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Research Article

Nephroprotective activities of antioxidants of Couroupita guianensis Aubl flowers extract against chloramphenicol induced nephrotoxihcity in Mice

DR. Laxman Landge[1], Prof. Ajit T. Kalse[2]

[1]Department of Biology, K. J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai, India.
[2]Zoology Research Laboratory, Department Of Zoology, Nanasaheb Y. N. Chavan, Arts, Science and Commerce, College Chalisgaon, Dist. Jalgaon, Maharashtra, India
[1]laxmanlandge2009@gmail.com

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Abstract: Development of remedies providing protection from chloramphenicol induced oxidative stress and nephron-toxicity would be the nice clinical significance. The present study was designed to investigate nephroprotective activities of antioxidants of *Couroupita guianensis* Aubl flowers extract on chloramphenicol induced nephrotoxicity in Mice. Twenty four Swiss Albino mice were divided into four equal groups as follows: I-Control group, II-Toxic group, Chloramphenicol 500 mg/kg body weight (oral); III- Extract group, 200 mg/kg body weight (oral) FE of CG; IV-Prophylactic group, Chloramphenicol 500 mg/kg (oral) + 200 mg/kg (oral) FE of CG. The treatments were administered once in a day for 14 days. It is followed by one day fasting to the mice. After 15 days biochemical and histopathological analysis were conducted to evaluate oxidative stress and nephrotoxicity. Serum level of blood urea nitrogen (BUN), creatinine, superoxide dismutase (SOD), catalase (CAT), total glutathione (GSH) and lipid peroxidase (LPO) were evaluated. The mice treated with chloramphenicol alone showed increase in BUN and LPO, while the level of creatinine, SOD, CAT and GSH were declined. The treatment of mice with FE of CG showed improvement of kidney function, as a result of decreased biochemical indices and oxidative stress parameters associated with chloramphenicol induced nephron-toxicity. The histopathological examination of mice kidneys showed slight improvement in the structure. Hence the natural antioxidants of FE of CG may protect the kidney against chloramphenicol induced nephro-toxicity and oxidative stress in mice. It is concluded that the antioxidants in the FE of CG has swietenine, sapropterin, usnic acid, lupeol and gamma tocopherol (vit. E) etc are useful to share the electron with free radicals in order to stop the role of free radicals to damage the kidney.

Keywords: *Couroupita guianensis* Aubl, Chloramphenicol, antioxidants, renal profile parameters, flower extract.

1. Introduction

As we know the entire world is overcoming the problem of the 'pandemic' disease "corona" due to changing the life style of the human being. The patients have to take antibiotics as the name of Azithromycin, Amoxycilin, Favipiravir (Fabi flu) in the heavy dose in order to recovery in the patients and stopped the spreading of disease, besides these one has to take NSAIDS drugs for antipyretic as well as to relief against the body pain. However all these medicinal drugs caused deposition of uric acids in the joints, diarrhoea, trouble in sleep, nausea, altered liver enzyme activities, etc.

However indiscriminate uses of antibiotics as well as chemotherapeutic drugs lead to health issues like nephron-toxicity like glomerulonephritis, inflammation of renal tubules, etc. The patients must take some antioxidants content medicines in order to recovered against disease as well as to be normalised the body organs as they are damaged their internal structure.

The research drug was Chloramphenicol that was discovered after being isolated from Streptomyces venezulae in 1947 (Pongs, O. 1979) It is an antibiotic which is useful for the treatment of various bacterial infections like eye ointment to treat conjuctivitis, used to treat meningitis, plague, cholera, thyphoid fever, etc.

Couroupita guianensis Aubl is an important medicinal plant. This study focused on evaluation of kidney protective function of *Couroupita guianensis* Aubl against chloramphenicol induce renal changes like kidney damage in the form of dialated tubules with regressed blood vessels and vacuolated glomeruli.

2. Materials and methods

I Plant material and authentication

The flowers of plants collected from local region of Mumbai and the plant is authenticated by BLATTER HERBARIUM, ST. Xavier's college, Mumbai-400001, India.

II Preparation of plant extract

The powdered form of flowers of *Couroupita guianensis* extracted with methanol as the reagent (95 % v/v) for about 18 hour by using soxhlet apparatus. The extract was filtered and the filtrate was concentrated under reduced pressure using rotary evaporator to obtain the extract as solid as residues. The extraction value (% w/w) of methanol was 18 (M. Sugumaran, et. al., 2008).

III Animals- mice

The animals used for the studies of toxicity and for efficacy were healthy Albino Swiss mice (*Mus musculus*), weighing between 30-35 gm obtained from Haffkins Institute, Parel (E), Mumbai- 400012. Under the Animal Maintenance permit Registration Number Invochem Laboratory, 226, "Gauri" Commercial Complex, Station Road, Vasai Road (E), Dist. Thane-401210; CPCSEA Registration No. 851/C/04/CPCSEA, from the ministry of Social Justice and Empowerment, Government of India. After procurement, the male and female mice were kept in same cage. The cages were provided with rice husk bedding and were cleaned daily. The house was maintained at 28±2^{o} c and exposed to 10-12 hours of day light and a relative humidity of 30-70 %. The animals were provided with drinking water ad libitum and fed on commercially available feed supplied by AMRUT FEED.

IV Drug- chloramphenicol

Chloramphenicol was procured from Mehta Pharmaceutical Limited, 315, Janki Centre, Plot No. 29, Shah Industrial Estate, Off Veera Desai Road, Andheri (W), mumbai, India. It is kept in below room temperature. Chloramphenicol is beneficial to control the growth of gram positive and gram negative bacteria, however chloromycetin at high concentrations results in renal-toxicity **(**Saba et al., 2000). Therefore to study an extent of toxicity of chloramphenicol, there should be low, medium and high dose of drug given to the mice. It can be determined by LD_{50} of chloramphenicol such as ¼ th of LD_{50} was the low dose, ½ of LD_{50} was medium dose and ¾ th of LD_{50} was the high dose of chloramphenicol that was given to mice for the study. LD_{50} of chloramphenicol is 2300 mg/kg body weight of mouse according to Pfizer material safety data sheet, 2007.

V Experimental protocol

Group I (6 mice) were used as controls. Group II (6 mice) received low dose of chloramphenicol i.e. 500 mg/kg. Group III (6 mice) received 200 mg of leaf extract of *Couroupita guianensis* (F E of C G). Group IV (6 mice) received low dose of chloramphenicol i.e. 500 mg/kg and 200 mg/kg of flower extract of *Couroupita guianensis.*

VI Blood sample collection and analysis

Blood sample was collected by puncture of retro- orbital vein and put the blood in EDTA vial for all renal analysis like blood urea nitrogen (BUN**),** creatinine, other biomarkers are glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO).

VII Histopathological studies

The animals were sacrified to remove the kidney. The kidney was fixed in Bouin's solution for 12 hrs and then embedded in paraffin's wax using conventional methods (Galighor A. E., et al., 1976), cut into 5 µm thick sections and stained haematoxylin – eosin dye. The sections were then observed for histopathological changes.

Statistical analysis

The results of antirenaltoxicity activity were presented as the mean ± SE of 6 mice each group. Results were analyzed statistically using analysis of variance (ANOVA) two ways without replication followed by 'f' test. Values of $P < 0.05$ were considered significant.

3. Results

Biochemical analysis

Table 1 shows the effect of FE of CG on renal parameters of mice like BUN, creatinine, others are GSH, SOD, CAT and LPO. All values like BUN and LPO were increased in toxic group, whereas the other values i.e. GSH, SOD and CAT were decreased at chloramphenicol given to mice. However, all the values were significantly get recovered due to administration of FE of CG.

Table – 1

Renal observations after treatment and recovery with the help of flower extract of *Couroupita guianensis* Aubl. in *Mus musculus.*

Groups	BUN mg/dl	Creat. Mg/dl	SOD U/mg	CAT OD/mg	GSH µg/mg	LPO n moles/g
Control	17.23±1.5	0.53±0.09	35.6±7.2	3.8±0.44	4.78±0.77	117.3±2.7
Chloramphenicol	27.58±7	0.48±0.09	30.2±7.92	2.22±0.3	2.28±0.43	263±54.6
FE of CG	16.53±1.8	0.47±0.14	24.5±7.98	3.71±0.5	3.76±0.73	125.5±8.7
Chloramphenicol + FE of CG	16.9±1.3	0.52±0.07	25.48±8.9	2.22±0.5	2.34±0.32	202±27.7

P values < 0.05 by 'f' test. The values are expressed as Mean ±SE from 6 mice in each groups. FE of CG flower extract of *Couroupita guianensis*.

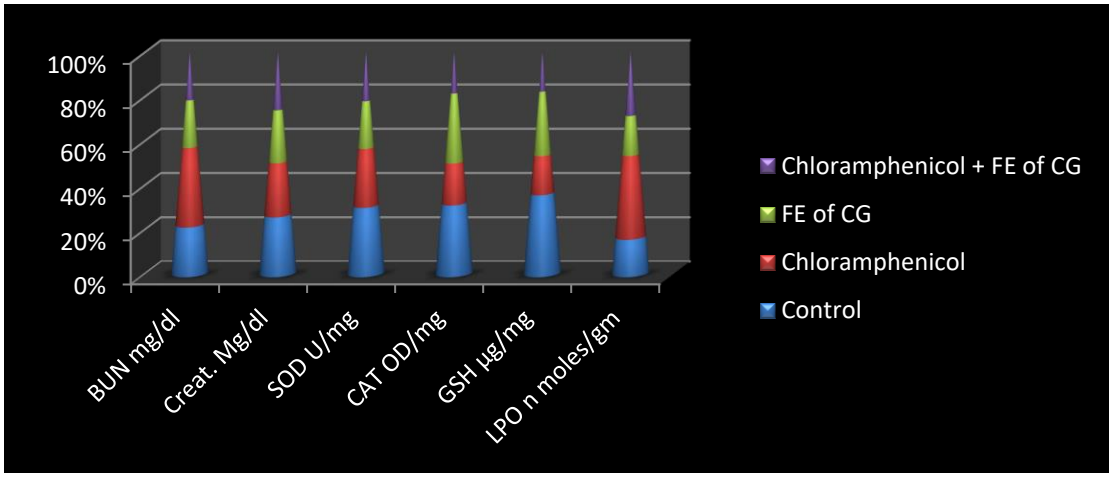
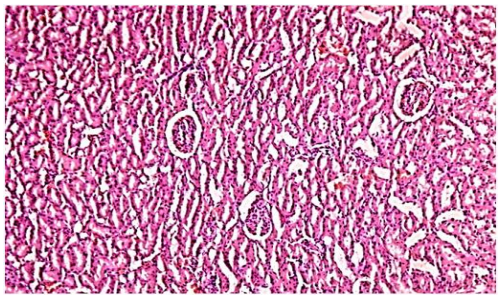


Fig.1-Effect of FE OF CG on kidney function markers of mice with chloramphenicol induced renal changes (values are mean ±SE from 6 mice/group. P values < 0.05 compared control group with others).

Histopathalogical analysis-

A



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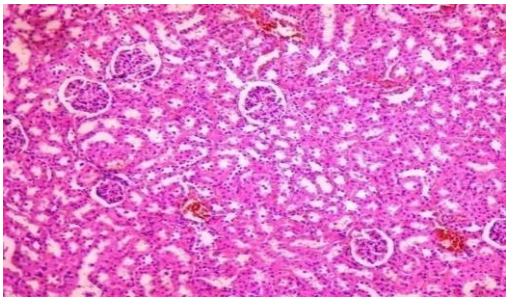

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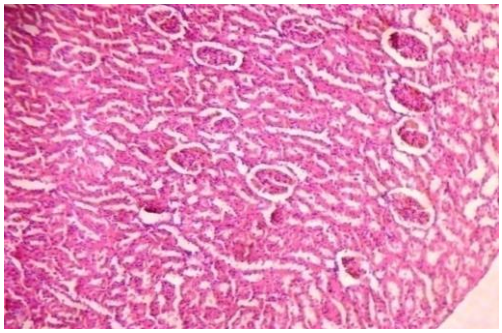

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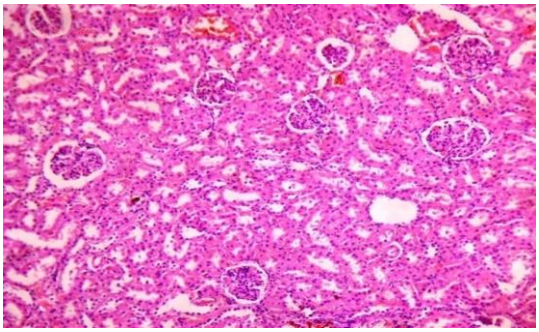

Fig-2 Photomicrographs of kidney sections (H & E × 200): (A) Renal cortex of control group presented normal glomerular structure. (B) Renal cortex of chloramphenicol treated group showed glomerular regression and dialated tubules. (C) Reanal cortex of FE of CG group showed slight atrophy of glomeruli and dialation of tubules. (D) Renal cortex of chloramphenicol treated + FE of CG group showed vacuolar glomeruli and slight dialation of renal tubules.

4. Discussion

The phytocompounds present in the plants are good source of the exogenous antioxidants. They scavenged the damaged cells as share an electron in order to balance an electron which is stolen by free radicals from the phospholipid plasma membrane. Free radicals as well as ROS are produced during the toxicity of body organs by toxicants. The perpose of the research study was damaged the tissue by toxicants in the form of chloramphenicol. Protection of impared part of body organs with plant origin antioxidants from *Couroupita guianensis* Aubl were swietenine, sapropterin, usnic acid, lupeol and gamma tocopherol (vit. E).

The present study was aimed to investigate the nephroprotective ability of *Couroupita guianensis* Aubl against chloramphenicol induced nephron-toxicity in mice. Serum urea nitrogen is used to determine renal function but it is less sensitive than urea clearance tests (Faulkner, 1976**)**. However, it is needed to collect both blood and urine over a timed period which is difficult to conduct in experimental animals. The blood urea nitrogen may not be changed significantly until 50% of renal function is impaired (Hayes, 1994). Elevation of BUN and significant fall in the creatinine has been reported with the toxic use of chloramphenicol. The elevation in BUN and serum creatinine and significant fall in creatinine clearance has been reported with the toxic use of gentamicin which was in agreement with the current findings for gentamicin treated animals. The protective role of *Cinnamomum tamala* extract can easily be concluded from current results (Bennette WM, et.al., 1980).

Over production of reactive oxygen system initiates the process of lipid peroxidation in cell membranes and destruction of cell components as well as cell death (Gutteridge et.al., 1990**)**. Hence the level of LPO is elevated in prophylactic group also. The creatinine level is not recovered significantly due to the increased the value of LPO in chloramphenicol + FE of CG group. BUN value is declined in FE of CG group while it is slightly recovered in chloramphenicol + FE of CG group. SOD, CAT and GSH values were very declined while

LPO level was elevated in prophylactic group.

Histopathological examination of kidneys treated with chloramphenicol confirmed the occurance of chloramphenicol induced renal damage in kidneys (figure 2). The histopathological observation illustrated that more or less the dialation of renal tubules in extract as well as prophylactic groups. But the renal tubules are highly dialated in toxic-chloramphenicol group. Cronin and coworkers documented that glycosuria in gentamicin treated animals could be due to proximal tubular necrosis (Cronin RE, et.al., 1980) on treatment in extract of *Cassia occidentalis* leaf recovered the tubular structure. The glomerular structure was also highly regressed in toxic-chloramphenicol group while slight or more atrophy of glomeruli was occurred in extract and prophylactic groups respectively.

Significant increase in urinary protein excretion may be due to the presence of hyaline and renal casts in the tubules which may lead to cellular degeneration. Renal casts may block the tubules and lead to renal damage (Houghton et.al., 1986 and Solez 1983).

The overall effect as per the statistical analysis of these parameters suggested that the flower extract of Couroupita guianensis Aubl was nephrocurative as well as nephroprotective and was significantly effective against chloramphenicol intoxification.

5. Conclusion

The *Couroupita guianensis* flower contains flavonoids, terpenoids, alkaloids, etc. The methanolic extraction of flower of *Couroupita guianensis* demonstrated significantly GSH and CAT values that were declined by chloramphenicol induced kidney toxicity and reduced lipid peroxidation. The methanol extraction of *Couroupita guianensis* flower reduced the increased values of blood urea nitrogen and lipid peroxidation due to presence of active phytocompounds in plant. The further studies should be conducted to know the benefits of phytocompounds to protect the other organs too.

6. Acknowledgements

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