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Research Article

# EVALUATION OF EFFECTS OF RUTIN ON OXIDATIVE STRESS IN DIABETIC RAT

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**ABSTRACT**

Oxidative stress which usually results from excessive production of ROS and/or diminished activity of antioxidants have been implicated as a major contributor to the etiology of severe pathologies, including diabetes. Moreover, increasing evidence shows that excess ROS acts as negative regulators of insulin signaling leading to insulin resistance, a known metabolic abnormality associated with diabetes. Oxidative stress is currently suggested as a mechanism underlying diabetes and diabetic complications. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus, are thought to be the etiology of chronic diabetic complications. In recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications. We observed a significant increase in Superoxide dismutase (SOD) and Catalase activity with the exception, an increase in activity of LPO (Lipidperioxidase) as compared to the control subjects. Rutin improved SOD and Catalase activity in diabetic rat with gastropathy when compared with the normal rat treated with vehicle.

**Keywords:** Diabetes, Rutin, SOD, LPO, Catalase

## INTRODUCTION

Free radicals which are atomic or molecular chemical species with unpaired electrons are highly unstable and can react with other molecules by giving out or accepting single electron. Oxidation processes are one of the most important routes for producing free radicals in food, drugs and even living systems. These unstable molecules are capable of causing cellular damage, which leads to cell death and tissue injury. Free radicals are linked with the majority of human diseases like ageing, atherosclerosis, cancer, diabetes, liver cirrhosis, cardiovascular disorders, etc.[1]

Oxidative stress which usually results from excessive production of ROS and/or diminished activity of antioxidants have been implicated as a major contributor to the etiology of severe pathologies, including diabetes. Moreover, increasing evidence shows that excess ROS acts as negative regulators of insulin signaling leading to insulin resistance, a known metabolic abnormality associated with diabetes.[2]

Free radicals may play an important role in the causation and complications of diabetes mellitus. In diabetes mellitus, alterations in the endogenous free radical scavenging defense mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury. [3, 4]

Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus, are thought to be the etiology of chronic diabetic complications. In recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications. Free radicals are continually produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both. Implication of oxidative stress in the pathogenesis of diabetes is suggested, not only by oxygen free-radical generation, but also due to nonenzymatic protein glycosylation, auto-oxidation of glucose , impaired glutathione metabolism, alteration in antioxidant enzymes , lipid peroxides formation and decreased ascorbic acid levels . In addition to GSH, there are other defense mechanisms against free radicals like the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) whose activities contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals. [5, 6, 7]

### Biomarkers of oxidative stress: in vivo Diabetes studies

#### Lipid Peroxidation

Hydroperoxides have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes such as malondialdehydes that will damage cell membranes. Peroxyl radicals can remove hydrogen from lipids, producing hydroperoxides that further propagate the free-radical pathway. The damage caused by LPO, is highly detrimental to the functioning of the cell[8]

#### Glutathione Levels

Reduced glutathione is a major intracellular redox buffer that may approach concentrations up to 10 mM. Glutathione functions as a direct free-radical scavenger, as a co substrate for glutathione peroxidase activity, and as a cofactor for many enzymes, and forms conjugates in endo- and xenobiotic reactions. [9]

#### Catalase

Catalase, located in peroxisomes, decomposes hydrogen peroxide to water and oxygen. Documented changes in catalase activity in chemically induced diabetic animals. For example, catalase activity is consistently found to be elevated in heart and aorta, as well as brain of diabetic rats. In contrast to decreased renal, hepatic and red blood cell catalase activity, catalase activity in liver and kidney of diabetic animals is increased.[10]

#### Superoxide Dismutase (SOD)

Isoforms of SOD are variously located within the cell. CuZn-SOD is found in both the cytoplasm and the nucleus. Mn-SOD is confined to the mitochondria, but can be released into extracellular space. SOD converts superoxide anion radicals produced in the body to hydrogen peroxide, thereby reducing the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite[11]

### Diabetes is characterized by immune system dysregulation and oxidative stress

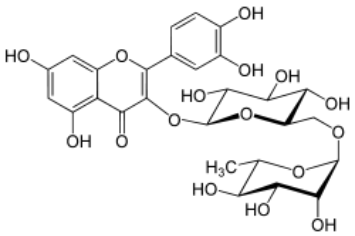
Latent autoimmune diabetes of adults (LADA) is a condition in which Type 1 diabetes develops in adults. Adults with LADA are frequently initially misdiagnosed as having Type 2 diabetes, based on age rather than etiology. Pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 diabetes. Many people destined to develop type 2 diabetes spend many years in a state of pre-diabetes which has been termed "America's largest healthcare epidemic.[12] This

altered redox equilibrium accentuates inflammatory injury, in which antioxidants may therefore have a therapeutic role. Oxidative stress plays a major role in the pathogenesis of diabetic macro- and microvascular complications. Ascorbate is of particular importance as it protects serum lipids from oxidation as reflected in plasma oxidized cholesterol. It is associated with both blood lipid and HbA1C reduction, perhaps reflecting its pro-repair and antioxidant functions. Improving glycemic control enhances the action of ascorbate since high glucose concentrations can reduce ascorbic acid uptake by as much as 40% [13,14]

**Drug Profile**

Rutin

**Structure**



2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)β-D-glucopyranosyloxy]-4*H*-chromen-4-one

**Other Names:-**Rutoside, Phytomelin, Sophorin, Birutan, Eldrin, Birutan Forte, Rutin trihydrate, Globularicitrin, Violaquercitrin

**Properties:-**

Molecular formula :$C_{27}H_{30}O_{16}$

Molar mass:610.517 g/mol

Exact mass:610.153385 u

Appearance :Solid

Melting point:242 °C, 515 K, 468 °F

Solubility in water: Insoluble

Rutin, also called rutoside, quercetin-3-O-rutinoside and sophorin, is the glycoside between the flavonoids quercitin and the disaccharide rutinose (α-L-Rhamnopyranosyl-(1→6)β-D glucopyranose). In fava d'anta, the synthesis is done via a rutin synthase activity.[15]

**Occurrences**[16]

Rutin is a citrus flavonoid glycoside found in buckwheat, the leaves and petioles of Rheum species, and asparagus. Rutin is also found in the fruit of the fava d'anta tree (from Brazil), fruits and flowers of the pagoda tree, fruits and fruit rinds (especially citrus fruits (orange, grapefruit, lemon, lime)) and berries such as mulberry, ash tree fruits and cranberries. Its name comes from the name of Ruta graveolens, a plant that also contains rutin. It is sometimes referred to as vitamin P, although not strictly a vitamin. Rutin is one of the primary flavonols found in *Clingstone* peaches[17]

**Chemical relatives**

Rutin (quercetin rutinoside), as well as quercitin, is a glycoside of the flavonoid quercetin. As such, the chemical structures of both are very similar, with the difference existing in the Hydroxyl functional group. Both quercetin and rutin are used in many countries as medications for blood vessel protection, and are ingredients of numerous multivitamin preparations and herbal remedies. Rutin is found in many plants, especially the buckwheat plant Fagopyrum esculentum Moench, Family Polygonaceae[18]

**Health effects**[19, 20, 21, 22]

- Rutin inhibits platelet aggregation, as well as decreasing capillary permeability, making the blood thinner and improving circulation.
- Rutin shows anti-inflammatory activity in some animal and *in vitro* models.
- Rutin inhibits aldose reductase activity Aldose reductase is an enzyme normally present in the eye and elsewhere in the body. It helps change glucose into the sugar alcohol sorbitol.
- Rutin also strengthens the capillaries, and, therefore, can reduce the symptoms of haemophilia. It also may help to prevent a common, unpleasant-looking, venous edema of the legs; however, a double-blind clinical study on the effect of buckwheat tea containing rutin did not show a significant effect above placebo.[38]
- Rutin, as ferulic acid, can reduce the cytotoxicity of oxidized LDL cholesterol and lower the risk of heart disease.[23,24]
- There is also some evidence that rutin can be used to treat hemorrhoids, varicosis, and microangiopathy. Rutin is also an antioxidant; compared to quercetin, acacetin, morin, hispidulin, hesperidin, and naringin, it was found to be the strongest However, in other trials, the effects of rutin were lower or negligible compared to those of quercetin.[25]

**MATERIALS AND METHODS**

SD rats of female sex were used. They were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle). Animals were allowed to take specified amount of standard laboratory feed and water *ad libitum*. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC).

**Methods**

**Induction of Diabetes using Alloxan rat model**

Rats were made diabetic by intraperitoneal injection of alloxan (hydrate) at a dose of 120 mg/kg. Alloxan was first weighed individually for each animal according to the body weight and solubilized with 0.5 ml of normal saline. It was then injected to 18 hr. fasted diabetic group rats to induce hyperglycemia and was maintained during the time of the study by the reinforcement of 100 mg/kg alloxan (*ip*) at day 12 and 21 after the first administration.[26,27]

**Collection of Blood samples**

All rats fasted for 18 h prior to the determination of blood glucose levels on day three, 15 and 57. During this time interval, 1.0 ml of blood was withdrawn from the retrorbital plexus under light ether anesthesia and centrifuged at 3000 rpm to separate plasma and cells. The plasma was used to estimate glucose levels.[28]

**Determination of blood glucose**

The plasma glucose levels were estimated using the glucose oxidase-peroxidase (GOD-POD) method with the glucose GOD-POD kit. Blood glucose levels were expressed as mg/dl. The rats showing a fasting glucose of more than 170 mg/dl three days after the first administration of alloxan were considered diabetic.[29]

**Evaluation of oxidative stress**

**Preparation of tissue homogenate**

After receiving the treatments for 56 days, the rats were sacrificed using deep ether anesthesia on the 57th day. The stomach were removed and thoroughly washed with ice-cooled 0.1 M phosphate buffered saline (PBS) containing 0.1 mmol/L phenylmethanesulfonyl fluorides. The individual tissue was blotted dry and homogenized in 0.1 M PBS in an ice bath to prepare a 10% suspension. This suspension was then centrifuged at 16000 × g for 1 h in a cooling centrifuge at 0℃. The supernatant was employed to assess the parameters of oxidative stress after estimating the protein content.[30, 31]

**Lipid peroxidation (LPO)**

To 2 ml, 5% suspension of separated RBC in 0.1 M phosphate buffered saline, 2 ml of 28% trichloroacetic acid was added and centrifuged. One ml of 1% thiobarbituric acid was added to 4 ml of supernatant, heated in boiling water for 60 min and cooled

immediately. The absorbance was measured spectrophotometrically at 532 nm. The lipid peroxidation was calculated on the basis of the treated experimental group.[32, 33]

**Superoxide dismutase (SOD)**

It was estimated in the 10% tissue homogenate to 50 µl of the tissue homogenate, 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol were added. An increase in absorbance was recorded at 420 nm for 3 min by spectrophotometer (Schimadzu 1601, Japan). One unit of enzyme activity is 50%inhibition of the rate of autoxidation of pyrogallol as determined by change in absorbance/min at 420 nm.The activity of SOD is expressed as units/mg protein.[34]

**Catalase (CAT)**

Catalase activity was determined in erythrocyte lysate using Aebi's method with some modifications. 50 µl of the lysate was added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and 1ml of 30 mM $H_2O_2$. Catalase activity was measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of $H_2O_2$, 43.6 M $cm^{-1}$ was used to determine the catalase activity. One unit of activity is equal to one millimoles of $H_2O_2$ degraded per minute and is expressed as units per milligram of protein.[35]

**Statistical Analysis**

Data were analyzed using Graph Pad Prism version 5.00 for Windows (Graph Pad Software, San Diego, CA, USA). Significance was analyzed using ANOVA and SEM. unless otherwise indicated; data are presented as the mean values (± SEM). The groups of experimental rats were compared to the appropriate normal groups. Differences were considered significant when $p < 0.05$.

**RESULTS**

- In table 1 and fig.2 shows that tissue levels of lipid peroxidation, in terms of MDA, were found to be significantly ($p < 0.0001$) of diabetic rats. After 4 weeks Rutin treatment in diabetic rats reduced lipid peroxidation
- In table 1 and fig.5 shows that tissue levels of SOD, were found to be significantly ($p < 0.0001$) of diabetic rats. After 4 weeks Rutin treatment in diabetic rats increased SOD
- In table 1 and fig.8 shows that tissue levels of catalase, were found to be significantly ($p < 0.0001$) of diabetic rats. After 4 weeks Rutin treatment in diabetic rats increased catalase and increased antioxidant enzyme levels to near normal
- Treatment of Rutin significantly restored LPO and antioxidant enzyme levels.

**Table 1: Oxidative stress**

| Groups | Treatment | LPO | SOD | CAT |
|---|---|---|---|---|
| Non-diabetic | Normal | 32.88±0.9754 | 180.6±0.7259 | 203.2±0.8451 |
| | Normal+d1 | 24.75±0.8539 | 189.5±0.6455 | 215.5±0.6455 |
| | Normal+d2 | 19.50±0.6455 | 196.5±0.6455 | 226.± 2.273 |
| Diabetic | Alloxan+vehicle | 71.37±0.2814*** | 113.3±1.622* | 119.8±1.789** |
| | Alloxan+d1 | 31.85±0.5420* | 151.4±3.094* | 179.2±2.812* |
| | Alloxan+d2 | 22.88±0.2681* | 155.6±2.655** | 192.0±0.8121** |

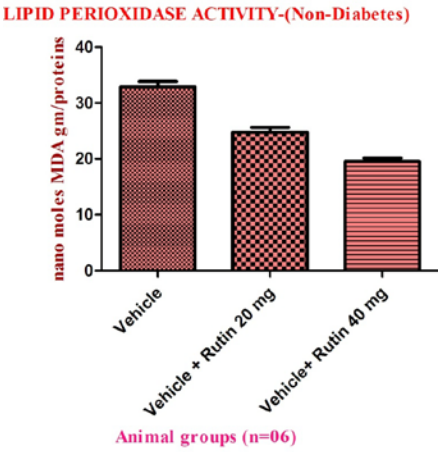



**Fig. 1: Effect of Rutin on LPO in Nondiabetic rat**

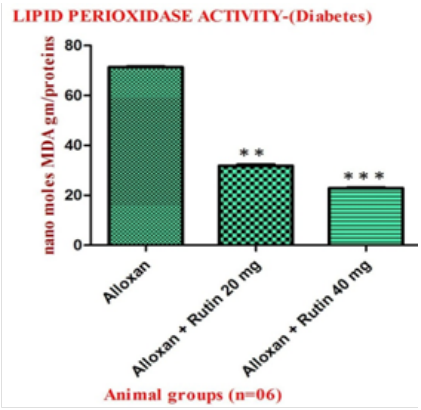



**Fig. 2: Effect of Rutin on LPO in Diabetes rat**

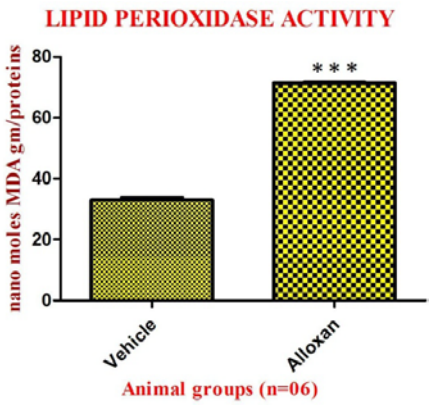



**Fig. 3: Effect of Alloxan on LPO in Normal rat**

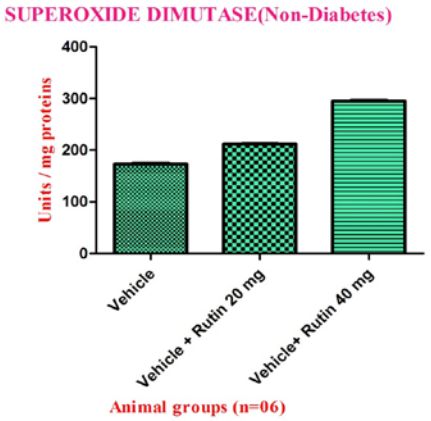



**Fig. 4: Effect of Rutin on SOD in Nondiabetic rat**

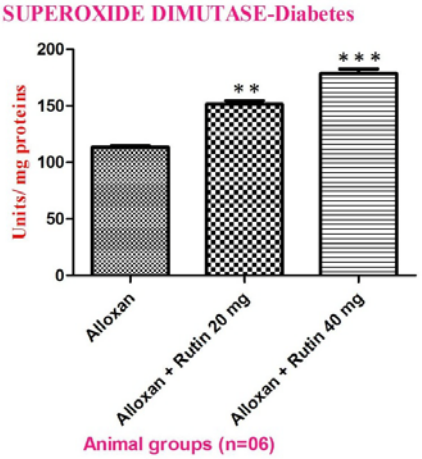



**Fig. 5: Effect of Rutin on SOD in Diabetes rat**

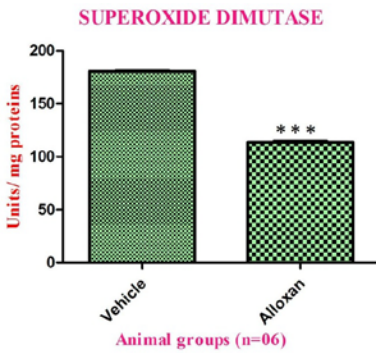



**Fig. 6: Effect of Alloxan on SOD in Normal rat.**

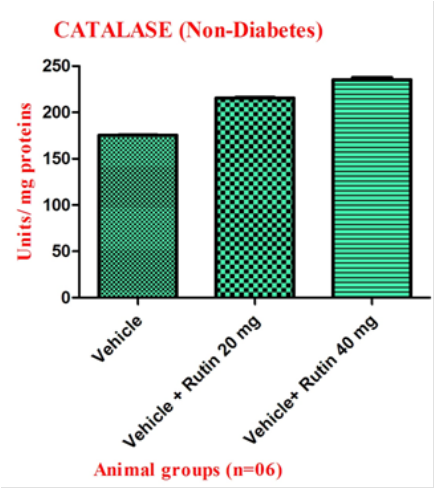



**Fig. 7: Effect of Rutin on Catalase in Nondiabetic rat**

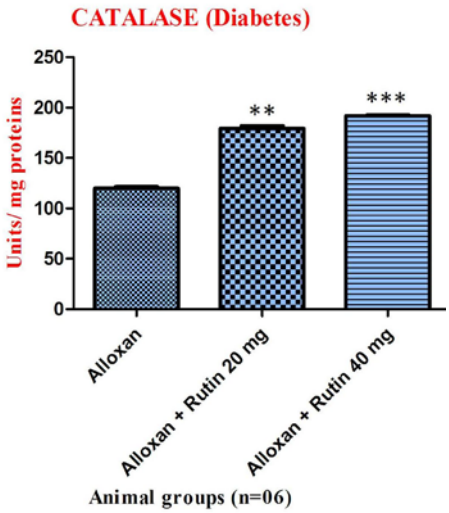



**Fig. 8: Effect of Rutin on Catalase in Diabetes rat**

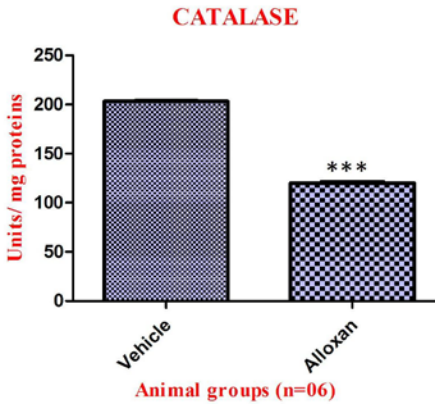



**Fig. 9: Effect of Rutin on Catalase in Normal rat**

## DISCUSSION

It was observed a significant increase in Superoxide dismutase (SOD) and Catalase activity with the exception, an increase in activity of LPO as compared to the control subjects. However the fasting blood sugar (FBS) levels were found significantly increased in the present study, Rutin improved SOD and Catalase activity in diabetic rat with gastropathy when compared with the normal rat treated with vehicle. Rutin is one of these candidate inhibits aldose reductase activity. It was observed that as per fig.No.6 and & 9 Alloxan decreased the SOD time and Catalase activity, which was increased significantly in fig no 5 and 8 in which 20 mg and 40 mg Rutin was administered respectively. The comparative study clearly indicates in fig 5 and 8 that the SOD and Catalase activity was increased significantly in diabetic rat with gastropathy.

Rutin decreased LPO activity in diabetic rat with gastropathy when compared with the normal rat treated with Alloxan decreased in activity of LPO is indicated in fig.no 3 and increase in LPO activity is indicated in Fig no 2 when Rutin was administered to Diabetic rat. The comparative study clearly indicates in fig 2 that the LPO activity was decreased significantly in diabetic rat with gastropathy.

**CONCLUSION**

Results obtained from this study indicates that, daily use of Rutin as a natural product supplement, may be a new choice for diabetic patients, as it bears a therapeutic potential to treat alloxan-induced gastropathy. These activities may possibly be due to presence of anti-oxidant activity which indirectly helped to decrease the levels of glucose, prevent the alteration of lipids and increase antioxidant status in diabetic gastropathy. Thus, improvement of gastrointestinal functions such as gastric emptying and intestinal transit may be a new tactic in diabetic condition with gastroparesis. However further clinical investigations are still required

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