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IN VITRO ANTI-INFLAMMATORY (MEMBRANE STABILIZATION) AND ANTIOXIDANT POTENTIAL OF RUTIN

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ABSTRACT: Rutin is commonly found in buckweed seeds; broccoli, fruits, especially citrus fruits, red apples and berries. The chemical name for rutin is quercetin-3-rutinoside or rutoside. This bioflavonoid is responsible for endowing the fruits and vegetables with colour and flavour. This compound offers numerous health benefits. In the present study the anti-inflammatory and antioxidant potential of rutin was assessed. For anti-inflammatory studies, RBC's were collected from healthy volunteers and the hemoprotective activity of the drug was carried out at various concentrations. Likewise antioxidant potential of the drug was determined by DPPH assay and ORAC assay. The results showed that rutin has a positive effect on both the parameters. The probable reasons governing the facts are discussed in the light of previous literature.

KEYWORDS: Rutin, HRBC membrane stabilization, DPPH and ORAC.

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1. INTRODUCTION

In recent years, flavonoids and other phenolic compounds of plant origin have received increasing attention, especially in the field of pharmaceutical sciences and medicine, due to their potential to prevent a number of chronic and degenerative diseases including cancer and cardiovascular diseases [1], [2], [3], [4], [5]. Flavonoid compounds have been shown to exert a wide range of antioxidant properties *in vitro*, and most of their health-promoting effects have been attributed to their antioxidant action including their ability to scavenge reactive oxygen species (ROS), chelate metal ions and terminate free radical reactions [6]. Flavonoids act as antioxidants, direct radical scavengers,

metal ion chelators, carcinogen inactivators, modulators of gene expression, DNA repair, hormones (including neurotransmitters) and anti-hormones, inhibitors of enzyme and inducers of apoptosis [7], [8], [9], [10].Rutin, a common dietary flavonoid with a wide range of pharmacological activities is present in many plants, fruits, vegetables and red wine [11], [12], [13]. Different studies have represented the biological effects of rutin, such as anti-oxidative, anti-inflammatory, anti-hypertensive, anti-carcinogenic, cytoprotective, anti-platelet, anti-thrombic, anti-diabetic, anti-adipogenic, neuroprotective, hormone therapeautic, anti-apoptotic and cardioprotective activities [14], [15], [16], [17]. Flavonoids comprise the largest group of plant polyphenols that are found ubiquitously in significant quantities in vegetables and fruits as wells as in plant-derived food products and beverages such as tea and wine. The average daily intake of total flavonoids is estimated to be a few hundred of milligrams (expressed as aglycones), which indicates a substantial exposure of humans to these phytochemicals [7]. Many researchers recognize buckwheat as a functional food due to its high nutritional value and health promoting activities. Buckwheat is rich in flavonoids and contains the antioxidative compounds vitamins B1, B2, and E, and the chemical compounds rutin, quercetin, and tannins [18].The anti-inflammatory property may also be beneficial in treating the inflammation of the tissues of the mouth (mucosistis) following cancer treatment [19]. In view of this, the present study has been taken to determine the anti-inflammatory and antioxidant potential of rutin, to elucidate it as a potential drug for various ailments.

2. MATERIALS AND METHODS

Rutin compound was purchased from Alpha Aesar, Great Britain, and used for the present study.

In vitro Anti-inflammatory Potential

Membrane Stabilization Assay

Rutin was subjected to human red blood cell (HRBC) membrane stabilization assay to study its anti-inflammatory potential according to the method of Gandhidasan *et al*. (1991) [20]. Blood was collected from healthy volunteers and mixed with equal volume of Alsever solution. The sample was then centrifuged at 3000 rpm for 5 min. and packed cells were washed with isosaline (0.85%, pH 7.2) and a suspension was made with isosaline (10% v/v). This was taken as HRBC suspension. The assay mixture contained 1.0 mL of phosphate buffer, 2.0 mL of hyposaline, 0.5 mL HRBC suspension and 1.0 mL of various concentrations of rutin (62.5, 125, 250, 500 and 1000 mM). For standard, 1.0 mL diclofenac solution (62.5, 125, 250, 500 and 1000 mM) was added to the assay mixture instead of rutin. For control solution, instead of hyposaline, 2.0 mL of double distilled water was added. The mixtures were incubated at 37°C for 30 min. and centrifuged at 3000 rpm for 5 min. The absorbance of the supernatant solution was read at 560 nM. The percentage haemolysis was calculated by assuming the haemolysis produced by control as 100 percent. The percentage of HRBC membrane stabilization was calculated using the following formula:

$$\text{Percentage Membrane Stabilization} = 100 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

In vitro Antioxidant Potential

The antioxidant potential of rutin was evaluated by DPPH radical scavenging assay [21], [22], and ORAC assay [22], [23], [24].

DPPH Assay

1.0 mL of DPPH in methanol, 1.0 mL of methanol and 1.0 mL of various concentrations (12.5, 25, 50, 100 and 200 μM) of rutin were incubated for 10 min. in dark condition. Similarly, a control containing 1.0 mL of methanol, instead of rutin and a standard containing 1.0 mL of L-Ascorbic acid and a reagent blank containing 1.0 mL of double distilled water were used. The absorbance was measured against reagent blank at 517 nm and calculated as follows:

$$\text{Per cent Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

ORAC Assay

To a 96-well plate, 25 μL of the Trolox (Standard) and rutin at various concentrations (12.5, 25, 50, 100 and 200 μM) were added in separate wells, followed by addition of 150 μL of the fluorescein solution to each well. The contents in the wells were mixed thoroughly and the plate was incubated for 30 min. at 37ºC. Then, 25 μL of AAPH solution was added into each well to initiate free radical formation. The reaction mixture was thoroughly mixed by pipetting to ensure homogeneity. The standard and sample wells were read immediately with a fluorescent microplate reader at 37ºC with an excitation wavelength of 480 nm and an emission wavelength of 520 nM and the results were calculated by the microplate reader itself as per the formula.

$$\text{Antioxidant capacity} = \text{Sum Sample} - \text{Sum Blank} / \text{Sum Trolox} - \text{Sum Blank}$$

Statistical Analysis

The data of all assays with six replicates were subjected to statistical analysis and the mean value along with its respective standard error was calculated. The per cent change between rutin and diclofenac for membrane stabilization studies, control and experimental value for both rutin and ascorbic acid for DPPH assays and between rutin and standard trolox for Orac assay was calculated. The data were analyzed statistically using 'Two Way Analysis of Variance (ANOVA)' [25]. The data together with tables and graphs/bar diagrams are presented in appropriate places in the text.

3. RESULTS AND DISCUSSION

In vitro Anti-inflammatory Potential

Membrane Stabilization Assay

The data on anti-inflammatory potential of rutin tested with HRBC membrane stabilization assay are presented in Table 1 and Fig. 1. The per cent hemoprotection was directly proportional to the

concentration. The values are slightly increased from 2.286 ± 0.128 at 62.5 µM to 95.803 ± 0.108 at 1000 µM. When compared with standard (Diclofenac), the membrane stabilization was higher in rutin. All the data when subjected to two way ANOVA, depicted that the values are significant at P<0.001 level. The results thus reveal that rutin has hemoprotective potential.

Table 1: HRBC membrane stabilization of rutin

Sr. No.	Concentration (µM)	HRBC Membrane Stabilization (%)	
		Rutin	Diclofenac
1	62.5	2.836 ± 0.128*(+98.51)	1.428 ± 0.553*
2	125	39.313 ± 0.255*(+10.43)	36.600 ± 1.701*
3	250	94.222 ± 0.075*(+0.04)	94.185 ± 0.150*
4	500	95.227 ± 0.115*(+0.13)	95.105 ± 0.131*
5	1000	95.803 ± 0.108*(+0.09)	95.718 ± 0.055*

Values are mean ± S.E. of six individual observations.

Values in parantheses are per cent change over standard diclofenac.

+ Denotes per cent increase over standard diclofenac.*Values are significant at P<0.001.

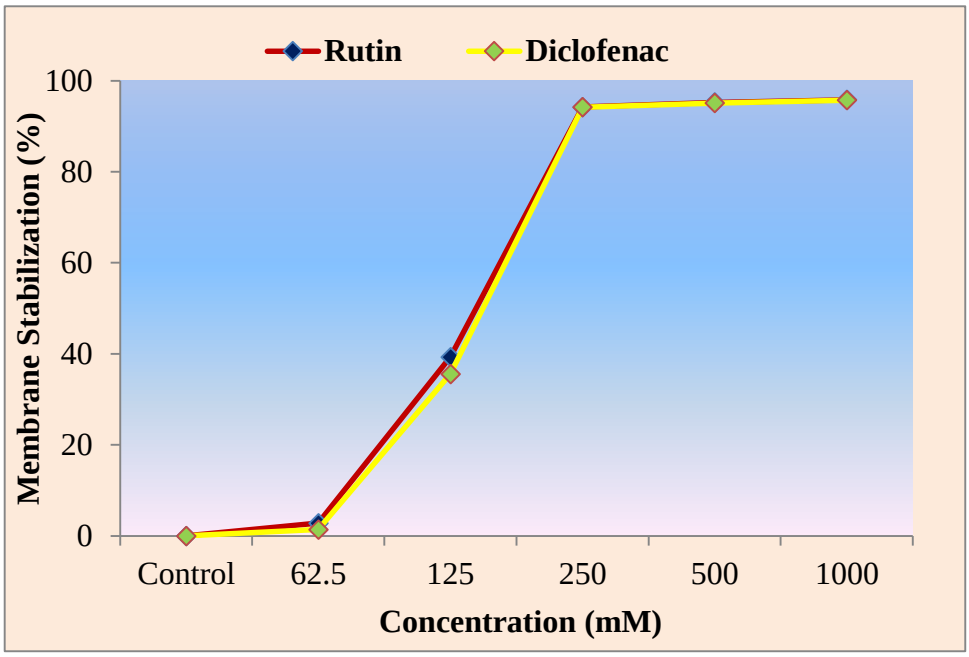

Fig. 1: HRBC membrane stabilization of Rutin

***In vitro* Antioxidant Activity**

DPPH Assay

DPPH assay was recorded as different concentrations like 12.5, 25, 50, 100 and 200 µM. At the starting concentration of 12.5 µM -52.557% DPPH inhibition was recorded in rutin, whereas in standard only -31.834% of DPPH inhibition was recorded. As the concentration increased, inhibition also increased, showing a maximum inhibition of -86.867% at 200 µM concentration of rutin,

whereas standard showed -72.786% inhibition (Table 2 and Fig. 2). The data when subjected to two way ANOVA, revealed that the values were significant at P<0.001 level. The changes in DPPH activity were significant within various concentrations of rutin as well as within standard. The results *in toto* indicated that rutin has more antioxidant potential than standard (L-Ascorbic acid). The calculated IC_{50} value for rutin was 9.488 and for standard was 46.833. The value pragmatically shows that rutin has more antioxidant potential than that of the standard.

Table 2: DPPH inhibition of rutin

Concentration (µM)	Rutin	L-Ascorbic acid
Control	1.212 ± 0	1.212 ± 0
12.5	0.575 ± 0.017*(-52.558)	0.826 ± 0.039*(-31.834)
25	0.446 ± 0.013*(-63.174)	0.792 ± 0.042*(-34.639)
50	0.221 ± 0.008*(-81.793)	0.579 ± 0.016*(-52.228)
100	0.152 ± 0.004*(-87.445)	0.439 ± 0.036*(-63.751)
200	0.159 ± 0.004*(-86.867)	0.329 ± 0.053*(-72.786)
IC50	**9.488**	**46.833**

Values are mean + S.E. of six individual observations, Values in parentheses are per cent change over control.- Denotes per cent decrease over control.* Values are significant at P<0.001.

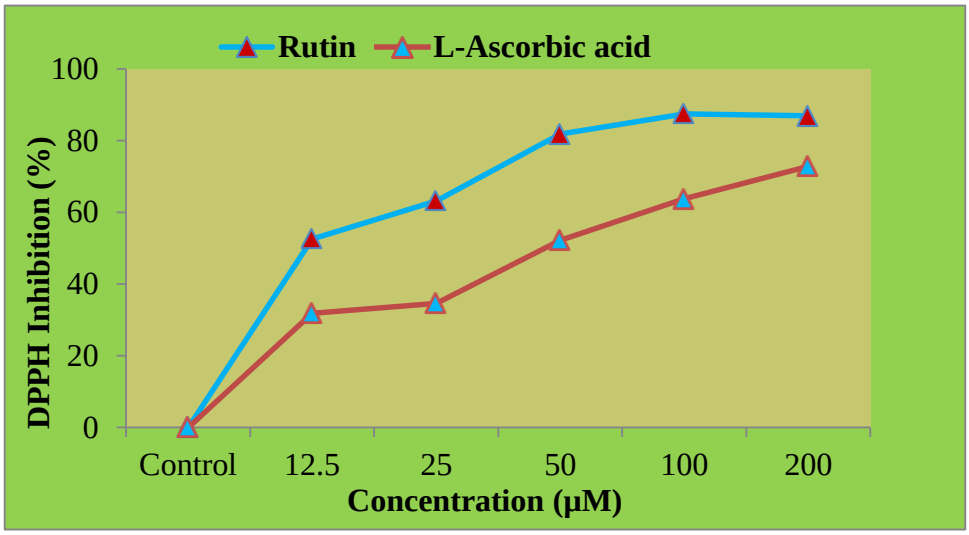

Fig. 2: DPPH assay of Rutin

ORAC Assay

Table-3 and Fig. 3 present on antioxidant potential of rutin when tested with ORAC assay. The data revealed that rutin has antioxidant potential as the NRFU value increased with an increase the concentration (12.5, 25, 50, 100 and 200 µM). The maximum NRFU value was recorded at 100 µM (79.08 ± 0.672). When the antioxidant activity of rutin was compared with that of Standard Trolox, a per cent increase was higher in rutin than that of the standard. As the concentration increased, the

antioxidant activity decreased in comparison with standard, and it was less than that of standard at 200 µM concentrations. When the data were subjected to two way ANOVA, the values were highly significant at P<0.001 level. The data altogether reveals that rutin has antioxidant potential even at lower concentrations.

Table 3: ORAC assay of rutin

Sr. No.	Concentration (µM)	Net Relative Fluorescence Unit (NRFU)	
		Rutin	Trolox
1	12.5	29.36 ± 0.858*(+68.639)	17.41 ± 0.429*
2	25	56.83 ± 1.017*(+85.114)	30.70 ± 1.239*
3	50	76.48 ± 1.032*(+27.53)	59.97 ± 1.708*
4	100	79.08 ± 0.672*(+14.81)	68.88 ± 0.510*
5	200	72.64 ± 0.962*(+2.339)	70.98 ± 0.784*

Values are mean ± S.E. of six individual observations.Values in parantheses are per cent change over standard trolox.+ Denotes per cent increase over standard trolox.*Values are significant at P<0.001.

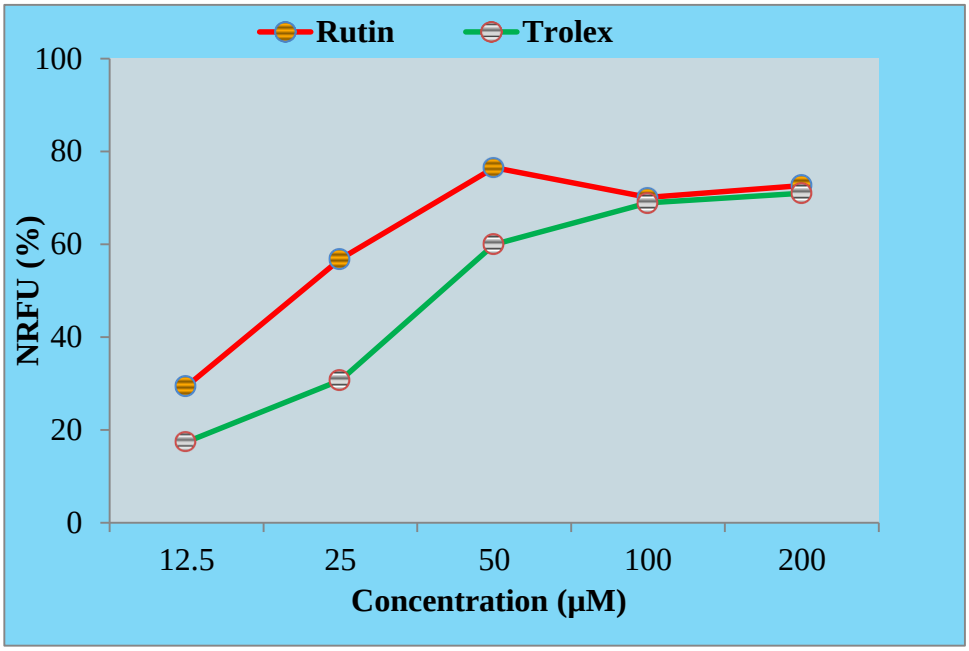

Fig. 3: ORAC assay of Rutin

DISCUSSION

Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. It is the result of concreted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti-inflammatory action [26], [27]. The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these

lysosomal enzymes or by stabilizing the lysosomal membrane [28]. Since HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. Anti-inflammatory activity of *Plectranthus amboinicus* containing flavonoids like quecertin, apigenin, luteolin, salvigenin, genkwanin, showed significant anti-inflammatory activity, which was concentration-dependent. In our study also, a concentration-dependent anti-inflammatory potential of rutin was observed, thus finding support from the above authors [27]. Anti-inflammatory drugs exhibit membrane stabilization effect by inhibiting hypotonicity-induced lysis of erythrocyte membrane, this erythrocyte membrane is analogous to the lysosomal membrane [29], and its stabilization implies that the drug may as well stabilize lysosomal membrane. Moreover, stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and protease, which cause further tissue inflammation and damage [30]. In the present investigation also, rutin might have exhibited membrane stabilization effect, thus reducing the lysis of erythrocyte membrane and proving that rutin has got anti-inflammatory potential. According to Hertog *et al*.[31], rutin (quercetin-3-rutinoside) is a glycoside of quercetin, and has been reported as one of the most commonly occurring flavonol glycosides in the human diet. The authors also added that quercetin has been reported to be one of the most predominant flavonol-type flavonoids. The average daily intake of quercetin accounts for approximately 70% of the total flavonoid intake in Western diet; with tea, onions and apples being the major contributors of flavonoids. Quercetin has been extensively studied as a pharmacological agent for its antioxidant properties and chemo-preventive effects. The protective effects of rutin against idarubicin-induced DNA damage, they recorded that rutin reduced the level of DNA damage almost completely at a higher concentration of 2 mM [5]. Similar results were obtained in our study also. In agreement with our results, the greater antioxidant capacities of aglycone flavonoids over conjugated forms against hydrogen peroxide (H_2O_2)-induced DNA damage in human lymphocytes [32]. Using vitamin C equivalent antioxidant capacity (VCEAC) assay, that glycosylated flavonoids were less effective antioxidants than their aglycone alone. In the present study also, a similar mechanism might be operating, thus proving that rutin has an excellent antioxidant potential [33]. The results indicate that rutin has anti-inflammatory and antioxidant potential and can be used as a drug for combating various ailments.

5. CONCLUSION

In conclusion, rutin compound has demonstrated promising anti-inflammatory and antioxidant potential by *in vitro* method. Increasing awareness, promotion and utilization of this polyphenolic compound (Rutin) will serve as a natural anti-inflammatory and antioxidant compound.

6. ACKNOWLEDGEMENT

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7. CONFLICT OF INTEREST

Declared none

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