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RESEARCH ARTICLE

Alloxan Induced Oxidative Stress and Impairment of Oxidative Defense System in rats

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ABSTRACT

Objective: To study the activity of statins and alloxan induced oxidative stress and oxidative defense system in rats.

Method and Materials: Either sex of Wistar rats were used for studying the activity of statins (Atorvastatin) and oxidative stress is induced by the induction of alloxan (100mg/kg) and statins acts as antioxidants which suppressed the oxidative stress. Animals were divided into five groups and each groups having six animals. Freshly prepared alloxan (100 mg/kg) i.p. were given to third group at first day and forth group at first day then followed by statin (30mg/kg), orally, up to seven day and fifth group at last day of treatment to produced oxidative stress. And investigate the defense mechanism of statins which suppressed the oxidative stress produced by the alloxan.

Result: With the help of investigation of biochemical parameters of different groups of animals we found out that atorvastatin showed significantly suppressed the oxidative stress produced by the alloxan and impaired the oxidative defense system in rats.

Conclusion: From the investigation it was found that alloxan produced the oxidative stress and statins impaired the oxidative defense system in rats.

Keywords: Oxidative stress, Atorvastatin, Alloxan.

1. INTRODUCTION

Oxidative stress indicates the intracellular accumulation of reactive oxygen species and nitrogen compounds, mainly the so called reactive oxygen species (ROS). The major ROS variants are hydrogen peroxide (H2O2), hypochlorous acid (HOCl), superoxide ($^{\cdot}O_2^+$), hydroxyl (OH), peroxyl ($RO2^+$) and hydroperoxyl ($HO2^+$) in mitochondrial respiration, ROS are generated in the electron chain, as a byproduct in the ATP generating process. This occurs in situation of enhanced oxidation of energy substract such as glucose and FFA, unless uncoupling compensates and prevents ROS formation.[1] The enzyme NAD (P) H oxidase play a key role to stimulate ROS formation and it can be activated by various cytokines. Statins are one of the most widely prescribed medications to patients in the high- risk group of developing cardiovascular diseases (CVDs) to lower total serum and cholesterol levels. Statins inhibit 3- hydroxyl-3-methylglutaryl coenzyme A (HMG- CoA) reductase in the cholesterol biosynthesis, thereby lowering serum low density lipoprotein (LDL) receptors in the liver, and in turn resulting in decreased levels of circulating total cholesterols. In addition to their major effects on serum low density lipoprotein (LDL) cholesterol, statins also exert wide variety of other effect on cellular metabolism. These effects of statins, known as pleiotropic effects include modulation of gene expression, reduction of endothelial dysfunction, inhibition of muscle proliferation, and induction of apoptosis. Several studies have suggested that statins' pleiotropic effects may provide

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greater protection against pathogenesis of CVDs than their cholesterol lowering effects.[2]

2. MATERIALS AND METHODS:

Procurement of drug

Statins was purchased from the local market of Mandsaur M.P.

Procurement and selection of animals

Either sex of wistar rats weighing between 180-200 gm were obtained from B.R.Nanata College of pharmacy Mandsaur Animal House. The animals were stabilized for 1 week; they were maintained in standard condition. They had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. All the experiments were carried out between 09:00 and 15:00 h. The experimental protocols were approved by Institutional Animal Ethics Committee of B. R. Nahata College of Pharmacy, Mandsaur, (M.P.).

Drugs and Treatment

According to protocol Atorva**s**tatin were given orally (30mg/kg) and alloxan dissolved in normal saline (100 mg/kg) and given intra peritoneal routes.[3]

Experimental Procedure-Rats were divided into five groups and each group having six animals, after induction of oxidative stress animals are divided into Group-1: Normal control, received normal saline daily, Group-2: Received Statin-daily dose 30 mg/kg orally up to seven day treatment, Group-3: Received Alloxan (100 mg/kg) i.p. single dose at first day, Group-4: Received Alloxan (100 mg/kg) i.p.at first day then followed by statin daily and Group-5: Received Statin (30 mg/kg, orally) daily for seven day then single dose of alloxan injection.

Induction of oxidative stress- Oxidative stress is produced by induction of freshly prepared alloxan monohydrate (100 mg/kg) i.p. and given to third group and in fourth group followed by statin 30 mg/kg orally and induced alloxan at last day to fifth group.

Biochemical estimations

On the 8^{th} day of investigation, the animals were sacrificed by decapitation. The brains, liver, kidney were removed, rinsed in isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared with 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction for enzyme assay was obtained by centrifugation of the homogenate at 12,000 ×g for 20 min, at 4 °C.

Measurement of Lipid peroxidation: (brain, kidney, liver)

Took 0.5 ml homogenate and 0.5 ml Tris-HCL (P^H- 7.4) and incubated at 37^0C for 2 hours Then 1 ml 10% TCA (Trichloro acetic acid) was added then Centrifuged at 1000 x g for 10 min to 1 ml supernatant, 1 ml of 0.67% TBA (Thiobarbituric acid) were added, Kept the tubes in boiling water bath for 10 min, Cooled the solution and added 1 ml of distilled water then absorbance were measured at 532 nm using UV spectrophotometer.[4]

Estimation of Reduced Glutathione (GSH)

1 ml of homogenate was precipitated with 1 ml of 4% sulfosalicyclic acid by keeping the mixture at 4^0C for 1 hour immediately Centrifuged at 1200 xg for 15 min then 1 ml of supernatant, 0.2 ml of DTNB (Dithiobisnitrobenzoic acid) and 2.7 ml of phosphate buffer (0.1 M, P^H-8) were taken then the yellow color was measured at 412 nm using UV spectrophotometer[5]

Catalase Estimation

Catalase estimation was measured on the basis of breakdown of hydrogen peroxide (H_2O_2) at 240 nm. Assay mixture consisted of 3ml of H_2O_2, phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%) and change in absorbance recorded at 240 nm. The result was expressed as micromole H_2O_2 decompose/mg of protein/min.[6]

Statistical Analysis

The statistical analysis was done using GraphPad Prism software demo version 5 and result were expressed in mean ± SEM and data were compaired by one way ANOVA followed by Dunnett's test and $p < 0.05$ considered as significant , $p < 0.01$very significant and $p< 0.001$ is considered as highly significant.[7]

3. RESULT:

Determination of Lipid peroxidation, Catalase (CAT), Glutathione (GSH), and Superoxide dismutase (SOD): In liver homogenate normal animals (group 2) treated with statin showed significantly increase in GSH level while decrease in CAT, GSH and Lipid peroxidation. In oxidative stress animal treated with statin (group 4 and group 5) showed significant decrease in Lipid peroxidation while significant increase in CAT, GSH and SOD level as compared to oxidative stress control animals (group 3).

Determination of Catalase (CAT), Glutathione (GSH), and Superoxide dismutase (SOD): In Brain homogenate normal animals (group 2) treated with statin showed significantly increases the CAT while decrease the SOD and GSH level as compared to normal control group 1. In oxidative stress animals treated with statin (group 4 and 5) showed significant increase the CAT, SOD, GSH level as compared to oxidative stress control animals (group 3).

4. DISCUSSION:

Oxidative stress and tissue damage are common phenomena linked to exposure to toxic agents and occurring in several diseases, including diabetes. In our study the significant finding is that statin prevented the oxidative stress which is produced by the induction of alloxan and cause diabetes in our animals (rats). Alloxan administration produced a marked oxidative impact, as evidenced by the significant rise of lipid peroxidation products (TBARS) and the significant decline of

endogenous antioxidants, including GSH content, and SOD and CAT activities, in the liver and brain. The decrease in GSH level in both the liver and brain of alloxan-treated rats might be attributed to the inhibition of its regenerating enzyme glutathione reductase (GSHR), regression of the antioxidant recycling mechanism in diabetic and the direct reaction between GSH and ROS generated by alloxan .Additionally, tissue containing reduced SOD and CAT activities might have enhanced

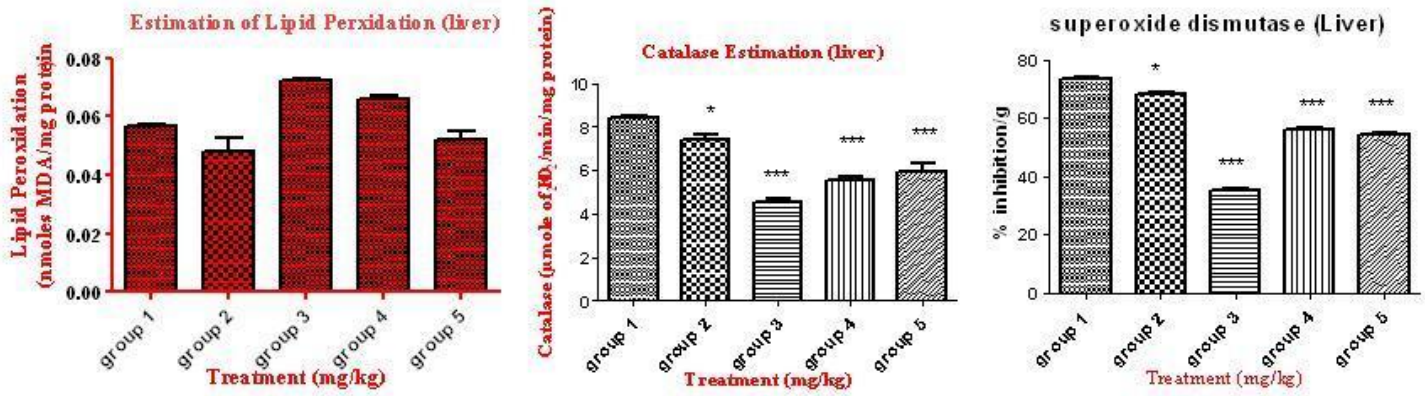

Fig 1: Effect of statin (30 mg/kg) on lipid peroxidation, SOD, Catalase level in various group of rats liver which produced oxidative stress by alloxon induction.

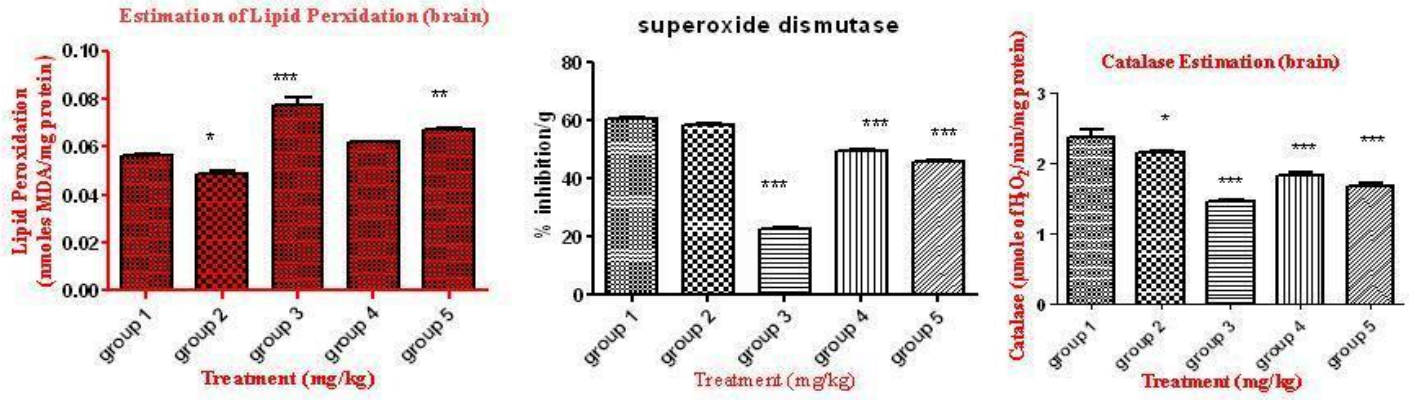

Fig 2: Effect of statin (30 mg/kg) on catalase, SOD, LP level in various group of rats brain which produced oxidative stress by alloxon induction

superoxide radicals and hydrogen peroxide which could potentially inhibit GSH-R activity and consequently decreased GSH in the liver and brain.

Atorvastatin reduces Rac-1 translocation and oxidative stress in endothelial cells exposed to high glucose and also Atorvastatin blunts vascular Rac-1 activity and oxidative stress in diabetes mellitus. Our experimental evidence further supports the concept that restriction of vascular oxidative stress is a fundamental goal in the treatment of diabetes mellitus.[8]

Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals.[9] Glucose oxidation is believed to be the main source of free radicals. In its enediol form, glucose is oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals.[10] The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals Superoxide anion radicals, can also react with nitric oxide to form reactive peroxynitrite radicals.

Antioxidant defense mechanisms involve both enzymatic and non enzymatic strategies. Common antioxidants include the vitamins A, C, and E, glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Other antioxidants include - lipoid acid, mixed carotenoids, coenzyme Q10, several bioflavonoid, antioxidant minerals (copper, zinc, manganese, and selenium), and the cofactors (folic acid, vitaminsB1, B2, B6, B12). They work in synergy with each other and against different types of free radicals.[11]

Vitamin E suppresses the propagation of lipid peroxidation; vitamin C, with vitamin E, inhibits hydro peroxide formation; metal complexing agents, such as penicillamine,bind transition metals involved in some reactions in lipid peroxidation and inhibit Fenton and Haber-Weiss-type reactions; vitamins A and E scavenge free radicals.[12] Due to induction of alloxan, oxidative stress is occurred in various group of our animals and due to effect of alloxan ROS are generated, and free radicals are produced which damage the cell membrane and cellular components which also leads to myocardial infarction and Alzheimer disease.[13] But statin produced antioxidant which suppresses the activity of free radicals which is generated by reactive oxygen species.[14]

5. CONCLUSION:

The statins, by inhibiting HMG-CoA reductase activity, reduce cholesterol and isoprenoid synthesis. They are being used primarily in preventing stroke and may increase bone formation and lower the risk of dementia. Due to induction of alloxan, oxidative stress is occurred in various group of our animals and due to effect of alloxan ROS are generated, and free radicals are produced which damage the cell membrane and cellular components which also leads to myocardial infarction and Alzheimer disease. But statin produced antioxidant which suppresses the activity of free radicals which is generated by reactive oxygen species.

6. ACKNOWLEDGEMENT

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Conflict of Interest: None Declared

