Int J Pharm Bio Sci 2015 Jan; 6(1): (P) 816 - 825

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANTIMUTAGENIC AND ANTIOXIDANT ACTIVITIES OF RUTIN

RAJINDER KAUR*, SAROJ ARORA AND A.K. THUKRAL

Department of Botanical and Environmental Sciences,
Guru Nanak Dev University, Amritsar 143005, Punjab, India

ABSTRACT

Rutin is a phenolic compound and found in many plant species. Its name is derived from Ruta graveolens a plant that contain this compound. Rutin has many health benefits like it inhibits platelet aggregation, inflammation, hemorrhoid development etc. It is also used in dietary supplements of human beings for its health benefits. Therefore, this study was planned to investigate its other medicinal uses i.e. antimutagenic and antioxidant activities. The antimutagenic activity of rutin was checked by using plate incorporation assay or Ames assay. Antioxidant potential was determined through various in vitro assays like DPPH free radical scavenging assay, lipid peroxidation, deoxyribose degradation (site-specific and non-site specific methods), reducing power, and chelating power assays. In both antimutagenic and antioxidant assays, rutin exhibited potent free radical scavenging and mutation inhibitory effects. This research paper is a part of my Ph.D. work.

KEY WORDS: **Rutin, Ames assay, free radicals, mutagens.**

***Corresponding author**

RAJINDER KAUR
Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, Punjab, *India*

INTRODUCTION

Free radicals such as superoxide anion, hydrogen peroxide, peroxyl radicals etc. play an important role in carcinogenesis by damaging the DNA molecules through strand breaks, modification of base pairs and DNA-protein cross-links [1,2,3]. Phenolic compounds are known for their antioxidant activities and decrease the oxidative stress induced carcinogenesis by attenuating the free radicals [4]. Rutin is present in many fruits, vegetables and beverages like apples, grapes, onion, tea and wine etc. [5]. It shows multiple pharmacological activities including anti-inflammatory, antiulcer, antibacterial, antidiabetic etc. [6,7,8]. Hence, the present study was designed to evaluate its antimutagenic and antioxidant activities using various standard in vitro assays.

MATERIALS AND METHODS

1 Materials

All the solvents and chemicals used in these experiments were of analytical grade and obtained from Merck, Mumbai, India. Rutin hydrate was purchased from Sigma Aldrich Company. The strains of Salmonella typhimurium TA98 and TA100 were procured from the Institute of Microbial Technology (CSIR), Chandigarh, India.

2 Antimutagenicity test

Antimutagenicity of rutin was checked using plate incorporation assay proposed by Maron and Ames [9]. In the present study, two strains of Salmonella typhimurium i.e. TA98 and TA100 were used. The experiments were carried out with (+S9 mix) and without metabolic activation (-S9 mix) system. NPD was used for TA98 and sodium azide was used for TA100 in experiments without S9, while 2-AF was used in experiments with (+S9) in both the tester strains. Experiments were performed by using fresh minimal agar medium, top agar and bacterial culture (density of 1-2x 109 CFU/ml). DMSO was used as solvent for the preparation of different concentrations (100-2500 µg/0.1ml). Mutagens are used in non toxic concentrations i.e. NPD (20 µg/0.1 ml), 2-AF (2.5 µg/0.1 ml) and sodium azide (20 µg/0.1 ml). The spontaneous reversion frequency of TA98 and TA100 was found for each experiment. Toxicity of rutin was checked against all the mutagens. The experiments were conducted in two modes i.e. co-incubation and pre-incubation mode. All the experiments were conducted in triplicates and percent inhibition of mutagenic activity was calculated as follows:

The inhibitory activity of rutin and standard was expressed as

Inhibitory activity (%) = [(a-b) / (a-c)]* 100
Where 'a' is the number of histidine revertants induced by mutagen alone (positive control), 'b' is the the number of histidine revertants induced by mutagen in the presence of rutin, and 'c' is the number of histidine revertants induced in the presence of rutin alone and solvent (negative control).The antimutagenic effect was considered strong when the inhibitory effect was more than 50%, moderate when 25-40% and less than 25% were considered weak and was not recognized as a significant result[10].

3 Antioxidant tests

The antioxidant potential of rutin was checked at various concentrations (50-1000 µg/ml).

(i) DPPH free radical scavenging assay

DPPH assay is the most widely used and simplest method to asses the free radical scavenging activity of compounds/extracts. The method described by Blois[11] was used in the present study. In this assay, deep purple color of DPPH decreases when antioxidants are present in the solution. Antioxidants donate hydrogen atoms or electron to DPPH free radicals resulting in decrease in absorbance at 517 nm. The radical scavenging activity was determined by using the following formula. % DPPH radical scavenging = (1- Absorbance of sample / Absorbance of control) ×100The samples were measured against blank (methanol).

(ii) Lipid peroxidation assay

The effect of rutin on lipid peroxidation was determined according to the method given by Halliwell and Guttridge [12]. In this experiment, lipid peroxidation was induced by ascorbate-Fe^{2+} in rat liver homogenate.

(iii) Deoxyribose degradation assay

The hydroxyl radicals scavenging effect of the rutin was checked as per method described by Halliwell et al.[13] and Arouma et al. [14]. Hydroxyl radical induces severe oxidative damage to the cells. In this method, hydroxyl radicals were generated through Fenton reaction system that degraded the deoxiribose sugar into fragments and generated pink chromogen on heating with TBA at low pH. The effect of rutin was checked in two modes i.e. site-specific and non-site specific mode.

(iv) Reducing power assay

The reducing ability of rutin was determined by the method of Oyaizu [15].

(v) Chelating power assay

The chelating ability of ferrous ions by rutin was checked as per method given by Dinis [16]. In this assay, antioxidants compete with ferrozine in chelation of Fe^{2+}. Ferrozine form a complex with Fe^{2+} and the presence of antioxidant in solution decrease the formation of ferrozine-Fe^{2+} complex by chelating Fe^{2+} resulting in decrease in colour that was checked spectrophotometrically.

3 Statistical analysis

The antimutagenic and antoxidant effects of rutin were statistically confirmed through mean, standard deviation (SD), linear regression, one-way and two-way analysis of variance (ANOVA). The differences ($p \leq 0.001$, $p \leq 0.01$) among means were compared by honestly significant difference (HSD) using Tukey's test.

RESULTS AND DISCUSSIONS

(i) Antimutagenic effects of rutin

Table 1 and 3 show the results obtained from plate incorporation method in two strains of *Salmonella typhimurium* (TA98 and TA100), either alone or in the presence of S9 mix. It is clear from the results that rutin exhibited 55.32% and 72.56% inhibitory activity at the maximum dose of 2500 µg/0.1ml/plate against NPD and 61.57% and 70.62% inhibitory activity against 2-AF in TA98 strain in co-incubation and pre-incubation modes respectively. When tested against sodium azide in TA100 strain of S. typhimurium, it showed 92.52% and 94.35% inhibition and against 2-AF it exhibited 99.25% and 99.76% inhibitory activity at the maximum dose tested in both co-incubation and pre-incubation modes respectively. All the results were found to be statistically significant in both one-way and two-way ANOVA (Table 2 and 4). Regression analysis of rutin on the percent inhibition of mutagenicity of direct-acting mutagen (NPD and sodium azide) and S9-dependent mutagen (2-AF) in TA98 and TA100 tester strain of *S. typhimurium* is given in Graph 1-4. Pre-incubation method was incorporated in these experiments so that even weak antimutagenic activity of rutin could be detected. In TA98 strain, no significant enhancement in percent inhibition was observed in experiments conducted in the presence of S9. But in TA100 strain, rutin showed a significant enhancement in the percent inhibition of mutagenicity induced by in-direct acting mutagens. In Ames assay, rutin was found effective in reducing the frame shift mutations induced by indirect acting mutagens in TA98 strain that could interfere with the metabolic activation of promutagens, by functioning as blocking agent [17]. Rutin also exhibited a strong effect against the damage induced by the direct-acting mutagens showing their protection from base pair substitution mutations in TA100. Strong inhibitory effect of rutin in pre-incubation mode explains its desmutagenicity effect, while inhibitory effect in co-incubation method explains its bioantimutagenicity effect.Desmutagens act as stage 1 inhibitors that interfere with fixation of DNA damage, while bioantimutagens act as stage 2 inhibitors that in acativate the mutagen before they could attack the DNA molecule [18]. It has been found that herbs and spices rich in phenols exhibit strong antimuagenic, carcinogenic and anti-tumor activities [19].

Table 1
Antimutagenic effect of rutin in TA98 tester strain of S. typhimurium.

Treatment	Dose (μg/100μl/plate)	TA98			
		Without S9 (-S9)		With S9 (+S9)	
		Revertants/plate	Percent inhibition	Revertants/plate	Percent inhibition
Spontaneous		27.33±1.15		27.67±2.52	
Positive control					
NPD	**20**	1018.00±31.24			
2-AF	**20**			2240.67±13.65	
Negative control	**100**	23.67±1.15		25.67±2.31	
	400	27.33±2.52		24.67±1.53	
	800	24.67±1.53		23.33±0.58	
	1000	23.00±1.00		26.00±2.00	
	1500	24.67±1.15		27.00±3.00	
	2000	26.33±1.53		25.00±1.00	
	2500	24.33±2.08		23.67±0.58	
Co-incubation	**100**	821.00±19.16	19.81±1.94	1627.33±8.33	27.69±0.40
	400	733.67±7.37	28.70±0.79	1543.33±18.88	31.47±0.84
	800	648.00±4.00	37.25±0.46	1258.67±8.50	44.29±0.37
	1000	619.33±7.57	40.07±0.72	1189.33±7.77	47.47±0.34
	1500	572.33±8.02	44.87±0.80	1076.00±23.81	52.61±1.13
	2000	516.00±5.57	50.62±0.62	943.33±7.77	58.55±0.33
	2500	468.33±6.43	55.32±0.54	675.00±6.56	61.57±0.44
Pre-incubation	**100**	755.00±7.81	26.45±0.82	1270.67±18.72	43.79±0.82
	400	700.33±6.03	32.07±0.69	1230.00±8.72	45.61±0.37
	800	567.67±11.93	45.34±1.27	1153.33±14.57	49.04±0.67
	1000	537.00±5.00	48.34±0.53	1035.33±12.34	54.42±0.55
	1500	440.67±11.06	58.12±1.18	908.33±5.69	60.19±0.29
	2000	362.33±21.73	66.12±2.28	754.33±11.93	67.08±0.57
	2500	297.00±11.79	72.56±1.13	675.00±6.56	70.62±0.28

Data shown are Mean±SD of experiments with triplicate plates/concentration/experiment.

Table 2
Statistical analysis of antimutagenic effect of rutin in TA98 tester strains of S. typhimurium.

One-way Anova	**TA98 (- S9)**	**TA98 (+S9)**
Positive control and co-incubation	F(7,16)=479.44***; HSD=40.10	F(7,16)=3950.07***; HSD=37.62
Positive control and pre-incubation	F(7,16)=669.71***; HSD=44.44	F(7,16)=4773.26***; HSD=34.58
Two-way Anova		
Co-incubation and pre-incubation		
Treatment	F(1,28)=950.77***	F(1,28)=2217.55***
Concentration	F(6,28)=1088.89***	F(6,28)=2944.61***
Treatment x Concentration	F(6,28)=32.69***	F(6,28)=137.31***
	HSD=32.285	HSD=37.829

Significant at ***p≤0.001

Table 3
Antimutagenic effect of rutin in TA100 tester strain of S. typhimurium.

Treatment	Dose (μg/100μl/plate)	TA100			
		Without S9 (-S9)		With S9 (+S9)	
		Revertants/plate	Percent inhibition	Revertants/plate	Percent inhibition
Spontaneous		240.00±3.00		244.67±6.66	
Positive control					
Sodium azide	**2.5**	1727.67±34.67			
2-AF	**20**			2479.00±47.16	
Negative control	**100**	236.33±17.04		244.00±8.54	
	400	237.00±1.00		237.00±5.29	
	800	231.67±11.72		245.00±7.00	
	1000	232.00±7.00		232.67±9.29	
	1500	229.33±4.93		231.00±5.57	
	2000	241.33±12.58		238.00±4.58	
	2500	236.33±24.01		227.33±7.77	
Co-incubation	**100**	515.00±22.87	81.31±1.08	382.00±11.79	93.83±0.83
	400	513.00±10.15	81.48±0.63	327.67±15.04	95.96±0.54
	800	457.33±28.50	84.92±1.91	302.33±9.07	97.43±0.20
	1000	457.00±19.52	84.95±0.94	275.67±15.01	98.09±1.00
	1500	400.33±8.50	88.59±0.77	272.67±10.60	98.15±0.71
	2000	354.67±8.14	92.38±0.41	261.00±7.21	98.97±0.29
	2500	348.00±13.53	92.52±0.84	244.33±8.33	99.25±0.36
Pre-incubation	**100**	527.00±17.35	80.53±2.06	340.33±12.50	95.69±0.89
	400	480.67±13.65	83.65±0.92	323.67±9.29	96.13±0.48
	800	451.33±24.34	85.33±2.29	311.00±5.57	97.05±0.16
	1000	420.33±8.08	87.41±0.94	241.67±11.02	99.60±0.90
	1500	363.67±19.86	91.04±1.56	239.33±6.81	99.63±0.54
	2000	353.00±22.54	92.49±1.81	244.00±11.27	99.73±0.32
	2500	320.67±22.03	94.35±0.93	232.67±16.04	99.76±0.85

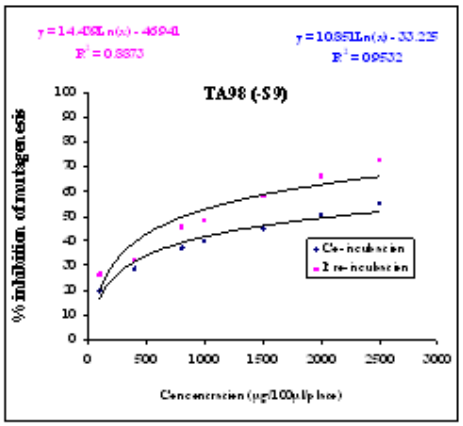
Data shown are Mean±SD of experiments with triplicate plates/concentration/experiment.

Table 4
Statistical analysis of antimutagenic effect of rutin in TA100 tester strains of S. typhimurium.

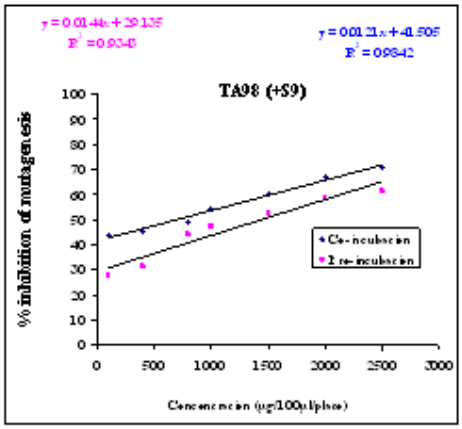
One-way Anova	**TA100 (-S9)**	**TA100 (+S9)**
Positive control and co-incubation	F(7,16)=1528.70***; HSD=57.83	F(7,16)=4584.92***; HSD=55.96
Positive control and pre-incubation	F(7,16)=1411.31***; HSD=61.13	F(7,16)=4788.44***; HSD=55.24
Two-way Anova		
Co-incubation and pre-incubation		
Treatment	F(1,28)=10.63***	F(1,28)=30.63***
Concentration	F(6,28)=91.52***	F(6,28)=102.43***
Treatment x Concentration	F(6,28)=1.72	F(6,28)=4.03**
	HSD=54.674	HSD=33.287

Significant at ***p≤0.001, **p≤0.01

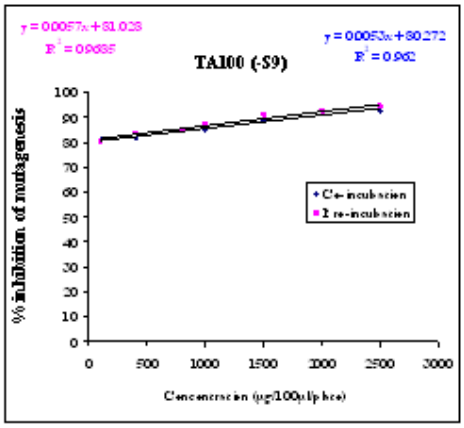
Graph 1-4
Regression analysis of rutin in TA98 and TA100 tester strain of S. typhimurium in Ames assay



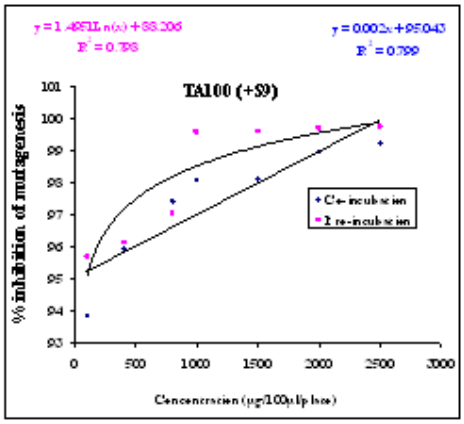
Graph 1



Graph 2



Graph 3



Graph 4

ii) Antioxidant effects of rutin

The results of antioxidant effect of rutin in different antioxidant assays are given in (Table 5 and Graphs 5-10). Rutin showed highest DPPH radical scavenging activity of 88.13% at 850 µg/ml concentration. In lipid peroxidation assay, rutin exhibited 95.57% inhibition at highest concentration tested. In deoxyribose degradation assay, rutin showed good hydroxyl radical scavenging ability in both site-specific as well as non-site specific deoxyribose degradation assay. Rutin showed 62.18% inhibition at 1000 µg/ml concentration in site-specific deoxyribose assay, while 77.89% inhibition in non-site specific deoxyribose degradation assay. Rutin exhibited moderate activity in reducing power assay. It showed maximum reducing power of 55.66% at 1000 µg/ml. It showed maximum percent inhibition of 30.96% at 1000 µg/ml in chelating power assay. One-way ANOVA for rutin represents statistically significant differences among mean percent inhibition values at $p \leq 0.01$ in all the assays (Table 6). The antioxidant properties of phenols are attributed to free radical scavenging ability, hydrogen or electron donation, chelation of metals etc. and these abilities depend upon arrangement of functional groups about the nuclear structure especially number and configuration of H-donating hydroxyl groups [20]. In the present study, rutin showed a strong radical scavenging effect in DPPH assay. It may be attributed to hydrogen and electron donating capacity of rutin by converting free radical to stable form [21]. The reducing ability of rutin was further confirmed in reducing power assay in which presence of antioxidants causes reduction of ferricyanide ions to ferrocyanide ions. Rutin showed a strong

reducing ability which may be due to the presence of hydrolysable hydroxyl groups attached to ring that can donate more electron or hydrogen atoms to free radicals. Therefore, it could be stated that most efficient free radical scavenger can also act as a powerful reducing agent. In lipid peroxidation assay, rutin showed potent antioxidant effect by reducing the process of lipid peroxidation induced by Fe^{2+}/ascorbate system in rat liver microsomal preparation. The rutin exerted this inhibitory effect on the lipid peroxidation may be either terminating the chain reaction as a result of hydrogen donation or due to the chelation of Fe^{2+} ions. When metal ion chelating ability of rutin was checked it acted as weak chelator for Fe^{2+} ions. It means rutin may lack required structure that contributes to chelation. Wang et al. [22] suggested that metal chelation reaction is dependent on the affinity of an antioxidant towards Fe^{2+} ion in relation to ferrozine. Therefore, binding constant and concentration of antioxidant also affect the chelating ability of the tested compound. The hydroxyl radical scavenging ability of rutin was cheked in deoxyribose degradation assay. In this assay, rutin exhibited a stronger concentration dependent inhibition of deoxyribose oxidation in both site-specific as well as non-site specific modes. Rutin showed more pronounced effect in non-site specific assay. In this assay, although EDTA chelate Fe^{3+} and form Fe^{3+} - EDTA complex but this complex is also effective in generation of hydroxyl radicals through superoxide driven Fenton reaction resulting in deoxyribose degradation [13]. It is therefore suggested that in non-site specific assay rutin showed its effect by scavenging hydroxyl radicals and protected the deoxyribose from hydroxyl radical mediated damage, while in site-specific assay, rutin exhibited its effect by chelation of Fe^{3+} resulting in amelioration of metal catalyzing formation of hydroxyl radical by interfering with the site-specific Fenton reaction [14,23,24].

Table 5
Antioxidant capacity of rutin in various in vitro antioxidant assays.

S. No.	Concentration (µg/ml)	DPPH Assay	Lipid Peroxidation Assay	Site-Specific Deoxyribose Assay	Non-Site Specific Deoxyribose Assay	Reducing Power Assay	Chelating Power Assay
		Inhibition (%) Mean ± SD					
1	**50**	32.89±0.52	73.95±0.35	26.77±0.45	33.33±0.30	13.76±0.12	2.75±0.54
2	**250**	84.27±0.25	78.53±0.93	37.43±0.19	57.34±0.65	44.79±0.24	13.82±0.72
3	**450**	87.04±0.45	85.64±0.58	42.20±0.70	65.54±0.26	48.45±0.09	24.26±0.72
4	**650**	87.75±0.19	89.84±0.26	48.99±0.45	69.60±0.40	51.27±0.03	28.00±0.27
5	**850**	88.13±0.44	91.90±0.35	54.99±0.10	75.65±1.04	53.94±0.09	29.14±0.28
6	**1000**	88.05±0.38	95.57±1.15	62.18±0.19	77.89±0.40	55.56±0.09	30.96±0.55
7	**IC_{50} Value**	102.02	17.61	672.14	185.99	617.79	2229.97

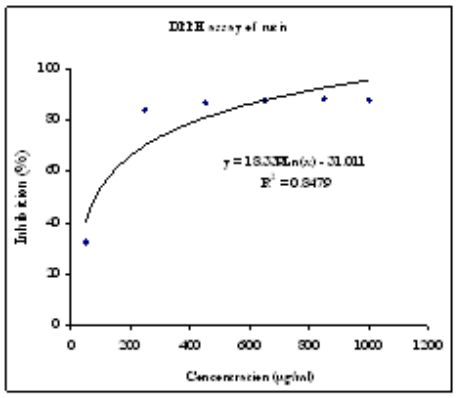
Data shown is Mean±SD of experiments performed in triplicate.

Table 6
Statistical analysis of antioxidant effects of rutin in various in vitro antioxidant assays.

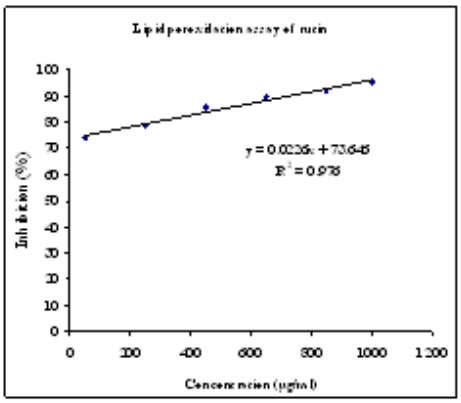
	DPPH Assay	Lipid Peroxidation Assay	Site-Specific Deoxyribose Assay	Non-Site Specific Deoxyribose Assay	Reducing Power Assay	Chelating Power Assay
F-ratio $_{(5,12)}$	9622.26***	434.32***	2986.88***	2457.01***	46235.03***	4036.86***
HSD	1.07	0.89	1.10	1.57	0.34	0.82
Regression Equation	y = 1.5451Ln(x) + 81.15	y = 0.0226x + 73.646	y = 0.035x + 26.475	y = 14.718Ln(x) - 24.283	y = 13.704Ln(x) - 36.849	y = 9.775Ln(x) - 36.629
R-value	R=0.9208**	R =0.9879***	R =0.9944***	R =0.9989***	R =0.9770***	R =0.9861***

Significant at $^{***}p \leq 0.001$, $^{**}p \leq 0.01$

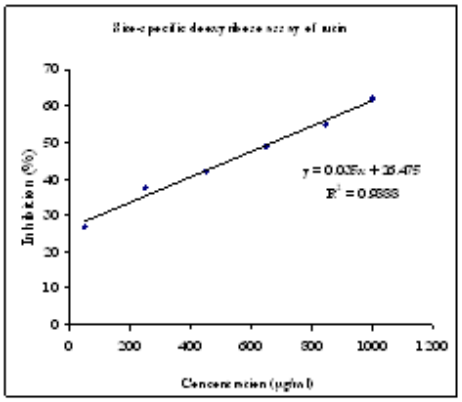
Graph 5-10
Regression analysis of antioxidant capacity of rutin in different in vitro antioxidant assays.



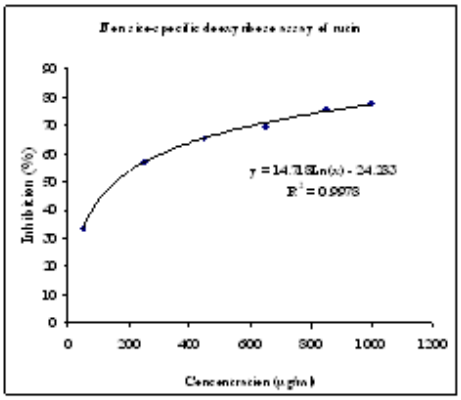
Graph 5



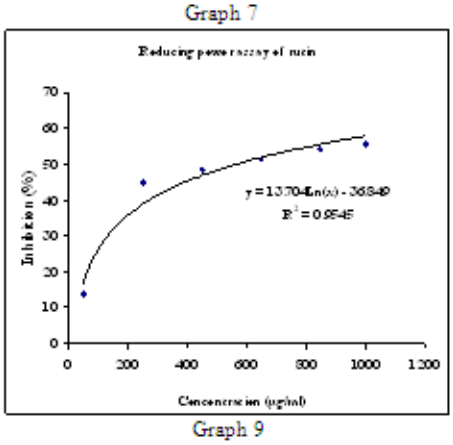
Graph 6



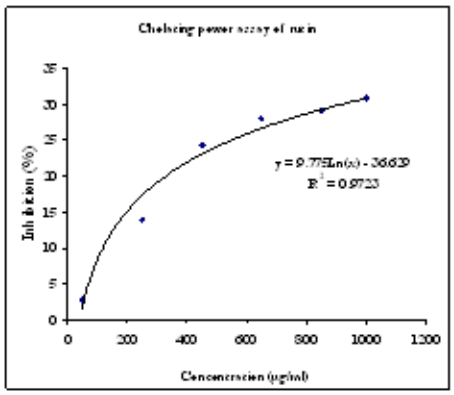
Graph 7



Graph 8



Graph 9



Graph 10

CONCLUSION

In conclusion, rutin exhibited strong antimutagenic effect against damage induced by direct acting and in-direct acting mutagens in TA98 and TA100 strains of *S. typhimurium* in both presence as well as absence of metabolic activation system in co-incubation and pre-incubation modes. In antioxidant assays, the effect of rutin may be attributed to its electron or hydrogen donating power or hydroxyl scavenging ability. Kim et al. [25] suggested that compounds that have antioxidant properties also reduce mutations induced by mutagens. Nath et al. [26] also suggested that a strong correlation exist between total phenols and antioxidant activity. However, further studies are needed to confirm the mechanisms of antimutagenic and antioxidant abilities and in vivo safety of rutin as therapeutic agent for treating free radical and mutagen mediated disorders.

ACKNOWLEDGEMENT

This work was financially supported by University Grants Commission, New Delhi (F. No.39-975/2010 (SR) dated 12-01-2011.

REFERENCES

1. Kasai H. and S. Nishimura, Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Res., 12: 2137-2145, (1984).
2. Floyd R.A., Watson J.J., Wong P.K., Altmiller D.H. and R.C. Rickard, Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. Free Radic. Res. Commun., 1: 163-172, (1986).
3. Jakus V., The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. Bratisl. Lek. Listy., 101: 541-551, (2000).
4. Yoshioka K., Deng T., Cavigelli M. and M. Karin, Antitumor promotion by phenolic antioxidants: Inhibition of AP-1 activity throughinduction of Fra expression. Proc. Natl. Acad. Sci. 92: 4972-4976, (1995).
5. Hertog M.G.L., Feskens E.J.M., Hollman P.C.H., Katan M.B. and D. Kromhout, Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet, 342: 1007-1011, (1993).
6. Janbaz K. H., Saeed S. A. and A. H. Gilani, Protective effect of rutin on paracetamol- and CCl4-induced hepatotoxicity in rodents. Fitoterapia, 73: 557-563, (2002).
7. Narasimhanaidu K. and S. M. P. Ponnaian, Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in Streptozotocin-induced diabeticWistar rats. Basic Clin. Pharmacol. Toxicol., 98, 97-103, (2006).
8. Sattanathan K., Dhanapal C. K., Umarani R. and R. Manavalan, Beneficial health effects of rutin supplementation in patients with diabetes mellitus. J. App. Pharm. Sci., 1: 227-231, (2011).
9. Maron D.M. and B.N. Ames, Revised method for Salmonella mutagenicity test. Mutat. Res., 113: 173-215, (1983).
10. Ikken Y., Morales P., Martinez A., Marin M.L., Haza A.I., and M.I. Cambero, Antimutagenic effect of fruit and vegetable ethanolic extract against N-nitrosamine evaluated by the Amest test. J. Agric. Food Chem., 47: 3257-3264, (1999).
11. Blois M.S., Antioxidant determinations by the use of a stable free radical. Nature, 26: 1199-1200, (1958).
12. Halliwell B. and J.M.C. Guttridge, Free Radicals in Biology and Medicine. In: B Halliwell and Guttridge J.M.C. (Eds.). Japan Scientific Societies Press, Tokyo, Japan, 1989.
13. Halliwell B., Gutteridge J.M.C. and O.I. Aruoma, The deoxyribose method: a simple ‘‘test tube’’ assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem., 165: 215–219, (1987).
14. Aruoma O.I., Grootveld M. and B. Halliwell, The role of iron in ascorbate dependent deoxyribose degradation. J. Inorg. Biochem., 29: 289-299, (1987).
15. Oyaizu M., Studies on product of browning reaction prepared from glucose amine. J. Nutr., 44: 307-315, (1986).
16. Dinis T.C.P., Madeira V.M.C. and L.M. Almeida, Action of phenolic derivatives (acetoaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavaengers. Arch. Biochem. Biophys., 315: 161-169, (1994).
17. Horn R.C. and V.M. Ferrao Vargas, Antimutagenic activity of extracts of natural substances in the Salmonella/microsome assay. Mutat Res., 18: 113-118, (2003).
18. Ramel C., Alekperov U.K., Ames B.N., Kada T. and L.W. Wattenberg, Inhibitors of mutagenesis and their relevance to carcinogensis. Mutat. Res., 168: 47-65, (1986).
19. Surh Y.J., Anti-tumor promoting potential of selected spice ingredients with

antioxidant and anti-inflammatory activities; short review. Food Chem. Toxicol., 40: 1091-1097, (2002).
20. Cao G., Sofic E. and R.L. Prior, Antioxidant and prooxidant behaviour of flavonoids: structure-activity relationship. Free Radical Bio. Med., 22: 749-760, (1997).
21. Jao C.L. and W.C. Ko, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. Fish. Sci., 68: 430-435, (2002).
22. Wang H., Gao X.D., Zhou G.C., Cai L. and W.B. Yao, In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaries fruit. Food Chem., 106: 888-895, (2008).
23. Arouma O.I., Methodological considerations for characterizing potential antioxidant actions of bioactive componentsin plant foods. Mutat. Res., 523-524: 9-20, (2003).
24. Guttridge J.M.C., Ferrous salt promoted damage to deoxyribose and benzoate: The increased effectiveness of hydroxyl radical scavengers in the presence of EDTA. Biochem. J., 243: 709-714, (1987).
25. Kim S.B., Kim I.S., Yeum D.M., Y. Park, Mutagenicity of Millard reaction product from d-glucose-amino acid mixtures and possible roles of active oxygen's in mutagenicity. Mutat. Res., 254: 65-69, (1991).
26. Nath M.M., Santosh Ch. And K. M. Chetty, Antioxidant activity and its correlation of different solvent extracts of male cones of Cycas beddomei Dyer, endemic taxa to Seshachalam Biosphere Reserve. Int. J Pharm. Bio Sci., 4: 1394-1403, (2013).