Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1780-1783
Received: 12-06-2020
Accepted: 10-08-2020

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Evaluation of nephroprotective effect of rutin against toxic effect of cyclophosphamide

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DOI: https://doi.org/10.22271/phyto.2020.v9.i5y.12596

Abstract
Cyclophosphamide (CYP) is an alkylating anti-tumor drug. CYP has many adverse effects, partly due to oxidative stress induction in various tissues. Rutin is one of the natural flavonoids with strong antioxidant properties. The aim of this study was to determine the nephrprotective effect of rutin against toxic effect of cyclophosphamide. The study was performed on swiss albino mice. Thirty male mice were divided into six group. The first group received normal saline for 14 days. The second group received only cyclophosphamide drug 25 mg/kg., i.p. on 0 to 14 days. The third group received amifostine drug prior 1hour before the administration along with cyclophosphamide (25mg/kg) respevtively for 14 days. The group fourth and five received 40mg/kg and 80mg/kg of rutin after the administration of cyclophosphamide (25mg/kg) respectively for 14 days. Cyclophosphamide administration significantly (P<0.05) decreased the levels of antioxidant markers such as superoxide dismutase (SOD), catalase (CAT) and increased lipid peroxidation (LPO). Cyclophosphamide elevated the levels of hematological variables like RBCs, WBCs, Hb and biomarker enzymes like Albumin, Creatinine and Total protein. Treatment with Rutin significantly (P<0.05) reversed the status serum biomarkers, hematological variables and antioxidant markers in cyclophosphamide induced nephrotoxicity. The results concluded that Rutin has shown nephoprotective effect against toxicity induced by cyclophosphamide in mice.

Keywords: Nephroprotective, rutin, cyclophosphamide, mice, oxidative stress

Introduction
Cyclophophamide (cytoxan) is an alkylating anti-tumor drug. The chemical formula of cytoxan is C7H15Cl2N2O2P. It was identified by X- ray diffraction [1]. Cytoxan is a member of oxazophorine group and other members are Ifosfamide & Trofosfamide [2]. Cyclophosphamide given at low doses act as either an anti-angiogenic or an immune-stimulatory agent in combination with immunotherapies in the treatment of cancer [3]. Flavonoids are the subgroup of polyphenols which are extensively dispersed in plants such as citrus fruits, berries, onions, parsley, legumes, green tea, red wine, sea buckthorn, and dark chocolate [4]. Rutin is a flavonoid that is widespread in the plant kingdom [5]. Rutin synthesized through the phenylpropanoid metabolic pathway, involves the transformation of the amino acid phenylalanine to 4-coumaroyl-CoA. 4-coumaroyl-CoA combined with malonyl-coA to produce true backbone of flavonoids. The biosynthetic pathway continues through series of enzymatic modifications to produce rutin [6]. It has a wide range of pharmacological properties that have been utilized in human medicine and nutrition. It is also used as an antimicrobial, antifungal, and anti-allergic agent [7]. Amifostine is a pharmacological antioxidant used as a cytoprotectant in cancer therapy [8]. Amifostine is thought to protect the normal tissues to tumor tissue against oxidative damage generated by cancer therapies by becoming concentrated in higher levels in normal tissues. The degree to which amifostine though accumulates in tumors and protects against cancer therapies [9].

Material & Method
Material
Cyclophoshamide were purchased from zydus onco science, Amifostine were purchased from DRDO and Rutin were purchased from himrishi herbals. Tris Hcl from Hopax. Sodium hydroxide were purchased from Agro chemicals. Monosodium phosphate, Disodium phoshpahte, sodium carbonate were purchased from shubhchem industries. EDTA, Hydroxylamine, nitro blue tetrazonium, Tritron-x 100 were purchased from Triveni chemicals. All the other chemicals were purchased from merck (sulfosalicylic acid, TCA, TBA, Hydrogen peroxide, copper sulphate, sodium potassium tartarate, Bovine serum albumin, Folin-phenol reagent, potassium iodide and sodium chloride).

Animals

Male swiss albino mice (20-30gm) were obtained from the central animal facility of ShriRam College of Pharmacy, Banmore and were maintained in polypropylene cages on rodent pellet condition of controlled temperature (22±2oC) and acclimatized to 12/12 h light/dark cycle. Free acess to food and water were allowed until 2 hour before the experiment. The care and maintenance of the animals were as per approved guidelines of the "Committee for the purpose of control and supervision of experiments on animals (CPCSEA)". Food and water were provided 2 hour after the experiments. All experiments on animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation. (891/PO/Re/S/OS/CPCSEA)

Methods

Grouping of Animals

The animals were divided into five groups. N=5

- Group 1: Normal saline
- Group 2: Cyclophosphamide (25 mg/kg i.p.)
- Group 3: Amifostine (20 mg/kg i.p.) + Cyclophosphamide (25 mg/kg i.p.)
- Group 4: Cyclophosphamide (25mg/kg i.p.) + Rutin (40 mg/kg oral)
- Group 5: Cyclophosphamide (25 mg/kg i.p.) + Rutin (80 mg/kg oral)

Animals were divided into five groups of 6 animals each. The first group received normal saline for 14 days. The second group received only cyclophosphamide drug 25 mg/kg., i.p. on 0 to 14 days. The third group received amifostine (20mg/kg) drug prior 1hour before the administration along with cyclophosphamide (25mg/kg) respevtively for 14 days. The group fourth and five received 40mg/kg and 80mg/kg of rutin after the administration of cyclophosphamide (25mg/kg) respectively for 14 days. After 14 days of dosing animals were anaesthetized with ether for collection of blood from retro orbital sinus. Various hematological and biochemical analysis were carried out.

Hematological Evaluation of Blood

The hematological variables, i.e. RBC, WBC, and Hb were measured.

Biochemical Test

Lipid Peroxidation assay [10]

Lipid peroxidation was measured by the method of (Willa, 1965). In which 0.5ml homogenate and 0.5ml Tris Hcl (p^H 7.4) were mixed and incubate at 37ºC for 2hrs then 1ml 10% TCA ice-cold were added to the mixture. The was then centrifuge at 1000 for 10min. 1ml supernatant added to 1ml of 0.67% TBA and kept the tubes in boiling water bath for 10mn. After cooling the mixture 1ml of DDW was added and the optical density (OD) was measured at 532nm.

Reduced Glutathione [11]

Reduced glutathione was measured by the method of (Ellman, 1959). In which 1ml 0.75 ml of PMS or homogenate of sample were taken and 0.75 ml of 4% sulphosalicylic acid were added to it and the mixtrure was centrifuge at 1200g for 5min at 4 ºC. To 0.5ml of supernatant 4.5ml of 0.01 M DTNB reagent were added and optical density (OD) was measured at 412nm.

Superoxide dismutase (SOD) assay [12]

1. Solution A: 50Mm Na2Co3 (0.52g/100ml) in 0.1Mm EDTA (0.003g/100ml) (PH 10.8)
2. Solution B: 96Mm NBT in solution A (0.008g/ 100ml)
3. Solution C: 0.6% Triton X- 100 (v/v) in solution A
4. Solution D: 20Mm Hydroxylamine Hcl adjust PH to 6 with NaOH

After making solutions, for test 2ml NBT were taken and mixture of 0.5ml Hydroxylamine Hcl + 0.1ml of PMS of homogenate were added to it. Change in optical density (OD) were measured at 560nm for 2mn at 30/60 sec intervals. DDW for blank.

Catalase assay [13]

Catalase was measured by the method of (Luck, 1971). In which 50ml of phosphate buffer were taken and 12.5Mm H_2O_2 were added to phosphate buffer. Then diluted 0.16ml of H_2O_2 (30% w/v) to 100ml with phosphate buffer and pipetted directly in to cuvette. For test: 3ml H_2O_2- Phosphate buffer- 0.05ml of supernatant and for blank: 3ml H_2O_2+ Phosphate buffer. Change in optical density (OD) were measured at 240nm for 2mn with 30/60 sec intervals.

Protein estimation [14]

Protein estimation was measured by the method of (Lowry, 1951). In which 0.1ml of supernatant was distilled with 0.9ml of DDW and 5ml of working alkaline solution which contains (48ml of 25 sodium carbonate in 0.1M NaOH + 1ml of 1%(w/v) copper sulphate + 1ml of 2% sodium potassium tartrate). Then the mixture was incubate at room temperature for 10mn. 0.5ml of folin-phenol reagent were added. Then incubated at room temperature for 30mn and optical density (OD) measured at 750nm against reagent blank.

Protein estimation (Biuret-test)

To 0.1ml of homogenate, 2.9ml of NaCl and 3ml of working reagent (20ml of stock was diluted to 100ml with 0.2N NaOH) were added. Then kept at room temperature for 10mn and optical density (OD) were measured at 540nm.

Statistical Analysis

Statistical evaluations were made using one-way ANOVA followed by Dunnet's test. A probability of 0.05 and less was taken as statistically significant. The analyses were carried out using stigma stat for windows version 2.03(SPSS Inc. USA).

Result & Discussion

Table 1: Effect of Rutin on serum level albumin, creatine and total protein in CYP induced nephrotoxicity in mice

Treatment	Albumin (mg/dl)	Creatinine (mg/dl)	Total Protein (mg/dl)
Normal saline	3.63 ± 0.10	1.57 ±0.04	50.60 ±0.09
Cyclophosphamide	8.74 ±0.18	3.76 ±0.20	74.67 ±0.30
Amifostine + CYP	2.74 ± 0.12	1.63 ± 0.02	50.57 ± 0.10
CYP + Rutin 40mg/kg	7.70 ± 0.23	4.13 ± 0.09	65.55 ± 0.16
CYP + Rutin 80mg/kg	5.68±0.33	2.60±0.08	54.34±0.22

Table 2: *Effect of Rutin on antioxidants SOD, Catalase, GSH and lipid peroxidation in CYP induced nephrotoxicity in mice*

Treatment	SOD (Unit/mg protein)	Catalase (Unit/mg protein)	GSH (Unit/mg protein)	Lipid peroxidation (Unit/mg protein)
Normal saline	12.60±0.18	9.79±0.10	13.77±0.22	3.42±0.23
Cyclophosphamide Control	3.47±0.18	3.67±0.13	4.76±0.04	33.90±0.03
Amifostine + CYP	13.46±0.09	10.64±0.18	10.80±0.08	3.49±0.23
CYP + Rutin 40mg/kg	6.39±0.18	5.69±0.19	9.01±1.1	19.10±0.45
CYP + Rutin 80 mg/kg	8.47±0.26	7.30±0.12	6.88±0.60	15.99±0.05

Serum Enzyme Biomarkers

The CYP control group shows extremely significant increase in serum Albumin (ALB), Creatinine (CTN) and Total protein (TP) values compared to normal saline. Rutin (Ru 40 mg/kg and 80 mg/kg) treated mice significantly decreased the ALB, CTN and TP values in dose dependent compared to CYP control group.

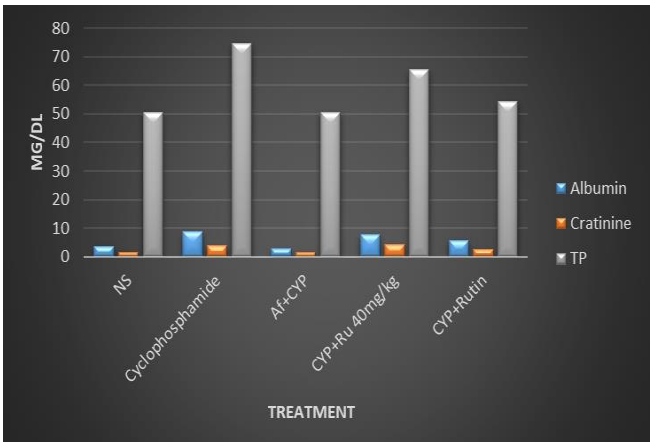

Fig 1: Effect of Rutin on Albumin, Creatinine & Total Protein level in CYP induced nephrotoxicity in mice

- $P<0.05$, compared to normal group (One way ANOVA followed by Dunett's test).
- $P<0.05$, compared to CYP control group (One way ANOVA followed by Dunett's test).

Hematological Parameters

CYP treated group show significant decrease in WBC count, Hb and RBCs count as compare d to normal group. Treatment with Rutin in test group was significantly increase WBC count, Hb and RBCs as compared to CYP treated group.

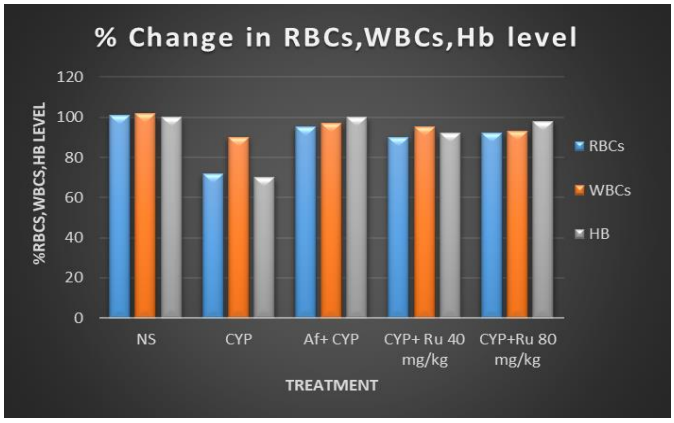

Fig 2: Effect of Rutin on % Hb, RBCs, and WBCs level in CYP induced nephrotoxicity in mice

- $P<0.05$, compared to normal group (One way ANOVA followed by Dunett's test).
- $P<0.05$, compared to CYP control group (One way ANOVA followed by Dunett's test).
- Control value for Hb: 14 ±0.06gm%, RBCs: 4.2±0.12 million/cm, WBCs: 6100±314/cm.

Effect of SOD and Catalase

SOD and catalase activity were reduced significantly in CYP control group compared to normal saline. Test group such as Rutin (40 mg/kg and 80 mg/kg) treated groups resulted extremely improvement in SOD and catalase activity compared to CYP treated group. Effectiveness of Rutin were found in dose dependent manner.

Effect on GSH

GSH levels was decreased significantly in CYP treated group as compared to normal group. The data showed that Rutin significantly improved the decreased GSH level as compared to CYP treated group. Effectiveness of Rutin were found in dose dependent manner.

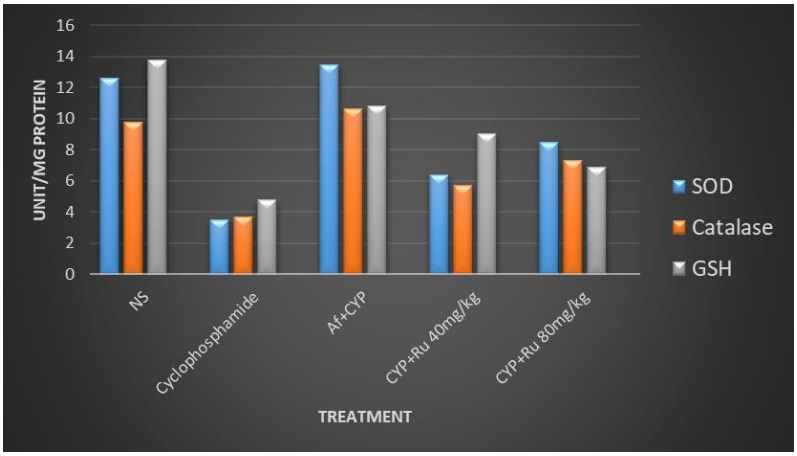

Fig 3: Effect of Rutin on SOD, Catalase & GSH level in CYP induced nephrotoxicity in mice

- *P*<0.05, compared to normal group (One way ANOVA followed by Dunett's test).
- *P*<0.05, compared to CYP control group (One way ANOVA followed by Dunett's test).

Effect on Lipid Peroxidation

CYP control group shows extremely significant increase in LPO levels compared to normal group. Rutin treatment in a dose dependent manner demonstrated significant reduction in LPO levels as compared to CYP control group.

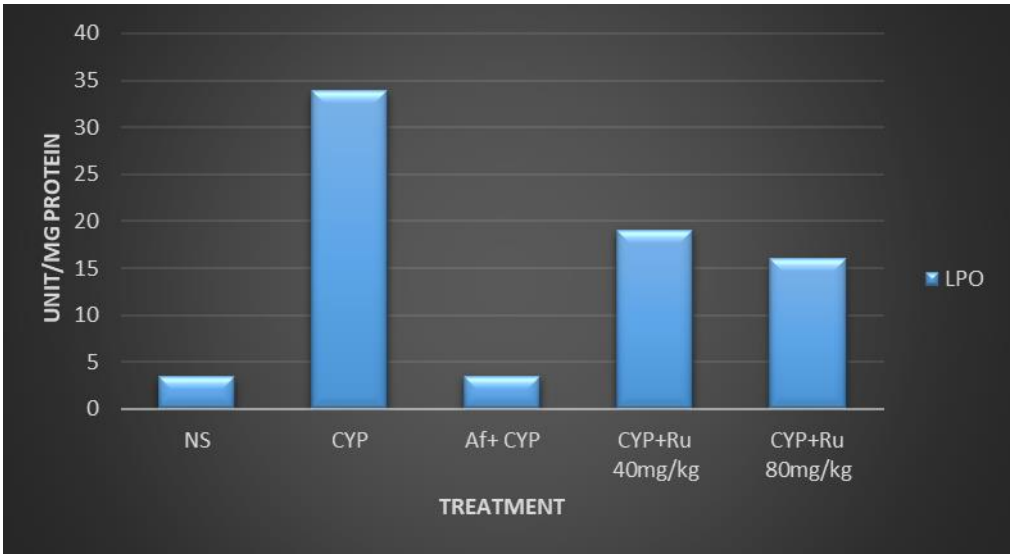

Fig 4: Effect of rutin on lipid peroxidation level in CYP induced nephrotoxicity in mice

- *P*<0.05, compared to normal group (One way ANOVA followed by Dunett's test)
- *P*<0.05, compared to CYP control group (One way ANOVA followed by Dunett's test)

Conclusion

From these studies, we can end that formation of free radicals can be prevented by an antioxidant. Herbal drugs are economic, easily available and without any side effects as medicinal herbs as the potential source of therapeutics have attained significant role in health system all over the world for both humans and animals. The herbal drugs are potential not only in disease condition but also for maintaining proper health. Rutin is an herbal derived antioxidant drug. Results concluded that Rutin has shown nephroprotective effect against cyclophosphamide toxicity.

Acknowledgements

The author would like to acknowledge the director of Shriram College of Pharmacy, Banmore Dr. Vinay Jain for allowing conduction of the present experiment.

References

1. Isabella Karle L, Jean Karle M, William Egan, Gerald Zon, Joan A. Brandt Absolute configuration of (+) - cyclophosphamide. A crystal and molecular structure determination by x-ray diffraction J Am. Chem. Soc.1977; 99(14):4803-07.
2. Sladek NE. Metabolism of oxazaphosphorines. Pharmacol Ther. 1988; 37:301-55.
3. Pietras K, Hanahan D. A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. J Clin Oncol. 2005; 23:939-52.
4. Liu P, Jackie S. Effects of Flavonoids on Proliferation of Breast Cancer Cells and Vascular Smooth Muscle Cells, A Thesis Submitted for the Degree of Master of Medical Sciences, The University of Hong Kong, 2007.
5. Hosseinzadeh H, Nassiri-Asl M. Review of the protective effects of rutin on the metabolic function as an important dietary flavonoid. J Endocrinol Invest. 2014; 37:783-8.
6. Li X, Kim JK, Park SY, Zhao S, Kim YB, Lee S *et al*. Comparative analysis of flavonoids and polar metabo-lite profiling of Tanno-original and Tanno-high rutin buckwheat. J Agric Food Chem. 2014; 62:2701-8.
7. Sharma S, Ali A, Ali J, Sahni JK, Baboota S. Rutin: therapeutic potential and recent advances in drug de-livery. Expert Opin Investig Drugs. 2013; 22:1063-79.
8. Keith I Block, Charlotte Gyllenhaal. Commentary: The Pharmacological Antioxidant Amifostine-Implications of Recent Research for Integrative Cancer Care. Integrative cancer therapies. 2005; 4(4):329-351.
9. Prasad KN. Multiple dietary antioxidants enhance the efficacy of standard and experimental cancer therapies and decrease their toxicity. Integr Cancer Ther. 2004; 3:310-322.
10. Wills ED. Mechanisms of lipid peroxide formation in animal tissues. Biochem. J. 1966; 99:667-676.
11. Ellman GL. Tissue sulfydryl group. Arch Biochem Biophys 1959; 82:70-77.
12. Kono Yasuhisa. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of biochemistry and biophysics. 1978; 186(1):189-195.
13. Luck H. Methods in enzymatic analysis. Academic Press, New York. 1974, 885.
14. Lowry, Oliver H, Nira J, Rosebrough A, Lewis Farr, Rose J Randall. Protein measurement with the folin phenol reagent. J Biol. chem. 1951; 193(1):265-275.