopal et al, 2002, Rajagopal et al, 2003). This plant was widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine (Bhagwati et al. 2003). The nephroprotective activity of ethanolic and aqueous extracts of the stem bark of Bridelia retusa was carried out in carbon tertrachloride treated female mice (Cordeiro2011).

The protective property of 70% ethanol extract was assessed by measuring the levels of body weight, blood urea and serum creatinine and tissue glutathione and lipid peroxidation in administered doses. The extract exhibited free radical scavenging activity in dose dependant manner (Kumar et al, 2011). The nephrotoxicity associated with a limiting side-effect of the antineoplastic, cisplastin and the amino glycoside, antibiotic Gentamycin is due to the involvement of oxidative stress via free radical formation. Nephroprotective induced by metal viz. cadmium chloride and mercuric chloride in rat models, however, this fraction has been found to enhance Gentamycin nephroprotective (Alam et al, 2005). nephrotoxicity associated as a limiting side-effect of the antineoplastic, cisplastin and the amino glycoside. Similarly cyclodextrin sulphates, poly aspartic acids etc also have been found to partially reduce Gentamycin induced renal damage (Gilbert et al, 1989).

MATERIALS AND METHODS

Collection of plants

Healthy and well grown leaves of selected plant Cassia auriculata were collected from the area in and around Chidambaram, Cuddalore district, Tamilnadu, India. The leaves were immediately brought to the laboratory using separate polythene bags. They were washed with tap water.

Preparation of plant extract

Forty grams of the powdered leaves were loaded in Soxhlet apparatus and fractionated in 125 mL of Methanol solvent. The fraction was evaporated at rotary evaporator at 40°C (Vogel, 1978).

Separation of bioactive compound

After the conformation of the antimicrobial activity, 5 kg of air-dried and powdered plant material of Cassia auricula, was exhaustively extracted with methanol using Soxhlet apparatus. Removal of the solvent from the combined methanol extracts under reduced pressure at 40°C gave a residue (40 g). This residue was used for column chromatography to separate the bioactive principle was analyzed for the identification of the compound using spectral analysis namely IR, ^{1}H NMR, ^{13}C NMR and mass spectrum, conformed as oleanolic acid (Senthilkumar,2011).

Nephro protective activity of Oleanolic acid

Animals

Male albino rats of Wistar strain approximately weighing 125-150 g were used in this study. The animals were housed in spacious polypropylene cages bedded with rice husk. Animal House, Annamalai University, Chidambaram. (Ethical committee clearance no: 742/ 02.09.2010)

Experimental animals

Body weights of the animals were recorded and they were divided into 3 groups of 6 animals each as follows

Group 1:
Normal control rats fed with standard diet and received daily intraperitoneal injection of isotonic saline for 8 consecutive days.

Group 2:
Rats, received daily i.p. injection of Gentamycin (GM) (80 mg/kg) for 8 consecutive days.

Group 3:
Rats were treated with oleanolic acid orally (through Intragastric tube) at the dose of 50 mg/kg body weight for every day in addition to injection of Gentamycin for 8 consecutive days.

Estimation of Malondialdehyde

Malondialdehyde was estimated by the thiobarbituric acid assay method (Buege, 1978). The sample was added with 2.0 mL of TCA-TBA-HCl reagent and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. The flocculants centrifuged at 1000 rpm for 10 minutes. The absorbance of the sample was read at 535 nm.

Determination of reduced glutathione

Reduced glutathione was estimated (Moron, 1979). 0.5 mL of sample was precipitated with 1 mL of 10% TCA and the precipitate was removed by centrifugation. To 0.5 mL of the supernatant, 1 mL of DTNB was added and the total volume was made up to 3 mL with phosphate buffer. The absorbance was read at 412 nm. The level of glutathione was expressed as µg/mg protein; µg/dL serum.

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Estimation of ascorbic acid (Vitamin C)
The level of ascorbic acid was estimated (Omaye et al, 1979). To 0.5 mL of mitochondrial / plasma sample, 0.5 mL of water and 1 mL of TCA were added, mixed thoroughly and centrifuged. To 1 mL of the supernatant, 0.2 mL of DTC reagent was added and incubated at 37°C for 3 h. Then 1.5 mL of sulphuric acid was added, mixed well and the solutions were allowed to stand at room temperature for another 30 minutes. The colour developed was read at 520 nm.

Estimation of urea
The serum urea was estimated (Berthelot, 1959). About 0.1 mL of enzyme was mixed with 1.0 mL of buffered enzyme reagent and incubated at 5 minutes at 37°C after 1.0 mL of colouring reagent was mixed and incubated for 5 minutes at 37°C, at last 1.0 mL of distilled water was added and mixed well. The absorbance of sample against reagent blank was read at 600 nm. Values were expressed as mg/dL.

Estimation of creatinine
Serum creatinine was carried out by alkaline pirate method (Boneses, 1945). One mL of serum was diluted with 3 mL of distilled water and precipitated the protein by adding 2 mL of sodium tungstate and 2 mL of 2/3 N H_2SO_4 which was added drop wise with constant shaking and allowed to stand for two minutes and filtered it. Pipetted out 3 mL of protein free filtrate and add 1 mL of picric acid followed by 1 mL of NaOH. The colour intensity was read at 470 nm at 15 minutes.

Result
Significant rise observed in the level of MDA and decline the content of GSH in serum and kidney of group II Gentamycin treated rats as compared to group I control animals. Group III Gentamycin treated rats treated with oleanolic acid at 50 mg/kg. There was a significant reduction in the MDA level and improvement in the GSH content as compared with group II rats. The concentration of MDA was significantly higher in kidney of Gentamycin treated rats, as compared to normal control animal. These constituents were found to attain a near normal level in serum and kidney of Gentamycin and Oleanolic acid treated rats, conversely, vitamin C (Table.1).

Table 1. Effect of oleanolic acid on MDA and GSH in control and experimental rats

Parameters	Group I	Group II	Group III
Serum			
MDA (µmol of MDA formed/dL/serum)	2.56 ± 0.21	5.53 ± 0.51 [a*]	2.72 ± 0.19[a]
GSH (µg /dL)	12.38 ± 0.67	8.74 ± 0.43 [a*]	11.73 ± 0.61[a]
Kidney			
MDA (µmol of MDA formed/mg protein in tissues)	1.28 ± 0.24	8.28 ± 1.75 [a*]	1.36 ± 0.27[a]
GSH (µg /gm tissue)	7.36 ± 0.55	4.12 ± 0.30 [a*]	6.53 ± 0.42 [a]
Vitamin C (mg/gm tissue)	8.41 ± 0.63	4.24 ± 0.30 [a*]	7.84 ± 0.58 [a]

Values are expressed as mean ± SD for six rats. [*]as compared with group I normal rats (p< 0.001) [a]as compared with group II control rats (p< 0.001)

The Gentamycin treated group showed a significant rise in the serum urea as well ascreatinine levels (Table. 2).

Table2. Effect of oleanolic acid on urea and Creatinine in control and experimental rats

Parameters	Group I	Group II	Group III
Urea (mg/dL)	20.64 ± 1.51	106.47 ± 7.74 [a*]	23.73 ± 1.77[a]
Creatinine (mg/dL)	0.69 ± 0.012	1.00 ± 0.02 [a*]	0.64 ± 0.017[a]

Values are expressed as mean ± SD for six rats. [*]as compared with group I normal rats (p< 0.001)
[a]as compared with group II control rats (p< 0.001)

Discussion
In the present study, based on the antimicrobial properties of the medicinal plant, Cassia auriculata was found to be more effective against all the organisms tested, so it was selected for the further studies. The bioactive compound and was isolated and was identified as oleanolic acid by different spectral studies viz., IR, ^{1}H and ^{13}C NMR. The results demonstrated that there is an increase in renal cortical lipid peroxidation in GM-treated rats. In this context a marked increase in the concentration of serum and kidney MDA were observed in Gentamycin induced rats when compared to control rats (Ramasamy et al, 1986).

Creatinine is an end product of muscle catabolism, which is removed at a constant rate by the kidneys. The concentration of creatinine in serum is the most widely used and commonly accepted measure of renal function in clinical medicine (Perrone 1992, Mason,2004).

Conclusion
The studies strongly suggest that the administration of oleanolic acid of Cassia auriculata extract will have significant nephro protective activity in the in vivo condition which can be further studied in the human models.

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