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Research article

Effect of supercritical fluid extract of *Zingiber officinalis* in the management of diabetes and its related complications

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ABSTRACT

Diabetes mellitus is referred as diabetes which is the metabolic disorder. Major symptoms of diabetes include increased urination & thrust, head ache, high degree of sudden weight loss. Due to increase in blood glucose levels it causes trigger of various pathways which ultimately leads to various diabetic complications. Pathways like which includes Polyol Pathway, Hexosamine Pathway, Activation of PK-C (Protein Kinase-C), & formation of Advance Glycation End Products. For the diagnosis of diabetes two major tests are done blood glucose level and Glycated hemoglobin content (HbA1c). Ayurveda is the super bank for various molecules to treat any disease. Here we have chosen the super critical fluid extract of Ginger that is *Zingiber Officinale*. In our study we have used Streptozotocin induced diabetes mellitus model by using rats. Our result showed promising effects in the streptozotocine induced diabetes and immunomodulatory activity was also shown by the extract. Overall in conclusion it *Zingiber Officinale* supercritical fluid extract can be considered as source of multitalented molecules which can be proven better for the management of diabetes and its related complications.

Keywords: Diabetes, STZ, Immunomodulatory effect, Ginger, *Zingiber Officinale*, Supercritical fluid

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INTRODUCTION

Diabetes mellitus is referred as diabetes which is the metabolic disorder. Due to increased blood glucose levels all the types of diabetes causes the diabetic complications like diabetic hepatopathy, diabetic neuropathy, and diabetic cardiomyopathy. Major symptoms of diabetes include increased urination & thrust, head ache, high degree of sudden weight loss, blurry vision, numbness in extremities, loss of energy, dryness of skin, muscle pain, fatigue, high rate of infection etc. Foot ulcers, chronic kidney disease, impairment of the neurons, damage to the eyes site and cognitive weakening are the major long term complications. In type 1 diabetes there is failure of production of Insulin by beta islets of Langerhans which is also known as “(IDDM) insulin-dependent diabetes mellitus" or "juvenile diabetes. The major molecular basis of this type of diabetes is unknown but it is considered to be autoimmune disorder. In type 2 diabetes insulin resistance is major cause and due to this body cells fails to retort to insulin appropriately. As the illness reach to advancements, a deficiency of insulin may also ripen. Type 2 diabetes referred to as "(NIDDM) non-insulin dependent diabetes mellitus" or "adult-onset diabetes" [1].

Due to increase in blood glucose levels it causes trigger of various pathways which ultimately leads to various diabetic complications. Pathways like which includes Polyol Pathway, Hexosamine Pathway, Activation of PK-C (Protein Kinase-C), & formation of Advance Glycation End Products. For the diagnosis of diabetes two major tests are done blood glucose level and Glycated haemoglobin content (HbA1c). Blood glucose levels testing are done for two stages fasting blood glucose level and after meal blood glucose level. Fasting blood glucose level must be less than 110 mg/dL and postprandial means after meal it should not be more than 150 mg/dL. HbA1c levels are mainly major of control over diabetes. Levels of HbA1c should be less than 42 mmol/mol. limiting of extra sugar, and taking diet, proper exercise, maintaining good habits can prevent the diabetes. India is upcoming hub of diabetes. Though there are many antidiabetic agents are available in the market still there is no answer to diabetic complications [2]. This kind of scenario is may be due to improper control of blood glucose levels as well as effect of sugar through various pathways on cells of body. As per reports of WHO and American diabetes association metformin is the drug of

choice which molecularly act by inhibiting the liver's production of glucose. As per evaluations of WHO diabetes lead to 1.5 million deaths in 2012, making it the 8th disease foremost reason of death. Though additional 2.2 million bereavements worldwide were resulted due to higher blood glucose levels and the augmented jeopardies of cardiovascular disease and other linked complications (e.g. Immune dysfunction, kidney failure), which repeatedly principal cause of premature demise and are often registered as the fundamental cause of death credentials to rather than diabetes. So here we want a multitalented molecule which will cover many facets of diabetes and mainly will be effective in the management of diabetic complications.

Infections in Diabetic people are increasing day by day. In many research studies it is found that macrophages, CD4+ Cells, Neutrophils and dendritic cells are supressed due to which humoral and cellular immunity is badly hampered. To counter these kind of ill effects one must study diabetes on immunomodulatory basis. Here we have chosen the super critical fluid extract of Ginger that is *Zingiber Officinale*. Ginger covers Anti-diabetic, Antihypertensive as well as immune boosting activity which will be helpful in various diabetic complications. Different animal models claim different mechanisms which are quite comparable to several diabetic conditions of patient. Here we would like to test and evaluate comparative status of various marketed and herbal drugs in lowering of blood glucose levels and immunomodulatory activity [3].

Zingiber Officinale is widely used in India as folk medicine and spice from ancient time. In many ancient texts ginger is described as Rasayana herb which rejuvenates the body nourishes the cells and mainly acts as Immunomodulator. Super critical fluid extract is done by using CO2 which get evaporated at room temperature and we get pure extract without any solvent residue.

MATERIAL AND METHODS

Experimental Animals

Fresh Wistar rats weighing 160-200 grams were carefully chosen. Faunae of either sex were housed under typical standard laboratory conditions of temperature 22±30C and relative humidity of 44-56% with free admittance to standard diet pellet and water by using auto filter bottles. Has received ethical approval by the Institutional Animal Ethical Committee & number is CPCSEA/IAEC/2017/015.

Chemicals

Standard Marketed drugs were procured from local market, Glucose diagnostic kit (Bio lab India), Supercritical fluid extract of *Zingiber Officinale* was kindly provided by Nisarg Biotech Pvt. Ltd.

Acute toxicity Study

Acute oral toxicity studies were performed for super critical fluid extract of Ginger according to the OECD (Organization for Economic Co-operation and Development) guidelines. Male Rat (n = 6/each dose) were selected for acute toxicity study. The animals were fasted overnight with free access to water. Extract (suspended in 0.6% Carboxymethyl cellulose) was administered orally at a dose of 5 mg/kg. The general behaviour such as motor activity, tremors, convulsions, straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhoea and skin colour were observed for 3 days. If mortality observed in 4/6 or 6/6 animals, the dose administered was considered as toxic dose. However, if the mortality was observed in only one rat, then the dose was repeated with higher doses such as 100, 200, 500, 1000 and 2000 mg/kg. All combinations were found to be safe at 2000 mg/kg [4].

Induction of Diabetes

Diabetes was persuaded in overnight fasted Wistar rats by single dose Intraperitoneal injection of newly prepared STZ (Streptozotocine) at 65 mg/kg body weight (b.w) dissolved in 0.01 M citrate buffer with pH 4.5. The non-diabetic control rats were also received an injection of the plain citrate buffer. After 48 h STZ administration, from retro orbital plexus blood samples of the overnight fasted rats were collected to measure blood glucose levels.

The rats which were found to have permanent Diabetes Mellitus (Fasting Blood Glucose (FBG)> 250 mg/dl were considered diabetic and used in the study.[4] Animals were divided into six groups of eight rats in each as mentioned in Table No 1. All six groups received respective treatment mentioned in the table.

Table 1: Selected Groups and Dose respectively

Group & Drug	Drug Dose
Group I: Normal (NC)	-
Group II: Diabetic Control (DC)	-
Group III: Metformin (M)	100 mg/kg PO
Group IV: Metformin + Ginger CO2 Extract (MG25)	100 mg/kg P.O. + 25 mg/kg P.O.
Group V: Metformin + Ginger CO2 Extract (MG50)	100 mg/kg P.O. + 50 mg/kg P.O.
Group VI: Metformin + Ginger CO2 Extract (MG100)	100 mg/kg P.O. + 100 mg/kg P.O.

Collection of blood and determination of biochemical parameters

Rats were treated for 6 weeks. Blood samples were collected from tail vein in 0, 3 and 6 weeks after the administration of and blood glucose levels were determined by using GOD/POD. At the completion of the treatments, the animals were fasted overnight and then blood samples were drawn from their retro-orbital plexus. Instantly after blood samples pool, serum was isolated by centrifugation at 3000 rpm for 10 min by using Remi Lab Centrifuge and then analyzed for various biochemical parameters. The serum samples were stored at −80 °C in a freezer until they were analyzed. Glycated Hemoglobin concentration levels were measured by earlier described methods using diagnostic kits (Biolab Diagnostics Pvt. Ltd.) by auto analyzer [4].

In-vivo phagocytic activity

At 42nd day all the animals received an intravenous injection of carbon suspension (1:50 dilution of Indian ink) in a dose of 0.15 ml/100g. Blood samples were withdrawn at 0 and 15 minutes after carbon injection. Blood samples (50 µl) were mixed with 4 ml of 0.1% sodium carbonate solution and the absorbance of this solution was determined at 680 nm [5]. The phagocytic index (K) was calculated indicated by carbon clearance using the following equation:

K= (Log OD1 - Log OD2)/15

Where OD1 and OD2 were the optical densities at 0 and 15 min respectively

Evaluation of Tissue Necrosis factor alpha level

At 42nd day blood was also collected and centrifuged for 10 min at 10,000 rpm. The serum was collected and was used to detect the TNF-α level. TNF-a ELISA Kits were used & from collected serum, TNF-α level was measured according to manufacturer protocol [6-7].

STATISTICAL ANALYSIS

The data was statistically analyzed by using Prism version 6.01. Results are presented as mean ± SEM, data analyzed by one-way ANOVA followed by Dunnett's test. (Dunnett's test is used when we compare one group {usually the control treatment} with the other groups.) Level of significance at $P < 0.05$ probability level. (Differences were considered significant when $P \leq 0.05$.) The results were expressed as Mean ± SEM. Mean values were considered statistically significant when $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. When compared with diabetic control group. Comparisons were made between normal control to diabetic control using student t-test (# $p<0.05$, ## $p < 0.01$, ### $p < 0.001$).

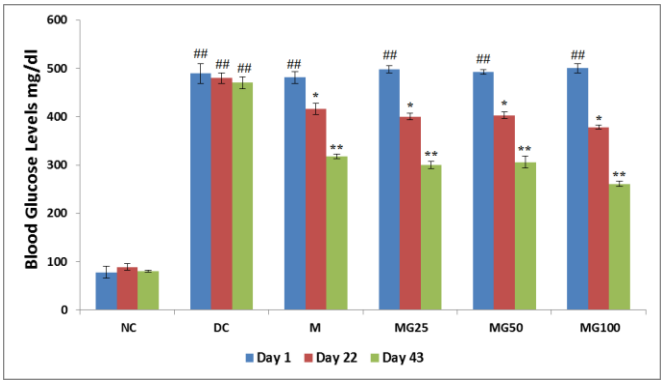
RESULT AND DISCUSSION

Acute toxicity

As per the OECD 425 guidelines acute oral toxicity was done and shown that supercritical fluid extract is safe up to the extreme dose of 2000 mg/kg body weight. The extract administration neither triggered any significant change in the behavior's nor the death of animals. 1/10th, 1/20th and 1/40th of the determined dose was fixed for further analyses. The animals were divided into six groups of eight animals each.

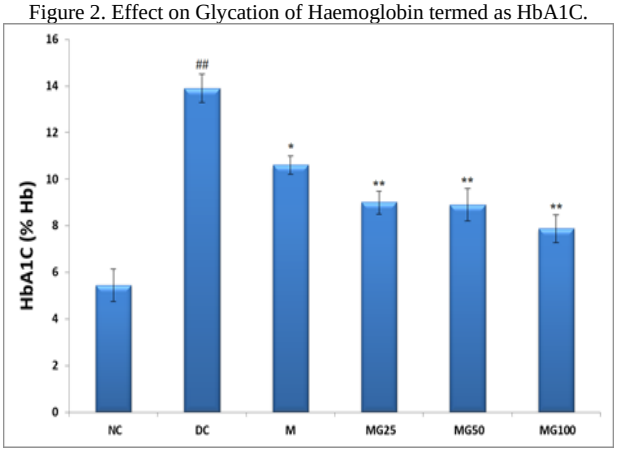
After intra peritoneal injection of STZ (Streptozotocin) in rats there was significant upswing of blood glucose levels which is statistically shown as highly significant ## $p < 0.01$. All the groups glaringly presented significant activity in terms of the reduction of fasting blood glucose levels as Metformin is given in combination with supercritical fluid extract [8]. Metformin is known for its secretogouge action. Metformin has been widely described as it acts through in cooperation with AMPK (AMP-activated protein kinase) dependent and AMPK-independent mechanisms; by inhibition of mitochondrial respiration but then again also possibly by inhibition of mitochondrial major enzyme known as glycerol phosphate dehydrogenase, and a mechanism linking the lysosome directly. Metformin recovers body from glycaemia by acting on the liver via activation of AMPK, to abundant further composite portrait of its sparkling on several mechanisms of actions [9].

Figure 1. Effect of Fasting Blood glucose levels



Results are presented as mean ± SEM. (n=8), data analyzed by one-way ANOVA followed by Dunnett's test.

Figure 2. Effect on Glycation of Haemoglobin termed as HbA1C.



Results are presented as mean ± SEM. (n=8), data analyzed by one-way ANOVA followed by Dunnett's test.

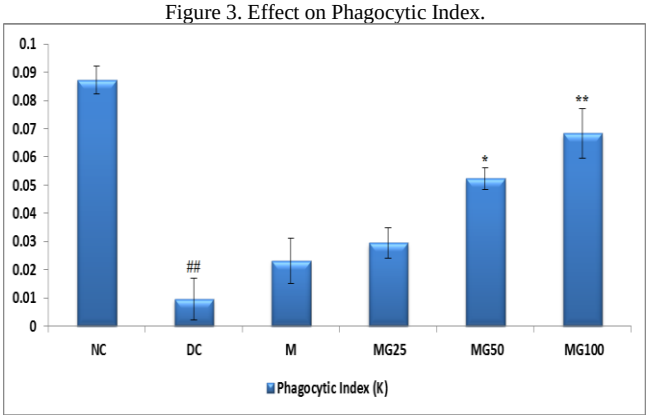
As due to no treatment and control over blood glucose levels in diabetic control group there was high levels of Glycation of hemoglobin. Here we can see the major difference and effect of combination of Metformin + Supercritical Fluid extract of Ginger which revealed highly significant activity as compared to plain metformin ($**p < 0.01$).

Zingiber Officinale are the rhizomes which are found dissident belonging to family Zingiberaceae. As a spice it is most widely consumed spices globally. It has a long antiquity of medicinal usage as herbal remedy to treat a variability of diseases counting constipation, indigestion (dyspepsia), nausea and vomiting, pain, and cold induced syndromes. As per the scientific research ginger also possesses anti-diabetic, anti-clotting, anti-cancer, anti- oxidant, and anti-inflammatory physiognomies, subsequently it can scavenge

superoxide anion and hydroxyl radicals too. *Zingiber Officinale* has multiple is quantity of potentially bioactive molecules, chiefly shogaols, gingerols, as well as few volatile oils as well as monoterpenes, sesquiterpenes, such as β-bisabolene and (-)-zingiberene. In tallying, phytochemical reports have shown that the main constituents of ginger are gingerol, shogaol, zingerone and paradol. Which was found to be 28%, 20%, 7% and 4% in our extract. In our study we found prominence of 6-gingerol and 6-shogaol are the major gingerol and shogaol present in the rhizome. *Zingiber Officinale* has been proven scientifically to possess anti-diabetic activity in a variety of studies. Other investigations have indicated the hyperlipidemic effects also [10].

According to new research *Zingiber Officinale*, wields its anti-diabetic actions due to invigorating actions on pancreatic β-cells islets of Langerhans, increases insulin sensitivity, insulin-like effects and peripheral application of glucose. Other mechanisms of actions include increased production and synthesis of hepatic glycogen through the enhancement of glycogen regulatory enzyme expression in the liver, stimulation of pancreatic insulin release, inhibition of carbohydrate metabolizing enzymes, and inhibition of glucose production in liver. However, further studies, especially in human subjects are consequently needed and the oral safety and care of various extracts further down protracted usage essential to be noted [11].

Figure 3. Effect on Phagocytic Index.

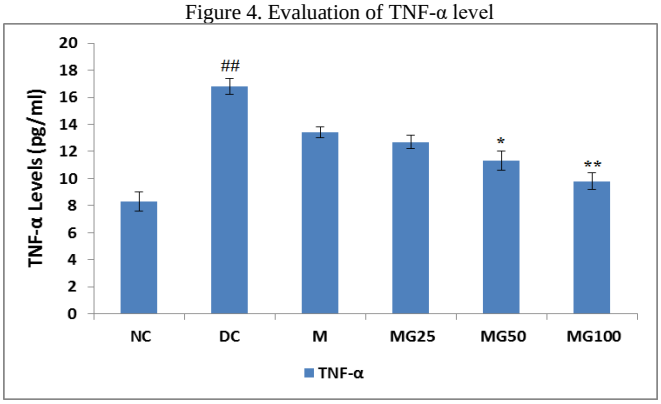


Results are presented as mean ± SEM. (n=8), data analyzed by one-way ANOVA followed by Dunnett's test.

Due to elevated blood sugar levels and no treatment there was drastic decrease in the phagocytic index in the Kupffer cell of liver which is statistically shown as highly significant ## $p < 0.01$ rise. Plain treatment with metformin was unable to hit the bull's eye this time and was unable to restore the levels of macrophage activation. This depicts the intense need of combination. In both MG50 and MG100 there was increased phagocytic index which proves the aim of the research. Both MG50 and MG100 groups have shown significant *$p < 0.05$ and highly significant **$p < 0.01$ effects. Oral hypoglycemic agents always have depicted negative impacts on immune cells. In this regards with published literature Biguanides (Metformin) have made known to decrease the Lysozyme levels and NO levels in diabetic rabbit. Subjects getting metformin have reduced vitamin B12 levels are well known now and vitamin B12 had important immunomodulatory actions on cellular immunity. Few publications mentions anomalies in the immune system in pernicious anaemia are restored by vitamin B12 replacement therapy. [11]

Many evidences have shown that and ideal molecule can normalize the functions of macrophages through the mechanism of AMPK, AMPK independent targets, NF-κB, ABCG5/8, Sirt1, FOXO1/FABP4 and HMGB1. On the basis of this foundation we can summarize the role of metformin-based combination drugs. Macrophages play a significant role in many diseases, and refining macrophage dysfunction may be a key mechanism for ideal anti-diabetic combination to enlarge its pharmacological silhouette.

Figure 4. Evaluation of TNF-α level



The levels of TNF-α were found to be increased in Diabetic group Both MG50 and MG100 groups have shown significant *$p < 0.05$ and highly significant **$p < 0.01$ decrease in levels of TNF-α. TNF-α causes rise in adipocyte lipolysis and has adverse effects on the insulin signalling pathway over by shifting the serine/ tyrosine phosphorylation of insulin receptor substrate. TNF-α causes decrease in glucose intake in peripheral tissues by directing GLUT4 (glucose transporter 4) and insulin signalling pathways. In the current study, oral combination of Metformin and super critical fluid extract of *Zingiber Officinale* have decreased the levels of TNF-α [12].

CONCLUSION

In conclusion, the present study depicted that combination of Metformin and super critical fluid extract of *Zingiber Officinale* significantly reduced the levels of Fasting blood glucose levels, Glycated haemoglobin and serum TNF-α and increased the phagocytic index of macrophages. By viewing these actions of *Zingiber Officinale*, it can be a better medication in combination with conventional drugs for diabetic patients to lessen the hazards of some subordinate chronic diabetic complications.

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CONFLICT OF INTEREST

All the authors mentioned here are contributed equally in this project and paper. So there is no conflict of interest.

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