Original Research Article

Investigation of antioxidant and chemoprotective potential of Rutin (a bioflavonoid) in Swiss albino mice

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Abstract

The present study is to investigate the role of Rutin in protecting against deleterious effects of doxorubicin by *i.p* administration in Swiss albino mice. Increasing concentrations of RTN significantly scavenged DPPH, Hydroxyl, Superoxide and ABTS radicals in a dose dependent fashion. The acute toxicity assessment assay showed no toxicity on days 1, 4, and 14 when mice were administered with 50 to 100 mg/kg of RTN. The $LD_{50(14)}$ value was calculated and it was found to be 195.05 mg/kg.b.wt. The optimum dose of RTN for chemo protection was selected by giving different doses of RTN before administration of DOX. The lowest mortality was observed in the animals treated with 10 mg/kg RTN. An increase in the drug dose up to 2.5 mg/kg did not significantly enhance survival compared to 10 mg/kg. Animals pretreated with *i.p* 10 mg/kg of RTN for three consecutive days before treatment with DOX showed dose-dependent increase in survival when compared with the DOX alone group. The $LD_{50/30}$ was found to be 11.2mg for DOX alone, while it was increased to 13.83mg after RTN treatment with DRF of 1.12. From the present investigation it can be concluded that Rutin showed a significant scavenging of free radicals generated *in vitro and* significantly reduced the deleterious effects of DOX and increased 30 day mouse survival with a DRF of 1.12.

Key Words: Acute toxicity; rutin; doxorubicin; mice, free radicals.

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Received Date: 12/10/2015 Revised Date: 28/11/2015 Accepted Date: 23/12/2015

Access this article online

Quick Response Code:

Website: www.medpulse.in

DOI: 28 December 2015

INTRODUCTION

Cancer, a comparatively rare disease in the past, has now become a fairly common ailment. The etiology of cancer still remains largely unknown and so is the cure[1]. Modern cancer research is directed towards the elucidation of molecular steps involved in carcinogenesis, the molecular events that specially lead to the development of cancer and understanding of various molecular events that are influenced by the drugs used for cancer treatment. The goal of the cancer therapy is to completely eradicate the neoplastic cells without causing any appreciable damage to the normal tissue of the host. This could be achieved by reversing the neoplastic cell state and/or removing the neoplastic cells completely from the host system. The, cancer is a dreadful disease, against which no unified treatment concept has emerged so far. The current proven methods of treating malignant diseases are surgery, radiotherapy, chemotherapy and immunotherapy. Chemotherapy is used at various stages of cancer. It is used to treat solid tumors (cancerous lumps) affecting organs such as the breast or bowel, as well as blood cancers such as leukemia. The goal of chemotherapy in people with early cancers is usually to kill the cancerous cells and to cure the condition. When cancer has spread to other organs, it may not be possible to cure the condition fully. In this case, the aim of chemotherapy may be to slow the progress of the disease and to extend the period of good quality life as long as possible. This is called palliative chemotherapy. All chemotherapy drugs work by attacking cells that are dividing rapidly. Chemotherapy drugs interfere with the division of these cells and may cause the complete eradication of cancer. Therefore, it is

How to site this article: B Naveen Kumar, T Kalpana. Investigation of antioxidant and chemoprotective potential of Rutin (a bioflavonoid) in Swiss albino mice. *MedPulse – International Medical Journal.* December 2015; 2(12): 970-974. http://www.medpulse.in (accessed 30 December 2015).

necessary to screen newer agents that are non-toxic and can protect. Dietary ingredients might be very useful, if they were found to protect against the deleterious effects of chemotherapeutic agents, as they will be easily acceptable, would not put an extra burden to the body and can be safely manipulated for human use without toxic manifestations. Chemotherapeutic drugs act on the cellular DNA apart from other cell components, irrespective of their nature i.e. neoplastic or non-neoplastic. Cancer chemotherapeutic agents mainly deliver their toxic effects through free radical formation and genome toxicity. Doxorubicin, a quinone-containing anthracycline is an important anticancer drug used in treating a wide spectrum of hematological malignancies and solid tumors. Iron plays an important role in DOX-induced free radical generation and oxidative damage[2]. Therefore, iron chelators may protect normal cells from DOX-induced acute toxicity. A great interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activity of these polyphenolic compounds[3]. Due to their radical-scavenging and iron-chelating properties, flavonoids can be considered as potential protectors against toxicity caused by doxorubicin. Rutin (quercetin-3-rhamnosyl glucoside), a natural flavone derivative Rutin's anti-inflammatory potential has been demonstrated in a number of animal studies[4]. In experimentally induced colitis, both pre- and post-induction treatment with rutin conferred significant preventive and healing effects[5]. The effect of rutin on chemoprotection has not been studied yet. Further, it is well known that chemotherapy produce adverse effect on the normal cells. The use of rutin may help to reduce the deleterious effects of chemotherapy in the cancer patients, undergoing treatment. However, detailed studies on its chemoprotective effect are lacking. Therefore, the goal of the present study is to investigate the role of Rutin in protecting against deleterious effects of doxorubicin by *i.p* administration in Swiss albino mice.

MATERIALS AND METHODS

RTN or doxorubicin hydrochloride (DOX) was dissolved in double distilled water (DDW) before use. The doses were expressed in mg/kg body weight (kg.b.wt.). RTN or DOX was administered intraperitoneally (*i.p.*). Four to six weeks old inbred mice of Swiss albino strain of either sex weighing 25 to 30 g were used. The animal experiments were carried with the prior approval from the Institutional Animal Ethics Committee.

Free radical scavenging by RTN

a. **Superoxide anion scavenging activity:** Phenolic compounds, particularly flavonoids and catechins, are important antioxidants and superoxide scavengers. Their scavenging efficiency depends on the concentration of phenol and the numbers and locations of the hydroxyl groups. This assay was based on the capacity of the sample to inhibit the photochemical reduction of NBT in the NADH–NBT–PMS system[6].
b. ***DPPH radical scavenging activity:*** The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule was determined by the method described earlier[7].
c. ***ABTS radical decolorisation assay:*** ABTS diammonium salt radical cation decolorisation test was performed using spectrophotometric method described by Miller and co-workers[8].
d. ***Hydroxyl radical scavenging activity:*** Hydroxyl radical scavenging assay was performed by the oxidation of deoxyribose using standard method described by Halliwell and co-workers[9].

Maximum tolerable dose (MTD) and acute toxicity and Dose Reduction Factor (DRF): The maximum concentration of RTN, which did not bring about death/severe toxic manifestations in the experimental animals, was considered as MTD. Acute toxicity of RTN was determined in terms of percent survival of animals according to the method of Prieur and co-workers[10] and with minor modification of Ghosh[11]. The animals were given freshly prepared single *i.p* dose of RTN (50, 100, 150, 200, 250 and 300mg/kg.b.wt.). After treatment, mortality was recorded and LD_{5014} was calculated. To study the optimum dose of RTN against DOX, animals were injected with *i.p.* injection of 50 mg/kg.b.wt. RTN consecutively for 3 days. And other group were injected with single *i.p.* injection of 20 mg/kg b. wt. DOX. Whereas, some of the animals were injected *i.p* with RTN (1, 2.5, 5, 10, 25 and 50 mg/kg.b.wt.) consecutively for 3 days, one hour after the last treatment, the animals of this group received 20 mg/kg.b.wt. doxorubicin hydrochloride. The animals of both the groups were also observed daily for up to 30 days post-DOX treatment. The dose reduction factor (DRF) was calculated.

RESULTS

Free radical scavenging activity of RTN: Increasing concentrations of RTN significantly scavenged DPPH radicals in a dose dependent fashion and maximally (74%) at a concentration of 120µg/ml (Figure. 1A). At higher concentrations (>120µg/ml) saturation was observed and did not exhibited any increase in scavenging activity. RTN significantly ($p < 0.05$) inhibited 2-deoxyribose degradation in a dose dependent manner (Figure 1B). Maximum inhibition was observed

at a concentration of 120µg/ml (75%). At higher concentrations (>120µg/ml) saturation was observed and did not exhibited any increase in scavenging activity. Figure 1C shows the ability of RTN to quench superoxide radicals in the PMS-NADH reaction mixture. The concentration of 120µg/ml RTN exhibited 70% inhibition and plateau thereafter at higher concentrations. The total antioxidant capacity of the RTN was calculated from the decolourization of $ABTS^{\bullet+}$, upon interaction with the RTN that suppressed the absorbance of the $ABTS^{\bullet+}$ radical and the results are expressed as percentage inhibition of absorbance as shown in Figure 1D. RTN resulted in a concentration dependent increase in free radical scavenging ability against ABTS in a concentration dependent manner, with a saturation point reaching a concentration of 120µg/ml.

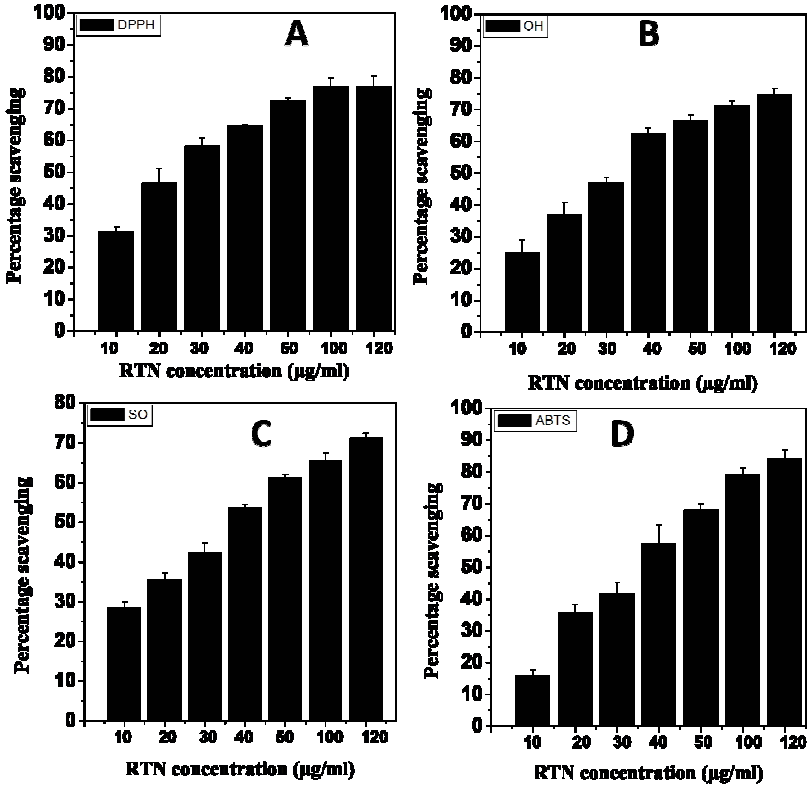

Figure 1: Effect of various concentrations of RTN on the scavenging of various free radicals generated *in vitro*. a) DPPH b) Hydroxyl c) $ABTS^{\bullet+}$ d) Superoxide anion.

Acute Toxicity Assessment: No toxicity was observed on days 1, 4, and 14 when mice were administered with 50 to 100 mg/kg of RTN. Tested RTN doses did not alter body weight and no adverse clinical signs were observed when compared with the DDW control group. However, further increase in the drug dose resulted in a corresponding decrease in the animal survival with a 50% reduction in survival at a dose of 200 mg/kg.b.wt. Animals injected with 250 mg/kg.b.wt. resulted in 70% mortality and no animals survived when the drug dose was raised to 300 mg/kg.b.wt. The $LD_{50(14)}$ value was calculated and it was found to be 195.05 mg/kg.b.wt (Table 1).

Table 1: Acute toxicity of oral administration of RTN in Swiss albino mice

RTN dose	(mg/kg.b.wt.)	Mortality (%)
50		0
100		00
150		40
250		70
300		100

Optimum dose of RTN for chemoprotection: The optimum dose of RTN for chemo protection was selected by giving different doses of RTN before administration of DOX. Treatment of mice with 50 mg/kg of RTN alone did not cause any drug induced mortality at 30 days. The majority of the animals in the DDW + DOX alone group died within in 30-days. Treatment of mice with RTN reduced mortality induced by DOX. Survival increase in all doses of RTN treated groups compared to the vehicle-

treated group. The lowest mortality was observed in the animals treated with 10 mg/kg RTN. An increase in the drug dose up to 2.5 mg/kg did not significantly enhance survival compared to 10 mg/kg. Since maximum survival was observed with 10 mg/kg of RTN, it was worth to consider as an optimal dose for radioprotection and further experiments were performed using this dose.

Dose reduction factor (DRF) in the mice treated with RTN: Animals pretreated with *i.p* 10 mg/kg of RTN for three consecutive days before treatment with DOX showed dose-dependent increase in survival when compared with the DOX alone group. The $LD_{50/30}$ was found to be 11.2mg for DOX alone, while it was increased to 13.83mg after RTN treatment. The dose reduction factor (DRF) of 1.12 was obtained (Figure 2).

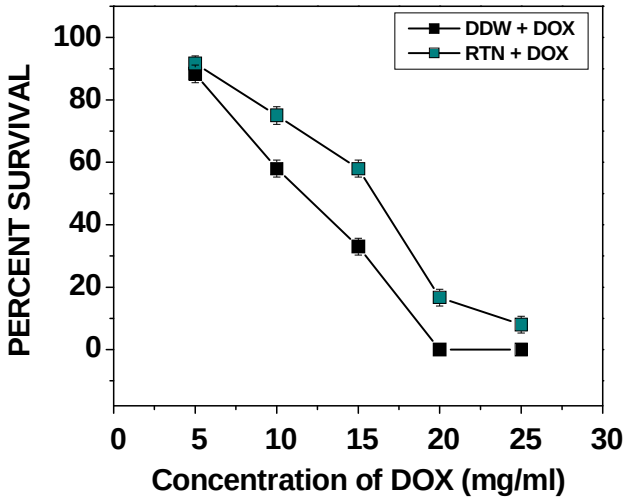

Figure 2: Dose response curves for 30 day survival of mice with or without RTN (10 mg/kg.b.wt.) before treatment with various concentration of DOX.

DISCUSSION

Cancer is the second largest killer disease in the world and more than 10 million people are diagnosed with this disease every year and results in 6 million deaths every year or 12% of deaths worldwide. It has been estimated that there will be 15 million new cases of cancer every year by 2020. Medical therapy of cancer has considerably expanded in the last decade. Therefore, it is essential to screen chemical agents that can protect the normal cells against DOX-induced cumulative toxicity. Chemicals that inhibit the cytotoxicity of anticancer drugs in various ways can act as good chemoprotective agents. Antioxidant and free radical scavenging properties of RTN may thus reduce the damage inflicted by chemotherapeutic agents. It is a well-established fact that the exposure of DOX in animals at cellular level can induce damage in the biologically important macromolecules such as proteins, lipids carbohydrates including DNA. The survival time of an animal following exposure to DOX is related to the primary target tissues affected. About 60% of the animals died within 10 days in this group, which may be due to the functional failure of the gastrointestinal tract. The remaining 40% animals died within the next seven days and death was as a result of DOX damage to the hematopoietic system in mice, pre-treatment of mice with RTN resulted in a dose dependent reduction in the DOX-induced mortality up to 10 mg/kg. Further, the decrease in the DOX dose delayed the DOX-induced mortality. About 80, 60 and 40% of animals survived after 2.5, 5, 10 and 15 DOX exposure respectively. However, more than 50% of the animals died within 10 days at 15mg/kg of DOX, which may be due to the gastrointestinal damage caused by DOX, and none of the animals survived beyond 30 days. We obtained 11.2mg as a $LD_{50/30}$ for DOX alone for our animal colony. When mice were administered with 10 mg/kg RTN before DOX, the $LD_{50/30}$ value increased to about 13.83mg. The dose reduction factor (DRF) value is calculated to be about 1.12. The DRF of 1.12 obtained in the present study for chemopotential of RTN was much lower than that of WR- 2721 (DRF of 2.7), a well known chemoprotector[12]. The chronic phase of DOX toxicity is probably mediated by preferred metabolic conversion of DOX to doxorubicinol. The DOX metabolism to doxorubicinol occurs by cytoplasmic NADPH-dependent aldose, aldehyde, and carbonyl reductases. The main mechanism of doxorubicinol toxicity is its interaction with iron and subsequent formation of ROS affecting biomacromolecules[2]. ROS can directly bind to DNA forming DNA adducts and alkali-labile sites on DNA. DNA strand breaks result following excision repair. It is therefore, conceivable that there is a close relationship between DNA adducts formation and DNA strand breaks. Under normal circumstances DNA damage always takes place but is kept to a minimum by the cell's protective mechanisms that include a repertoire of antioxidant species as well as efficient repair enzymes. However, under certain conditions, the fine balance between pro-oxidant species and protective mechanisms can be upset resulting in the circumstances termed "oxidative stress" as a result of this oxidative stress the integrity of cellular genome is adversely affected. The exact mechanism of chemoprotection by RTN is not known. However, it has been reported that flavonoid activities depend heavily on their antioxidant and chelating properties[13,14]. Being polyphenols, the flavonoids are excellent scavengers of free radicals due to high reactivities of their hydroxyl

substituents in a hydrogen atom. Both free radical scavenging and chelating properties are apparently responsible for the inhibitory effect of flavonoids on lipid peroxidation. DOX mediated toxicity is generally believed to be caused by the formation of oxygen free radicals including superoxide free radicals (15). Superoxide radicals can react with hydrogen peroxide to form highly reactive hydroxyl radicals via the iron catalyzed Haber-Weiss reaction. The secondarily derived hydroxyl radicals can cause protein and DNA damage (16). The oxygen radicals produced by DOX may interact with cellular DNA to induce DNA adducts, DNA strand breaks and alkali labile sites as observed in this study. These DNA lesions induced by DOX may be later converted into double strand breaks that subsequently become micronuclei after a cell division. This may be the reason for the increased micronuclei in the DOX treated group. Scavenging of DOX-induced free radicals may be one of the important mechanisms of chemoprotection by RTN, which is evident by a dose dependent scavenging of $^{\cdot}OH$, $O2^{\cdot-}$ and DPPH free radicals in vitro in the present study. RTN also inhibited the induction of $ABTS^{\bullet+}$ radicals in vitro efficiently and a maximum inhibition of $ABTS^{\bullet+}$ radicals (90%) was observed at the lowest concentration of 5 µM. These observations are in conformation with the earlier findings, where RTN has been reported to scavenge hydroxyl, superoxide free radicals and lipid peroxides[17]. To conclude, Rutin showed a significant scavenging of $OH^{\bullet}$, $O_2^{\bullet-}$, DPPH, $ABTS^{\bullet+}$ and NO (nitric oxide) radicals generated *in vitro* in a dose dependent manner indicating free scavenging potential of RTN. *In vivo* studies clearly demonstrated that RTN at dose of 10mg/kg.b.wt. significantly reduced the deleterious effects of DOX and increased 30 day mouse survival indicating protective potential of RTN against DOX induced toxicity.

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Source of Support: None Declared
Conflict of Interest: None Declared