Protective Effects of *Millingtoniahortensis* Linn. On Chloramphenicol-Induced Oxidative Stress and Nephrotoxicity in Mice

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Abstract. Chloramphenicol is an important antibiotic commonly used in undeveloped as well as developing nations in treatment of life-threatening bacterial infections. However,renal-toxicity is resulted by chloramphenicol at a high concentration Sabaet.al., [20].It is studied that the damaged in kidney caused due to the free radicals generated in the kidney and other body organs also. Theeffect of *Millingtoniahortensis* Linn. Extractwas examined by estimating the elements, such as, nitrogen, glutathione, serum creatinine, lipid peroxidation and the things like catalase along with SOD activities. In present research, nephrotoxicity is induced by chloramphenicol that ischaracterizedby significant increment of serum markers levels,for that LPO level raised andSOD level reduced along withGSH and CAT levels. Co-signification of methanolic extract with chloramphenicol was designed for significantly prevent both the functional and histological renal injury protection. It is concluded that the phytogenic antioxidants play an important significance in the ameliorating action for recovery of damaging effects caused by the Chloromycetin.

Index Terms: Antioxidants,Chloramphenicol, Flower extract,*Millingtoniahortensis* Linn, Renal profile parameters

I. INTRODUCTION

Chloramphenicol is an antibiotic come from the Streptomyces of bacteriumvenezuelae orgenerate artificially and it is effective forgram positive as well as gram negative bacterial infection. Chloramphenicol is an effective and well accepted broad spectrum of antibiotic. However, it has many features that demand careful usage in animals that is companion and that has led to prohibition of use this in food that are producing by animals in several states and countries, including Canada and USAKahn, [9].However, the high dose of chloramphenicol causes liver-toxicity due to formation of some radicals that are free or reactive oxygen species (ROS). Free radicals produce deleterious effect on lipid plasma membrane along withcellular components thus generating peroxidation of lipids that can leads to cell death Ryter et al., [18].

Kidney is the vital organ of body that is used to remove the toxic and waste material of body. They remove the metabolites when the people are administered with toxicants in the form of pollutants, medicinal drugs like analgesic drugs, antibiotics, etc. The significance of renal function is for the implementation of chloramphenicol and its metabolites in blood was researched byKuninet.al. [3]**.** they found that metabolitescan retained if the function was not significant. The metabolites are not eliminated due to the effect of free radicals which lead to alter the function of renal tubules.

In the body, Medicinal plants possess scavenging activity against the radicals are free to boost the energy of antioxidant for defence mechanism and that can have a significant role of unfavorable damaged tissue minduced by chloramphenicolK B H Kumar and Kuttan R, [13**]**.The study shows protective role of *Millingtoniahortensis* Linn. Against the chloramphenicol induced renal toxicity due to presence of antioxidants in the plants. The phytochemical screening of *Millingtoniahortensis*showed that along with tannins, saponins, flavonoids, terpenoids, alkaloids and phlobatanninsM., Faraz. et.al. [5],Harborne et.al. [7]and H.O., Edeoga, et.al., [4].This study focused on kidney protective function that are evaluated of*Millingtoniahortensis*Linn against chloramphenicol induced toxicity in the renal area like damage of the kidney in the form of dilated tubules with vascular glomeruli.

II. METHODS

Material of Plant and its authentication

The researcher has gathered the flowers of flowers of *Millingtonia hortensis* from the regional areas adjacent with the metropolitan of Mumbai. Moreover, 'BLATTER HERBARIUM' has checked the authenticity of the whole herb. The aforementioned Herbarium is situated in "ST. Xavier's college, Mumbai-400001, India". The flowers are collected from Songir, Dist. Dhule (M.S.) and washed in order to remove dust or sands etc., dried in sun for 7 days continuously and made a powder form. It is extracted by following method.

The 30-gm flower powder was taken in a container and adds 200 ml. methanols, then kept for 24 hours in the

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shaker, after that the formula was filtered through eight layers of cloth that is made of muslin and then the final extract was collected. The extraction process was repeated twice. The formula was extracted by reducing the pressure using rotary evaporator and then that filtrate was collect for the solution as solid as possible.S.Maneemegalai and P. Monika [19].

Animals- mice

For the study of efficiency and for toxicity the animal is utilized were healthy Albino "Swiss mice (*Musmusculus*)", that is weighted btween 30-35 gmobtained from'Institute of Haffkins', situated at "Parel (E), Mumbai- 400012'. While the acquisition of alpha and beta mice was completed, these were caged in a same space for further study. The enclosures were cleaned daily and equipped with facilities of rice covering. The house has been kept at a specific temperature of "28±2^{o} c" and it has also been exposed for 10-12 hours to light in a day.

Drug- chloramphenicol

Mehta Pharmaceutical Limited produced Chloramphenicol, situated at "315, Janki Centre, Plot No. 29, Shah Industrial Estate, Off Veera Desai Road, Andheri (W), and Mumbai, India". For further study, the temperature level is maintained at a level of sub room temperature. To control the growth of the bacteria such as, gram positive and gram-negative chloramphenicol is responsible, though Chloromycetincan producerenal-toxicity at a very high level. Therefore the researchers study chloramphenicol for toxicity to an extend version; that could be low, high or medium dosage of drug given based on the requirement of the mice. Another deciding factor include LD_{50of} chloramphenicol, such as,¼ the of LD_{50was}'low dosage', ½ of LD_{50} was'medium dosage' and ¾ the of $LD_{50\ was}$ the 'high dosage' of "chloramphenicol" that was administered to the mice in respect of the research purpose. As per the "Pfizer material safety data sheet, 2007', the dosage of chloramphenicol is "LD_{50}" while the mouse weighs is "2300 mg/kg".

Protocol for Experimental

"Group I (6 mice)" were utilised as controlers, for that"Group II (6 mice)" that received 500 mg/kg chloramphenicol. "Group III (6 mice)" received 200 mg/kg flower solution of*Millingtoniahortensis* Linn.(F E of M H).

Analysis the sample of blood

By puncture, the sample of blood specimen has been obtained from "retro-orbital vein" and that sample was kept on EDTA vial for analyzing renal functioning such as, "blood urea nitrogen (BUN)**",** "creatinine"**,** different biomarkers like, "speroxidedismustase (SOD)", "glutathione (GSH)", "lipid peroxidation (LPO)" and "catalase (CAT)".

Studies related to Histopathology

The animals were undergone sacrifice as their internal organs such as kidney was removed. After that the dissolution of kidney and fixed in "Bouin's solution" for twelve hrs and then kept in wax of paraffin by using a traditional method

Analysis Statistically

Results of this research show that it is caused by "antirenaltoxicity". Activites were presented as the mean value '± SE' associated with 6 group of every mice group. A statistical method was also employed with evaluating the results by means of performing the two way ANOVA test without any replication.

III. RESULTS

Analysis of Biochemical parameters

Serum biochemical findings of all groups have shown in table 1. The blood urea nitrogen, creatinine levels are elevatedwhereas the other values like SOD, catalase and GSH are decreased in chloramphenicol group. However, due to administration of "FE of M H" all the values were getting recovered validly.

Table – 1: Observations of Renal functions after the process of recovery and treatment by means of flower solution of *Millingtoniahortensis* Linn. In*Musmusculus.*

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	0.58±0.09	30.2±7.92	2.22±0.3	2.28±0.43	263±54.6
FE of M H	17.91±1.8	0.58±0.21	34.5±10.05	3.55±0.6	3.76±0.73	85.8±20.8
Chloramphenicol +FE OF MH	16.9±1.3	0.49±0.05	32.3±11.4	3.25±0.3	2.99±0.38	165±28.8

"P values are < 0.05 by 'f' test,the values are expressed as Mean ±SE from 6 mice in each groups". 'FE of CG' flower extract of *Millingtoniahortensis*Linn.

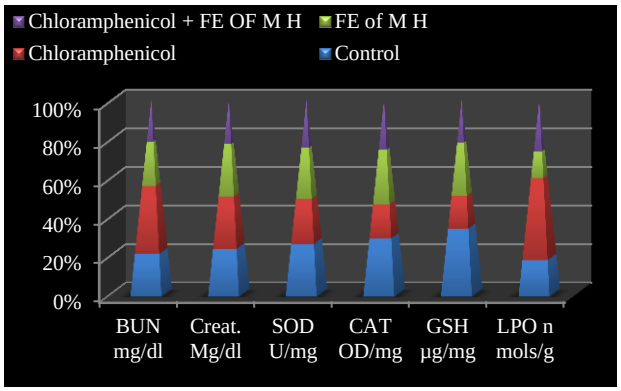
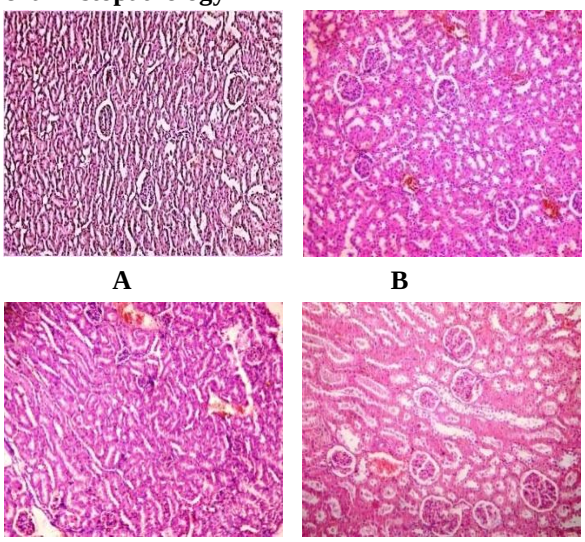


Fig.1- "Effect of FE OF M H on kidney function producers of mice with renal changes that are induced by chloramphenicol"

Renal histopathology



A **B**

C **D**

Fig-2 micro photographs of the inter-sections associated with kidney "(H & E × 200)": (A) control group of renal cortex that are represented by normal structure of glomerular. (B) Chloramphenicol treated group of renal cortex showed by dialated tubules and glomerular regression.

IV. DISCUSSION

Free radicals are the fundamental cause of various diseases. They cause biochemical damage in cells and tissues which results in several diseases such as hepatic disorders, renal failure, diabetes mellitus, ageing, cancer, etc.K.R. Kirtikar et al., [12].Therefore, the prevention of above mentioned diseases need antioxidants in the body or antiradical molecules in either body or in the form of antioxidants enriched Ayurvedic medicine. The aim of research study was damaged the tissue by chloramphenicol. Protection of impaired body with antioxidants from Millingtoniahortensis Linn.were BergeninSastry et al., [21], 2-4 methoxyflavoneluteolineOtto Wofbeis et al., [15],hypericinLiebes, et al., [14] and Gingolide-B van Beek, [22], specialised flavone hortensinN. Bunyapraphatsara et al., [2] and glucosidal alkaloid MillingtoninT., Hase et al., [8].

Main purpose involved with this work was to investigate the ameliorative significance of *Millingtoniahortensis* Linn. against chloramphenicol induced oxidative stress and renal toxicity in mice. Treatment with many synthetic and natural antioxidant elements has been useful in either amelioration or prevention of nephrotoxicity in experimental ratsI., Karahan et al., [11]. The study evaluated the efficacy of FE of MH, antioxidants and free radical scavenger on the chloramphenicol induced oxidative injury and renal damage by chloramphenicol in proven intoxicated rats. The significant elevation of blood urea nitrogen (BUN), creatinineand LPO has shown the toxic use of chloramphenicol. It was caused due to free radicals as well as reactive oxygen system (ROS) which were generated in the chloramphenicol group.

Mice received FE of MH extract alone for 14 days, there was nearly same the BUN level, while creatinine level was elevated. Antioxidantenzyme levels are same to that of control vehicalanimals except GSH and LPO level were decreased in extract group. Rats were treated with gentamicin alone significantly increase in LPO level while GSH and CAT activities were reduced in the kidneytissue similar results were observed by *Ozbek et al*., [16]and *Adaikpoh et al*., [1]. Co-administration of chloramphenicol and FE of MH caused significantly recovered the values of renal functional parameters, but the levels of GSH, CAT and SOD were slightly recovered while LPO was still uplifted in the same group of prophylactic.

Histopathologicalstudy revealed that the remarkable dialation in the renal tubules and necrosis in the glomeruli to from vacuolated glomeruli in the renal cortical part of kidney section (Fig.2-B).There were slight atrophy in glomeruli and renal tubules which were dialated slightly in FE of MH group (Fig. 2-C). When the study of kidney sections in prophylactic group were somewhat more dialated renal tubules and slight vacuolar glomeruli (Fig. 2-D) as compared to extract as well as control vehical groups. Rehman et al., [17] studied that histopathological observation illustrated remarkable tubular necrosis and cellular infiltrations along with sloghing of cells in tubular lumen of the kidneys of gentamicin treated groups in both nephro-curative and nephro-protective studies.

As per the statistical analysis of these diagrams, the overall study suggested that the flower solution or extract of*Millingtoniahortensis* Linn. Was nephroprotective and significantly affective against the intoxification of chloramphenicol.

V. CONCLUSION

The protective role of methanolic extract of flower of *Millingtoniahortensis*Linn.against chloramphenicol induced kidney damage was evaluated in this study. The protective potential may be due to scavenger and antioxidants like tannins, saponins, terpenoids, flavonoids, alkaloids and phlobatannins. Bergenin, 2-4 methoxyflavoneluteoline, hypericin, Gingolide-B, millingtonin, hortensin, etc are the active antioxidants involved to protect the kidney against chloramphenicol that damaged the kidney. Further study should be focused on the efficacy study of flower extract of *Millingtoniahortensis* Linn. against the various antibiotics that have been used in the corona condition.

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