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IN VIVO ANTI OXIDATIVE EFFECT OF POLYHERBAL FORMULATION OF FLAX SEED, FENUGREEK AND JAMUN SEED ON STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

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ABSTRACT

Oxidative stress is the main cause of the secondary complications in diabetes mellitus (DM). The aim of this study is to evaluate the antioxidative effect of polyherbal formulation (PHF) of hydroalcoholic extracts of flaxseed, fenugreek, jamun seed (1:1:1) and their individual drugs in Streptozotocin-Nicotinamide induced DM in wistar rats. The drugs were given orally at dose of 200mg/kg body weight (bw)for 30 days .On 31st day liver were isolated and homoginized in Tris-HCl buffer for measurement of antioxidants. The result shown that significant ($p<0.05$) increase in lipid peroxidation and decrease in suproxide dismutase, catalase, glutathione peroxidase, glutathione s transferase, glutathione reductase, vitamin C, vitamin E level in diabetic animals. The treatment with PHF and their individual drugs significantly reversed these changes. The PHF treated group shown greater percentage changes than their individual drugs treated groups. This concludes PHF has greater antioxidative effect and the synergism exist between their individual drugs.

KEYWORDS: **Antioxidants, PHF, Streptozotocin, Nicotinamide**

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INTRODUCTION

Diabetes mellitus is a global threatening metabolic diseases and it is being one of the major cause of increasing mortality. According to WHO, The diabetes prevalence has increased from 108 million to 422 million between 1980 to 2014.[1] The steady rise in blood sugar level leads to increased production of oxygen free radicals via auto oxidation of glucose and non enzymatic protein glycation and increased lipid peroxidation.[2] Free radicals are reactive oxygen species that contain one or more unpaired electrons which enter into the cell and induce cell damage by oxidizing the cell components and molecules.[3] Because of this oxidative stress the insulin resistance has developed in diabetic subjects.[2] The enzymatic and non enzymatic antioxidants protect our body cells from oxidative stress.[4] In the case of any pathological conditions that cause excess formation of free radicals, the level of the both enzymatic and non enzymatic antioxidants has been altered. Hence the level of the antioxidants in the body provide useful index of the oxidative stress. The management of the diabetes without side effects is one of main goal in the diabetic research. Herbal drugs are becoming popular in the treatment of diabetes due to less or minimum side effects and affordable cost. Now-a-days Poly herbal preparations are mostly preferred because of better therapeutic effect than the single herbal drug. *Linum usitatissimum* (flax seed) belong to the family Linaceae has been used for many diseases and reduce the post prandial blood glucose level and greater antioxidant activity in humans.[5,6] The *Trigonella foenum graecum* (fenugreek) and *Syzygium cumini* (jamun) are popular traditional herbs for the treatment of diabetes mellitus.[7-9]. The present study designed to see the antioxidative effect of poly herbal formulation of flax seed, fenugreek and jamun seeds when compared to their individual drug treatment in the wistar albino rats.

MATERIALS AND METHODS

Collection and identification of plant

Seeds belonging to the herbal formulation of flaxseed, fenugreek and jamun seed were collected from the local market, Puducherry, India and authenticated by siddha physician and nodal officer, Siddha unit, Dept of Indian System of Medicine &Homeopathy, Puducherry. The seeds were dried under shade and coarsely powdered before use.

Extraction of the plant material

Extraction of flax seed

Powdered flax seeds were defatted by petroleum ether (at 60-80°c) in the soxhlet apparatus. The marc was hydrolyzed with 1M sodium hydroxide for 1 hr at room temperature by constant rotation followed by extraction with 50% ethanol then acidified with 1m HCl upto the PH: 2-4. dry the filterate at 50°C[10]

Extraction of fenugreek and jamun seedsDried powdered seeds were extracted with 50%ethanol using soxhlet apparatus for 20-24 hrs. The extract was concentrated under vacuum at 50°c.

Experimental animal

Wistar strain albino rats weighing 180-220gm were used for this study. Before commencement of the experiments proper Institutional Animal Ethics Committee permission was obtained. Rats were housed under standard Laboratory conditions with food and water provided ad libitum.

Induction of diabetes mellitus

Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. The DM was induced in overnight fasted Wistar albino rats by a single intraperitoneal (i.p) injection of 60 mg/kg streptozotocin, 15 min after the i.p. administration of 120 mg/kg of nicotinamide Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hr after administration. Rats with fasting blood sugar levels around 160 to 300 mg/dl were selected for the study.[10]

Experimental groups

Group 1: Control Animals were administered saline (1ml) orally for 30 day
Group 2: Diabetic animal were administered saline (1ml) orally for 30 day
Group 3: Diabetic Animals were administered flax seed extract 200mg/kg bw for 30 days
Group 4: Diabetic Animals were administered fenugreek extract 200mg/kg bw for 30 days
Group 5: Diabetic Animals were administered jamun seed extract 200mg/kg bw for 30 days
Group 6: Diabetic animals were administered herbal formulation of FFJ (1:1:1) of 200 mg/kg bw for 30 days
Group 7: Diabetic animals were administered Metformin (200mg/kg bw) for 30 days

On the 31st day the animals were killed by cervical dislocation and the liver were isolated and homogenized with10% 0.1M Tris-HCl buffer pH7.4 for the measurement of enzymatic and non enzymatic antioxidant levels. Lipid Peroxidation (LPO) was estimated by the method of Ohkawa.[11] Superoxide dismutase (SOD) was estimated by the method of Marklund & Marklund.[12] Catalase (CAT) was estimated by the method using Sinha.[13]Glutathione peroxidase (GPx) was estimated by the method of Rotruck.[14] Glutathione s-transferase (GST) was assayed by the method of Habig and Jakoby.[15] Reduced glutathione (GSH) was determined by method of Moron et al 1979.[16] Vitamin E (α –Tocopherol) was estimated by the method of Baker and Frank (1951).[17] Vitamin C (Ascorbic Acid) was estimated by the method of Omaye.[18]

Statistical analysis

All the data were analyzed using one way analysis of variance (ANOVA) followed by tukeys multiple comparison. Results were expressed as mean ±S.D and the values $p<0.05$ were considered statistically significant.

RESULTS

Table 1
Effect of poly herbal formulation and their individual herbs on tissue lipid peroxidation & enzymatic antioxidants

Groups	Lipid peroxidase (LPO) (µ moles of MDA produced/mg of protein)	Suproxide dismutase (SOD) (units/mg protein)	Catalase (CAT) (μmoles of H_2O_2 utilized/min/mg protein)	Glutathione peroxidase (GPx) (μmoles of GSH oxidized/min/mg protein)	Glutathione-s-transferase (GST) (μmoles ofcDNB formed/min/mg of protein.)
Control	3.23±0.12	8.6±0.4	60.6±3.0	33.8±1.6	21.08±1.07
Diabetic	7.0±0.33*	5.66±0.26*	37.1±1.8*	17.6±0.8*	10.76±0.58*
Flaxseed extract	4.81±0.25*@	7.31±0.33*@	50.7±2.38*@	27.3±1.28*@	16±0.79*@
Fenugreek extract	4.85±0.24*@	7.15±0.33 *@	52.3±2.6 *@	27±1.35 *@	15.76±0.73 *@
Jamunseed extract	4.28±0.21*@$#	7.0±0.34 *@	49.9±2.4 *@	28.2±1.4 *@	16.1±0.79 *@
Herbal formulation	3.38±0.18 @$#&	8.11±0.38 @$#&	58±2.8 @$#&	31.9±1.4 @$#&	20.7±1.17 @$#&
Metformin	3.4±0.1 @$#&7	8.2±0.4 @$#&	58.9±2.7 @$#&	32.1±1.6 @$#&	21.28±1.23 @$#&

Values are expressed as mean±SD of six animals, Symbols *,@,$,#,&,+ represents the statistical significance (p<0.05) * –compared with control @ -compared with diabetic, $-compared with flaxseed extract, #- compared with frenugreek seed extract, #-compared with jamun seed extract, +-compared with herbal formulation

The effect of Poly herbal formulation and their individual herbs extracts on both lipid peroxidation and enzymatic antioxidant level were given in table 1 showed significant increase in lipid peroxidation and significant decrease in SOD,CAT,GPx,GST level in diabetes induced animals. The treatment with poly herbal formulation (PHF) and their individual herbs treated groups significantly decrease lipid peroxidation and increased the SOD, CAT, GPx, GST enzymes level. The percentage of changes observed was greater in PHF treated group than their individual herbss treated groups. The percentage reduction of LPO by flax seed, fenugreek seed and jamun seed extracts are 31.3%,30.65% and 38.8% respectively, but the polyherbal formulation treated group reduced 51.73% from the diabetic group. Similarly the percentage increase of SOD, CAT, GPx, GST by the individual herbs such as flax seed (29.1%,36.5%,54.75%,49.3%), fenugreek (26.1%,41.0%,52.6%,46.4%) and jamun seed (23.5%,34.5%,59.6%,49.8%) and the PHF is 43.23%,56.3%,80.7%and92.4% from the diabetic group. The changes observed in the poly herbal formulation treated group was similar to the control and standard drug metformin treated group.

Table 2
Effect of poly herbal formulation and their individual herbs on non enzymatic antioxidants

Parameter	Control	Diabetic	Flax seed extract	Fenugreek extract	Jamun seed extract	Herbal formulation	Metformin
Glutathione reductase (μgms of GSH/mg protein.)	4.73±0.24	2.35±0.13 *	3.80±0.17 *@	3.81±0.17 *@ $	3.85±0.18 *@	4.48±0.22 @$#&	4.5±0.21 @$#&
Vitamin C (μgms/mg protein)	2.65±0.13	1.26±0.05 *	1.66±0.08 *@	1.66±0.08 *@	1.80±0.08 *@ $#	2.15±0.1 *@$#&	2.33±0.12 *@$#&
Vitamin E μgms/mg protein)	2.01±0.09	1.0±0.05 *	1.65±0.05 *@	1.43±0.05 *@$	1.51±0.07 *@ $#	1.93±0.08 @$#&	1.9±0.1 @$#&

The comparison between groups and the statistical significance are as in table 1

The table 2 showed the effect of polyherbal formulation and their individual herbs on non enzymatic antioxidant. The glutathione reductase, Vitamin C, E levels were decreased in diabetic induced animals. The treatment with herbal formulation and their individual herbs significantly ($p<0.05$) increased the GSH, Vitamin C, Vitamin E level. The percentage increase of the GSH, Vitamin C, Vitamin E by the poly herbal formulation (90.7%, 69.73%, 91.7%) treated group was greater than their individual herbs such as flaxseed (61.7%, 31.57%, 63.6%), fenugreek (62.4%, 31.5%, 42.1%) and jamun seed (63.8%, 42.1%, 50.4%) extracts treated groups. Except vitamin C, the changes observed in the poly herbal formulation treated group was similar to the control and standard drug Metformin treated group.

DISCUSSION

The impaired metabolic events and sustained hyperglycemia in the diabetes mellitus are the main cause of oxidative stress.[19-20] The oxidative stress

means imbalance between the oxidants especially the reactive oxygen and nitrogen species and the level of the antioxidants. Oxidative stress caused by reactive oxygen species (ROS) that formed in excess or insufficient removal plays important role in the damage of cellular DNA, proteins, lipids and late diabetic complication.[21] It is believed that the negative regulation on insulin signaling and interpretation caused by ROS and RNS will be the main reason to develop insulin resistance in Type II diabetes mellitus.[22] The evidences showed that the decreased insulin level in diabetic rats increases the activity of the enzymes like fatty acyl coenzyme A which initiates auto oxidation of fatty acids and generate oxygen free radical.[23,24] and lipid peroxidation. The increased lipid level in the diabetes mellitus also causes the cells more susceptible to lipid peroxidation.[25] This affects the membrane function by altering the membrane fluidity and changing the activity of membrane bound enzymes. The enzymatic and non enzymatic antioxidants which includes, SOD, CAT GPx, GST, GSH, Vitamin C and Vitamin E are fight against ROS. The suproxide dismutase catalyses the suproxide ions into molecular oxygen and peroxide and play first line of defence in the free radical mediated cell injury.[26] The catalase which acts on hydrogen peroxide and converted into water and oxygen thus neutralizes it. [27] The vitamins C and E are act as antioxidants by detoxifying the free radicals and these vitamins levels were altered in oxidative stress. The enzymes Glutathione Peroxidase and Glutathione Reductase present in the cell metabolizes peroxide to water.[2] The alterations in these enzymatic and non enzymatic antioxidant level in the diabetes mellitus are the important biomarkers of oxidative stress. In this study the diabetes induced by STZ-Nicotinamide to resembles type II diabetes mellitus because STZ induced DNA damage stimulates DNA repair mechanism that consumes large quantities of nicotinamide adenine dinucleotide (NAD) the supplementation of Nicotinamide serves as a partial protection against excessive pancreatic beta cell damage caused by STZ. This study also showed that the treatment with poly herbal formulation and their individual herbs decreases lipid peroxidation and increases SOD, CAT, GPx, GST, GSH, Vitamin C and Vitamin E. this might be due to the presence of the phytochemical in the individual drugs of the PHF. The flax seed contains many phytochemicals in which lignan secoisolauricirecinol (SDG) has multiple functions. Lignans are phyto oestrogens that converted into mammalian lignin that are great antioxidants that prevent oxygen free radical production.[28] The seeds of the fenugreek and jamun seeds are rich in flavonoids, phenolics, saponins and tannins. [29] The flavonoids and polyphenols in fenugreek and jamun seeds believed to have antioxidant activity.[30] The flavonoids contain Functional hydroxyl groups which is responsible for antioxidative effects by scavenging free radicals and/or by chelating metal ions.[31,32] In this study the treatment with poly herbal formulation produce greater effect than the effect produced by their individual herbs .This proved that, the synergism present between the active chemical constituents of the individual drugs.

CONCLUSION

Though the active phytochemical constituents of individual plants have been well established, they usually present in minute amount and always, they are insufficient to achieve the desirable therapeutic effects. Scientific studies have revealed that greater effect were produced when these plants of varying potency given combine as compared to individual use of the plant and also the sum of their individual effect. This study concluded that, due to synergism the PHF of flaxseed, fenugreek and jamun seed had greater antioxidative effect. Futher study is recommended to find out the exact mechanism of action of antioxidative effect.

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