Effects of *Indravati Rasa* on lipid peroxidation of diabetes induced rats

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Abstract

Diabetes mellitus is a group of metabolic disorders. Ayurveda physicians have proved that diabetes mellitus may be controlled by some herbo-mineral preparations. Among there, *Indravati Rasa* has strongly been recommended for *Madhumeha* in various *Rasashastra* texts. Hyperglycaemia increases free radicals generation and free radical mediated injury leads to an increase production of malondialdehyde, a marker of lipid peroxidation. Circulating levels of malondialdehyde is higher in diabetic subjects. In this research, *Indravati Rasa* was evaluated for its possible effects on experimental diabetes mellitus and on lipid peroxidation in albino rats. Inbred Charles Foster albino rats 100-140.0 g were selected for this study. Streptozotocin in the dose of 70.0 mg / intra peritoneal produced significant increase in blood glucose and serum malondialdehyde at 48 h. *Indravati Rasa* in the dose of 133.0 mg/kg and 266.0 mg/kg was administered orally in two groups of rats. Analysis of data showed that *Indravati Rasa* attenuated the hypoglycaemic state, but it was statistically insignificant ($p>0.05$). *Indravati Rasa* (133.0 mg/kg) produced significant lowering of streptozotocin induced blood malondialdehyde level ($p<0.05$). The present findings suggest that the *Indravati rasa* at low doses (133.0mg /kg) possesses significant effect on lipid peroxidation.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia [1]. Diabetes mellitus represents a syndrome with disordered metabolism glucose due to either absolute deficiency of insulin secretion or reduction of its biologic effectiveness or both [2]. Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of langerhans in the pancreas leading to insulin deficiency. This type of diabetes can be further classified as immune-mediated or idiopathic [3]. The majority of type 1 diabetes is of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack. There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages [4]. Type 1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children.

Treatment of diabetes mellitus still remains one of the great challenges in the medical profession [5]. Ayurveda has elaborately mentioned that *prameha* is one of the *Maha roga* and *Madhumeha* is one sub type of *prameha* which is said to be incurable and it resembles closely to diabetes mellitus [6]. Ayurveda is a holistic and divine science. *Rasa shastra* is a branch of learning in Ayurveda pharmaceutics specially deals the medicine formulated with minerals, metals, precious stones, marine products and certain poisonous herbs. After a long time experience, ancient Ayurvedic physicians have proved that *Madhumeha* may be controlled by such herbo mineral preparations, among which *Indravati Rasa* is strongly recommended for *Madumeha* in various *Rasashastra* texts [7]. For the present study, *Indravati Rasa* was selected from *prameharoga Adhikara* of Bhaishajya Ratnawali [8] and it contains *Rasa sindura* (red-sulphide of mercury), *Vanga bhasma* (incinerated tin) and *Arjuna twak churna* (*Termenalia arjuna* Wight and Arn) in equal amount, this mixture is subjected to *bhavana* (triturating) [9] with *Shalmali mula swarasa* (juice of root bark of *Salmalia malabarica*) for seven days and finally it prepared in *vati* (pill) form. Dosage of *Indravati Rasa* mentioned in this text is one *masha* (1.0g) per day.

Most of herbo mineral preparations mentioned in *Rasashastra* are said to be potent in eliminating dreadful diseases and also rejuvenative, therefore the herbo mineral preparation *Indravati Rasa* has been selected for the study and effort has been made to evaluate for its possible

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hypoglycaemic effect and lipid peroxidation status in Type-1 Diabetes (Insulin Dependent Diabetes Mellitus - IDDM) experimentally. Increasing appreciation of the causative role of oxidative /injury in many disease states places great importance on the reliable assessment of lipid peroxidation. Malondialdehyde (MDA) is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products [10]. Lipid peroxidation refers to the oxidative degradation of lipids in cell membranes. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lies methylene-CH2-groups that possess especially reactive hydrogen [11].

Materials and Methods

Animals

Inbred Charles Foster albino rats of either sex weighing between 100-140.0 g were procured from central animal house, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. All the animals were kept in colony cages at an ambient temperature of 25±2°C, with 45-55% relative humidity and 10:14 light and dark conditions. The animals were kept on standard rodent feed and water *ad libitum*. All the experimental animals acclimatized in the department for 3 days before experiment. All the experiments were conducted after Institute Ethical Committee approval and the principles of laboratory animal care and use (NIH Publication N0 86-23, reviced 1985) guidelines were adhered throughout the study.

Drugs and Chemicals

Drugs

(1). *Indravati Rasa* (containing red-sulphide of mercury, Vanga Bhasma (Incinerated Tin), Arjuna Twak Churna (*Termenalia arjuna* Wight and Arn) in equal amounts and triturated with Shalmali moola swarasa (juice of root bark of *Salmalia malabarica*) for 7 days and prepared 250 mg sized pills were prepared).

(2). Insulin (Human Insulin – Regular) (Zydus Pharmaceuticals).

Chemicals

1. Streptozotocin (Sigma Aldrich U.S.A).
2. Citrate buffer (21.2g of citric acid monohydrate and 29.2 g of citrate were dissolved in one liter of carbon dioxide free water which served as stock solution) 280 ml of citric acid solution and 220 ml of trisodium citrate solution were mixed together.
3. Potassium chloride (KCl – 1.15%) was prepared by dissolving 1.15 g of KCl of 50 ml of distilled water was added to make the final volume of 100 ml.
4. Sodium lauryl sulphate (SLS 8.1%) was prepared by dissolving 8.1g of SLS in distilled water to a final volume of 100ml. Complete dissolution was achieved by gently heating in the water bath.
5. Acetic acid (20% pH 3.5): 20 ml acetic acid was mixed with 80 ml of distilled water and pH was adjusted to 3.5 by slowly adding 0.1 sodium hydroxide.
6. Thiobarbituric acid (TBA-O.8) (Merks comp): 800 mg of TBA was dissolved in 100 ml distilled water. Complete dissolution was achieved by slightly heating in the water bath.
7. n-Butanol/pyridine (15:1v/v) Solution of n-Butanol and pyridine in the ratio of 15:1was prepared by mixing 75ml of n-Butanol with 5ml of pyridine.
8. Tetraetoxypropane (TEP) was used as an external standard for obtaining the standard curve.
9. Potassium chloride, sodium lauryl sulphate, acetic acid, n-Butanol, pyridine and1, 1', 3, 3'–Tetraetoxypropane used in the experiment were of analytical grade.

Methods

Table 1: Grouping of animals and dose schedule

S. No	*Group*	*Drug dose*
1	Control vehicle (C)	2% gum acacia solution
2	Control streptozotocin (STZ)	70.0mg/kg i.p, single dose
3	*Indravati Rasa* (IVR- X dose)	133.0mg/kg/day,p.o
4	*Indravati Rasa* (IVR- 2X dose)	266.0mg/kg/day,p.o
5	Insulin (Human insulin regular)	4.0IU/kg,s.c/day

Drug administration

Indravati Rasa was suspended in 2% gum acacia and was given orally through aoro gastric tube. The dose of *Indravati Rasa* was selected on the basis of the human therapeutic dose (dose for 60.0 kg adult is 1g orally per day). The dose of *Indravati Rasa* in the experimental study was 10 times of human adult dose.

Induction of diabetes mellitus (IDDM) [12]

Diabetes was produced by intraperitonial injection of 70.0mg/kg streptozotocin in citrate buffer. Control animals received equal volume of buffer solution. Blood glucose was measured after 48 hours of streptozotocin injection to ascertain induction of IDDM. Blood was collected by puncturing the para orbital venous plexus with capillary tube. Blood glucose and serum

malondialdehyde levels were measured after 48hrs of streptozotocin injection and on day 21and day 28.

Estimation of blood glucose

The blood drop directly dropped on glucometer (Jhonson and Jhonson U.S.A) strip to measure the blood glucose level. Blood glucose level was expressed as mg/dl.

Estimation of serum malondialdehyde

Malondialdehyde estimation in the serum was carried out by using thiobarbituric acid (TBA).

Procedure

1.0 ml of serum was added to 2.0ml of the trisodium citrate acid - thiobarbituric acid - hydrochloric acid (TCA-TBA-HCL) reagent and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 g for 10 min. The absorbance of the sample was determined spectrophotometrically at 535.0nm, against a blank that contains all the reagents except serum.

Calculation

Malondialdehyde concentration of the sample was calculated using a TEEP standard curve and expressed as nmol/ml.

Statistical analysis

The data generated during the study was analyzed by employing paired and unpaired student 't' test.

Results

Table 2: Effect of *Indravati Rasa* on blood glucose level (mg/dl) in streptozotocin (STZ) induced albino rats (n=6)

Group	*Blood sugar level (mg/dl) mean, SEM*		
	Initial (48 hours after STZ injection)	*After Treatment*	
		After 21 days	*After 28 days*
Control (vehicle)	69.90 ± 2.83	69.32 ± 3.38	70.70 ± 2.76
Control (STZ)	4.24.56 ± 11.15	322.16 ± 17.44 (n=5)	322.15 ± 14.0 (n=4)
Indravati Rasa (single dose)	417.42 ± 9.79[a]	315.04 ± 10.50 [a] (n=5)	292.14 ± 7.73 [a] (n=4)
Indravati Rasa (double dose)	401.08 ± 4.51[b]	289.95 ± 6.01[a]	289.86 ± 7.17 [b] (n=5)
Insulin	404.62 ± 9.51[b]	254.18 ± 9.23[c]	198.13 ± 16.35[c]

a=p>0.05 b=p<0.01 c=<0.001

Table-3: Effect of *Indravati Rasa* on serum MDA level (n mol/dl) in streptozotocin (STZ) induced albino rats

Group	*Serum MDA level (n mol/dl) mean SD*		
	Initial (48 hours after STZ injection)	*After treatment*	
		After 21 days	*After 28 days*
Control (vehicle)	0.03 ± 0.02	0.03 ± 0.20	0.33 ± 0.20
Control (STZ)	6.90 ± 0.42	7.51 ± 0.28 (n=5)	8.12 ± 0.72 (n=4)
Indravati Rasa (single dose)	7.77 ± .304 [a]	7.50 ± 0.67 [a] (n=5)	6.88 ± 0.28 [b] (n=5)
Indravati Rasa (double dose)	7.16 ± .0.458[a]	7.17 ± .0.245[a]	6.89 ± .3.88[a] (n=5)

a=p>0.05 b=p<0.01 c=<0.001

Discussion

Analysis of the data showed that streptozotocin induced hyperglycaemia could be antagonized by insulin. *Indravati Rasa* in the dose of 133.0mg/kg and 266.0mg/kg per day were administered per orally in two groups of rats. 133.0mg/kg of *Indravati Rasa* could attenuate the streptozotocin induced hyperglycaemic status of rats only after 28 days. However, the reduction in blood glucose level was not statistically significant. But, *Indravati Rasa* 266.0 mg/kg dose also reduced hyperglycaemic status only after 28 days of treatment. The reduction in blood glucose level was statistically significant in this dose. The present findings in the light of conventional Ayurvedic use of individual components of *Indravati Rasa* justify the use of this novel product in experimental IDDM. Free radicals are known to be associated with diverse pathological conditions including diabetes mellitus, many degenerative diseases and ageing. An attempt was made to ascertain the status of lipid peroxidation in IDDM and possible role of *Indravati Rasa* in inhibition of lipid peroxidation. The present findings showed that *Indravati Rasa* at low doses (133.0 mg/kg per day) attenuated streptozotocin enhanced serum Malondialdehyde (MDA) level. Inability at higher dose of *Indravati Rasa* to produce similar effect is unknown to explain in the present experimental set up.

Analysis of IDDM and MDA data is suggestive of free radical involvement in the causation of IDDM. The ability of *Indravati Rasa* to antagonize streptozotocin induced hyperglycaemia and enhanced lipid peroxidation opens up a new window for *Indravati Rasa* in the armentarium of pharmacotherapy of IDDM.

Conclusions

Indravati Rasa is a herbomineral preparation is strongly recommended for the management of *Madhumeha* in rasa shastra texts. *Madhumeha* resembles closely to diabetes mellitus. Many of the reactions associated with hyperglycaemia may acutely and chronically increase the production of free radicals resulting in an oxidant antioxidant imbalance.

As a consequence of this imbalance free radical mediated injury ensures and this leads to an increased production of malondialdehyde a marker of lipid peroxidation. In this study experimental results reveals that *Indravati Rasa* is effective in streptozotocin. Insulin dependent diabetesmellitus (IDDM). The mechanism of anti hypoglycaemic effect might also be due to its influence on lipid peroxidation.

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