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Studies on the effect of lycored supplementation (Lycopene) on lipid peroxidation and reduced glutathione in pregnancy induced hypertensive patients.

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Abstract

The evidence of oxidative stress in pregnancy induced hypertensive subjects in comparison with normal pregnant women and lycored ingested pregnancy induced hypertensive subjects was evaluated by measuring the malondialdehyde and reduced glutathione. The level of malondialdehyde in pregnancy induced hypertensive subjects was significantly increased ($p<0.001$) from 4.35±0.74 nmol/ml to 6.50±0.75 nmol/ml and the level of glutathione decreased significantly ($p<0.01$)from 0.396±0.062 mg/ml to 0.283±0.0298mg/ml in pregnancy induced hypertensive subjects while the level of malondialdehyde in lycored ingested pregnancy induced hypertension patients was significantly decreased ($p<0.001$) by 40% and the level of reduced glutathione significantly increased ($p<0.001$) by 33.92% as compared to pregnancy induced hypertensive patients. These observations suggesting that pregnancy induced hypertensive patients are more susceptible to oxidative stress / damage while lycored might have beneficial role for pregnancy induced hypertensive patients since the imbalance between oxidant: antioxidants had improved.

Key words: Malondialdehyde (MDA), Reduced Glutathione (GSH), Oxidative Stress (OS), Pregnancy induced hypertension (PIH).

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Introduction

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to considerable extent [1]. During normal pregnancy, many gestational changes occur in the maternal body like enlargement of uterus with increase in blood supply, increase in cardiac output, increase in total blood volume and increase in interstitial fluid volume. In spite of tremendous physiological changes in maternal body, pregnancy posses over as a normal physiological process with no adverse mediated effect on woman's health during or after pregnancy. However pregnancy induced hypertension (PIH) is a multisystemic disorder affecting virtually every organ and system. The incidence of hypertension varies among different hospitals, regions and countries. In India, hypertension complicates approximately 10% -15% pregnancies and is a major contributor to maternal and fetal mortality and morbidity [2]. Pre-eclampsia and eclampsia are amongst those conditions in which hypertension essentially complicates the pregnancy with edema and /or albuminiria. Recently, the oxygen derived free radicals have been reported to play an important role in the pathogenesis of PIH. It is envisaged that increased free radical activity arises from increased production of free radicals or deficiency in protective antioxidant system [3,4]. It has been hypothesized that a placental oxidant – antioxidant imbalance, intensifies the release of lipid peroxidation into circulation. Vascular contact with circulating peroxidation products causes dysfunction of vascular endothelium by promoting peroxidative damage of endothelial cell membrane which ultimately initiates the maternal pathological changes [5,6]. Very recently, it was discovered that a new Carotenoid compound called lycopene, a red pigment which is present in large concentration in lycored tablet, is thought to play an important role in defense against chronic diseases like cancer, coronary heart disease etc. [7,8,9,10]. Lycopene is a Carotenoid compound, an acyclic isomer of β-carotene and does not show any pro vitamin A activity. It is highly unsaturated hydrocarbon containing eleven conjugated and two unconjugated double bounds. It is the most predominant carotenoid in human plasma present naturally in greater amount than β-carotene and other dietary carotenoids. This per-

haps indicates its great biological significance in human antioxidant defense system [11]. No much study has been done on the beneficial effects of lycopene in PIH patients. So, the aim of present work was to study the effect of lycored (Lycopene) on ROS induced oxidative stress in PIH patients by measuring local oxygen radical substance such as lipid peroxidation and antioxidant molecule glutathione (GSH).

Material and Methods

The present study was carried out in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynecology, Guru Teg Bahadhur Hospital, Govt. Medical College- Amritsar. The study included on ninety subjects. The subjects were selected from the patients attending antenatal clinics in Out Patients Department (OPD) of Guru Teg Bahadhur Hospital, Govt. Medical College- Amritsar. All the subjects were ranging 20 to 30 years with similar low socio-economic status and dietary habit. The subjects were divided into following three groups as follows:

Group 1: Normal pregnant women. (Control group)

Group 2: Pregnancy induced hypertensive women and

Group 3: Pregnancy induced hypertensive + Lycored ingested women.

Control group included one hundred subjects of normal pregnant women, who did not have any history of hypertension. PIH group (Group -2) included thirty hypertensive patients of pregnant women. The cases were selected on the ground of specified criteria [12] and in Group -3; an antioxidant lycored (containing 2000µg of lycopene) was given for one month at a dose of one bd (bis in di) to one hundred hypertensive pregnant women. After one month, the patients were clinically examined for blood pressure and blood samples were collected for the estimation of various biochemical assays.

Blood Sampling

Blood samples were drawn from all the subjects following a fast of 12 hours with a dry disposable syringe and needle, under all aseptic conditions by venepuncture in the anticubital vein in sterile, dry and acid washed vials and 10ml of blood was collected in ethylenediaminotetra acetic acid (EDTA) vial. Plasma was separated by centrifuging the blood at 3000rpm for 20 minutes at 4°C. This plasma was used for the estimation of malondialdehyde (MDA) and reduced glutathione (GSH). These biochemical assays were analyzed on the same day of blood collection from all the groups.

Biochemical Assays

Malondialdehyde (MDA):

MDA level in serum was estimated by measuring the pink colored chromophore formed by the reaction of thiobarbituric acid with malondialdehyde according to the method of Satoh, 1978 [13].

Reduced Glutathione (GSH)

GSH level in plasma was estimated by the method of Beutler et al 1963, using 5-5' dithiobis2-notrobenzoic acid [14].

Statistical Analysis

Numerical data were presented as mean values ± S.D. The statistical significance was evaluated by student's't' test.

Result and Discussion

MDA, representing lipid peroxidation was found to be significantly ($p < 0.001$) increased by 49.42% (from 4.35±0.74 nmol/ml to 6.50±0.75 nmol/ml) in PIH patients (Table 2). A significant increase in MDA level in PIH patients indicating the excessive formation of free radicals and activation of lipid peroxidation system. Free radical mediated lipid peroxidation leads to the formation of volatile breakdown products including aldehyde, ketone, alcohol etc and resulting in the irreversible damage to the placenta [15,16,17], whereas the level of MDA in lycored ingested PIH patients (group-3) was found to be significantly decreased ($p < 0.001$) by 40 % (from 6.50 ± 0.75 nmol/ml to 4.75 ± 0.67 nmol/ml) with respect to PIH patients (Table 2). This observation suggesting that lycored play an important role in maintaining the level of thiobarbituric acid reacting substance (MDA) to near normal by acting as a good chain breaking antioxidant. Lycored might react with peroxy radicals, which are formed in propagation phase of lipid peroxidation to form carbon centered radical. The carbon centered radical reacts readily and reversibly with oxygen to form a new chain carrying peroxyl radicals, which are more highly stable forms than reactive oxygen species and thereby inhibiting lipid peroxidation.

Glutathione is an important antioxidant metabolite, which counters oxidative stress by eliminating the compounds responsible for lipid peroxidation or by increasing the efficiency of NADPH that protects detoxifying enzymes [17,18]. A significant decrease ($p < 0.05$) by 28.53% was observed in GSH level in PIH patients with respect to control subjects (Table-2), and this could be due to its increased utilization in protecting 'SH' containing proteins from lipid peroxides. This observation of decrease in

GSH level is in agreement with the report that inverse relationship exists in GSH and lipid peroxidation [19]. Glutathione depletion to about 20% can impair the cells defense against the toxic action of xenobiotic and may lead to cell injury [20,21], while the level of GSH in lycored ingested PIH subjects was significantly increased ($p < 0.001$) by 33.92% as compared to PIH patients (Group-2) from 0.283 ± 0.0298 mg/ml to 0.379 ± 0.0621 mg/ml (Table 2). This could be due to the ability of lycored to protect the 'SH' groups from oxidative damage through inhibition of peroxidation of lipids.

The results obtained from the above study suggested that lycored offers protection to the PIH patients by preventing the inactivation of GSH thereby inhibiting the peroxidation of lipids and resulting in the maintenance of membrane integrity and cellular status.

Table 1: Demographic details of normal pregnancy, Pregnancy induced hypertension andpregnancy induced hypertension + Lycored women.

Parameters	**Group - 1 (Control)** (n=30)	**Group - 2 (PIH)** (n=30)	**Group 3 (PIH + Lycored)** (n=30)
Age (years)	29.40 ±2.55	28.46 ± 2.34	28.46 ±2.33
Gestational Age	30.30±3.91	22.11±2.26	22.11 ± 2.26
Systolic BP (mmHg)	120.33±2.40	149.73±2.96	130.40 ±1.40
Diasystolic BP (mmHg)	76.26±.61	99.90±3.00	80.20±2.70

Table 2: Plasma levels of Malondialdehyde and Reduced Glutathione in normal regnancy, Pregnancy Induced Hypertension and Pregnancy Induced Hypertension + Lycored women.

Biochemical assays	**Group 1** (Control)	**Group 2** (PIH)	**Group 3** (PIH + Lycored)
Malondialdehyde (nmol/ml)	4.35 ± 0.74[a]	6.50 ± 0.75 (49.42) [b]***	4.75 ± 0.67 (-40.00) [c]***
Reduced Glutathione (mg/ml)	0.396 ± 0.062	0.282 ± 0.0298 (-28.53) [b]**	0.379 ± 0.0621 (+33.92)[c]***

a: Mean ± S.D. of 30 observations.
b: Values in parentheses representing percent change with respect to Group-1.
c: Values in parentheses representing percent change with respect to (Group-2).
***p < 0.01, ***p < 0.001*

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