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RESEARCH ARTICLE

Effect of Leptin Analogue and Leptin Antagonist on Hypercaloric Diet Induced Oxidative Stress

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ABSTRACT

The objective of our study was to investigate tissue lipid peroxidation and antioxidant status in mice receiving leptin analogue and leptin antagonist along with hypercaloric diet for a period of 8 weeks. Significantly elevated levels of tissue MDA and lowered levels of reduced glutathione, superoxide dismutase (SOD) and catalse enzymes were observed in the lungs of mice fed with hypercaloric diet as compared with the control mice fed with a standard pellet diet. Subsequent to the treatment with leptin analogue and leptin antagonist was simultaneously administered along with hypercaloric diet for 14 days. Leptin administration significantly lowered the tissue MDA and elevated the activities of reduced glutathione (GSH), SOD and Catalase while opposite effect was reported with leptin antagonist in standard disease control mice. Thus leptin supplementation was found to be effectively improving hypercaloric diet induced oxidative stress.

KEYWORDS

Obesity, Asthma, SOD, MDA, Catalase, GSH

INTRODUCTION

Obesity is a nutritional disorder associated with several co-morbid diseases which includes hypertension, asthma, diabetes mellitus and hyperlipidemia. Each of these co-morbidities alone can increase the oxidative stress burden. Obesity is an inflammatory disorder characterized by higher levels of oxidative stress biomarkers compared with their leaner counterparts[1]. Also weight gain is one of the parameter significantly increases the concentration of these biomarkers[2]. There are multiple sources for oxidative stress in relation to obesity. Increased adipose tissue and visceral adiposity are significantly correlated with systemic levels of oxidative stress biomarkers[3]. Adipose tissue-mediated systemic oxidative stress and systemic inflammation may be secondary to increased leptin-to adiponectin ratio and increased levels of other adipokines, such as tumor necrosis factor[4].

Oxidative stress in obesity has a cumulative effect that favors the development of end-organ damage. It is unclear that whether obesity-mediated oxidative stress directly affects the lung or not. Evidence suggests that weight gain, diabetes, and poor glycemic control with

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obesity are associated with reduced lung function[5].

Compared with non-asthmatic subjects, subjects with asthma have increased systemic oxidative stress[6]. It is also found that children with asthma had higher plasma levels of MDA with healthy controls, and the MDA levels were higher in children with more severe asthma. This is partly explained by the fact that acute asthma exacerbations can worsen the degree of oxidative burden. In severe asthmatic, the increased airway concentrations of MDA, macrophages in bronchoalveolar lavage (BAL) fluid correlate with decreased levels of reduced glutathione (GSH), increased oxidized glutathione (GSSG). Present investigation was to prove that whether oxidative stress could potentially play a role in mediating obesity associated asthma.

At present there are limited treatment options available for these two co-morbid conditions. Even current treatments also have numerous side effects. Therefore there is need for identification of newer cost-effective treatment approach for asthma with obesity. Present investigation was done to find possible role of leptin as an anti-oxidant in pathogenesis of asthma with obesity. We investigated effects through high caloric diet induced obesity model and with ovalbumin induced asthma model in Swiss albino mice.

MATERIALS AND METHODS

Swiss albino mice of female sex weighing between 24 ± 6 g were obtained from the central animal house of faculty of pharmacy, Dharmsinh Desai University, Nadiad. The animal studies were approved by the Institutional Ethics Committee (DDU/FOP/06/17). Animals were kept individually in polypropylene cages in an environmentally controlled room of the animal house and maintained at a temperature of 25 ± 2°C with a 12 h dark and light cycle. 7 days of acclimatization were provided to animal. The animals were provided water and food ad libitum. Mice were fed with standard pellet diet or special high caloric diet according to the protocol. Composition of experimental diet (gm/kg diet) was according to Soni *et al.*[7].

Experimental Design

In this study, a total 60 mice were used and divided in to ten groups of 06 mice each.

Induction of Obesity State

High caloric diet control mice were maintained on the high caloric diet for eight weeks to induce obesity.

Induction of Asthma State

Normal control mice maintained on laboratory pellet chow diet for to eight weeks and then induction phase asthma was started. Mice were sensitized with Ovalbumin (OVA) conjugated to aluminum hydroxide as well as challenged with saline to induce asthma. The induction with Ovalbumin was done on day 1 to day 23 but challenge was for every 7th days for three weeks. During asthma induction phase mice were fed with laboratory pellet chow diet with water *ad libitum*.

Group No.	Group detail	No. of animal
I	Normal Control	06
II	Obese group	06
III	Asthmatic group	06
IV	Obese asthmatic group	06
V	Obese + Leptin	06
VI	Asthmatic + Leptin	06
VII	Obese asthmatic + Leptin	06
VIII	Obese + Leptin Antagonist	06
IX	Asthmatic + Leptin Antagonist	06
X	Obese asthmatic + Leptin Antagonist	06
Total		60

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Induction of Obesity and Asthma State

High caloric diet control mice maintained for up to eight weeks and then induction phase asthma was started. Mice were sensitized with Ovalbumin (OVA) conjugated to aluminum hydroxide as well as challenged with saline to induce asthma. The induction with Ovalbumin was done on day 1 to day 23 but challenge was for every 7th days for three weeks. During asthma induction phase mice were fed with high caloric diet with water *ad libitum.*

Leptin analogue (0.4 mg/kg, i. p. for 7 days) and leptin antagonist (3 mg/kg, p. o., for 7 days) were given intra-peritoneal or oral gavage respectively. At the end of experimental period the animal were anaesthetized with ketamine, following overnight fasting and assayed for antioxidant enzymes. Only lungs were excised out, washed thoroughly with saline and blotted dry. A 10% (w/v) homogenate was prepared in Tris-HCI buffer (0.01 M, pH 7.4) containing 0.25 M sucrose. The enzyme extract was obtained by centrifuging at 8000g for twenty minutes.

Measurement of Anti-Oxidant Parameters

Super Oxide Dismutase (SOD), Catalase, Reduced glutathione (GSH) and MDA were measured for the assessment of oxidative stress condition[8].

Estimation of Super Oxide Dismutase (SOD)

SOD activity was determined by use a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. The autoxidation of pyrogallol in the presence of EDTA in the pH 8.2 is 50%. The principle of this method is based on the competition between the pyrogallol autoxidation by $O_2^{\bullet-}$ and the dismutation of this radical by SOD[9].

(Cu-Zn) SOD activities are expressed as units/ml.

$$\text{(Cu-Zn) SOD Activity (U/ml)} = \frac{\text{\% inhibition of pyrogallol autoxidation}}{50\%}$$

One unit of (Cu-Zn) SOD activity being defined as amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation[10]. Absorption was read at the wavelength of 420 nm against Tris-EDTA buffer at zero time and after 1 minute of the addition of pyrogallol.

Estimation of Catalase

Catalase was assayed colorimetrically at 620 nm and expressed as moles of H2O2 consumed/min/mg protein as described by Sinha11. The reaction mixture (1.5 mL) contained 0.01 M pH 7.0 phosphate buffer (1.0 mL), tissue homogenate (0.1 mL) and 2 M H2O2 (0.4 mL). The reaction was stopped by the addition of dichromate-acetic acid reagent (2.0 mL, 5% potassium dichromate and glacial acid were mixed in 1:3 ratio).

Estimation of GSH

GSH activity was determined by the procedure of12. The assay solution contained 10% BSA, 50 mM Phosphate buffer (pH = 7.6), 2 mM NADPH, 20 mM GSSG. Absorbance at 340 nm was recorded at a temperature of 25 °C. The activity was calculated the using the molar coefficient for NADPH of 6.22 µ·mol−1 × cm−1 and expressed in U/gm tissue.

Estimation of Malondialdehyde (MDA)

Thiobarbituric acids (TBARS) content, a measure of lipid peroxidation, was assayed in the form of Thiobarbituric Acid Reacting Substances (TBARS) according to Ohkawa et al.13. Briefly, a reaction mixture consisting of 8.1% sodium dodecyl sulphate (0.2 mL), 20% acetic acid solution (1.5 mL) adjusted to pH 3.5 with sodium hydroxide and 0.8% aqueous solution of thiobarbituric acid (1.5 mL) was added to 10% (w/v) of PMS (0.2 mL). The mixture was brought up to 4.0 mL with distilled water and heated at 95 °C for 60 min. After cooling with tap water, distilled water (1.0 mL) and the mixture of n-butanol and pyridine (15:1 v/v, 5.0 mL) was added and the mixture centrifuged. The organic layer was removed and its absorbance was measured at 532 nm and compared with those obtained from MDA

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standards. The concentration values were calculated from absorption measurements as standard absorption.

Statistical Analysis

Statistical evaluation of analytical data was done by one-way analysis of variance (ANOVA) followed by Tuckey's test using statistical software GraphPad Prism 3.0. Data were expressed as mean ± standard error of the mean (SEM) and $P<0.05$ was considered statistically significant.

RESULTS

Endocrinological effect of leptin and leptin antagonist in the state of obese asthmatic condition were evaluated using various anti-oxidant parameters.

Effect of Leptin Analogue and Antagonist on

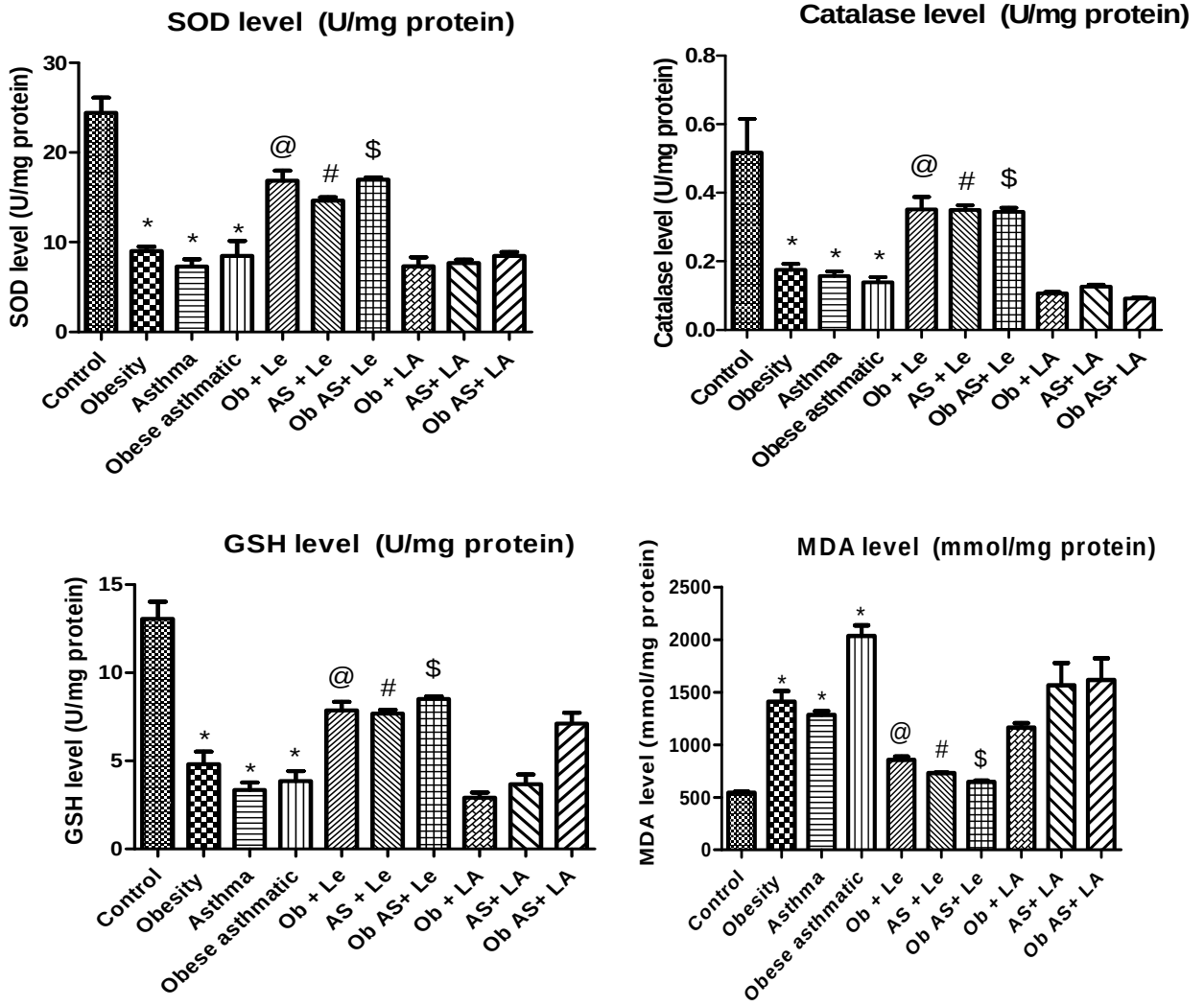

Figure 1: Effect of leptin analogue and leptin antagonist on antioxidant parameters like SOD, Catalase, Reduced glutathione (GSH) and MDA. All values are expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test (n=6)

*p<0.05 when compared with Control (Group-I)

@ p<0.05 when compared with standard control group of obesity (Group-II)

# p<0.05 when compared with standard control group of asthma (Group-III)

$ p<0.05 when compared with standard control obese asthmatic group (Group-IV)

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SOD

Seven day administration of leptin analogue in disease control groups reveal antioxidant enzymes such SOD, Catalase and Reduced glutathione (GSH) level significantly ($p<0.05$) decrease except MDA level increase, in asthma, obese and obese asthmatic group. While in case of leptin antagonist there is no any change in anti oxidant enzyme levels (Figure 1).

DISCSSION

Asthma symptoms such as dyspnea and wheezing appear as result of excess of thoracic and abdominal fat deposition[14]]. Obesity is disorder of inflammation and energy imbalance, occurring when calorie expenditure is less compared to high caloric food intake[15].

We investigated changes in anti-oxidant parameters in animals with obesity, asthma and obese asthmatic condition after exogenous leptin analogue and leptin antagonist. SOD, Catalase and GSH levels were significantly decreased in asthma animal, obese animals and obese asthmatic animals when compared with normal control animals. It has been previously reported that decreased level of anti-oxidant parameters indicate free radical generation and damage through them[16]. Furthermore, increased level of MDA also indicates lipid peroxidation. Therefore it may be suggested that reactive free radical aggravates the asthmatic condition.

In present study, it is observed that leptin administration improve the anti-oxidant parameters. Contrary administration of leptin antagonist non-significantly affects anti-oxidant parameters. So we conclude that leptin may be beneficial role in obesity associate asthma.

CONCLUSION

From our study we conclude that higher level of MDA levels with decline in SOD, catalasse and GSH level responsible for oxidative stress which is solely factor for aggravation of asthma in obese condition. This co morbid condition could be improve by leptin analogue. However further studies need to be undertaken to determine efficacy as well as combination of leptin analogue with standard drugs of asthma and obesity to improve these co-morbid condition.

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