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Original Research Article (Experimental)

Vasant Kusmakar Ras, an ayurvedic herbo-mineral formulation prevents the development of diabetic retinopathy in rats

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ABSTRACT

Background: Diabetic retinopathy is a slow progressing complication of diabetes mellitus with multifactorial aetiology affecting approximately 80% of diabetics worldwide. Chronic hyperglycemic milieu of Diabetes induces biochemical changes which contribute to the pathogenesis of Diabetic retinopathy.
Objective: The present study examined the protective effect of *Vasant Kusumakar Ras*, an Ayurvedic herbo-mineral formulation, in diabetic retinopathy.
Materials and Methods: Diabetes was induced in rats by intraperitoneal injection of streptozotocin (45 mg/kg). Rats were kept without any treatment for period of three weeks for induction of Diabetic retinopathy followed by treatment with *Vasant Kusumakar Ras* (11.25 mg/kg, p.o) for further 5 weeks. Fasting blood glucose levels, lipid profile and HbA1c were determined. Eye tissue homogenates were subjected to biochemical analysis to determine the levels of oxidative stress parameters (superoxide dismutase, catalase, reduced glutathione, lipid peroxidation), vascular endothelial growth factor and aldose reductase activity. Histopathological analysis of retinal tissue was conducted using Hematoxylin and Eosin staining.
Results: Vasant Kusumakar Ras treatment restored serum lipid profile which was altered in diabetic rats. Treatment with *Vasant Kusumakar Ras* significantly ameliorated the oxidative stress in eye tissue resulting in decreased lipid peroxidation and increase in endogenous antioxidant levels. Levels of aldose reductase and vascular endothelial growth factor in eye tissue were significantly decreased in *Vasant Kusumakar Ras* treated rats. Hematoxylin and Eosin staining indicated that the *Vasant Kusumakar Ras* treatment significantly restored the normal architecture of the retinal tissue.
Conclusion: Vasant Kusumakar Ras exhibits protective effect and prevents the development of Diabetic retinopathy through its effects on multiple biochemical pathways implicated in pathogenesis of Diabetic retinopathy.

1. Introduction

Diabetes is an epidemic which is spreading at an alarming rate worldwide. According to the International Diabetes Federation, approximately 420 million people suffer from this disorder currently, with figures projected to increase to 620 million by the year 2045. The major concern with diabetes is development of complications such as macrovascular-ischemic heart disease, related stroke, and peripheral artery disease and/or microvascular-related retinopathy, neuropathy, and nephropathy [1]. Diabetic retinopathy, is a slow progressing complication with multifactorial aetiology and affects approximately 80% of diabetics worldwide [2]. Chronic hyperglycemic milieu of *Diabetes mellitus* activates alternative pathways for excessive glucose metabolism which contribute to the pathogenesis of Diabetic retinopathy [3]. Current conventional therapy for treatment of *Diabetes mellitus* is inefficient

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in preventing the development of Diabetic retinopathy. The World Health Organization expert committee on Diabetes has recommended that traditional systems of medicine should be encouraged for prevention and treatment of diabetic complications, especially when the conventional pharmacotherapy is inefficient [4]. Vasant Kusumakar Ras is a well-known herbo-mineral formulation marketed worldwide for diabetes and associated symptoms. According to the Ayurvedic literature, *Vasant Kusumakar Ras* possess *Pramehgana (*anti-diabetic*), Ojovardhak* and *Rasayana* (improves immunity, rejuvenator) properties and thus can have a protective role in diabetes [5]. The aim of the present study was to confirm and elucidate the mechanism of protective action of *Vasant Kusumakar Ras* in prevention of Diabetic retinopathy in rodent model of *Diabetes mellitus*.

2. Materials and methods

2.1. Chemicals

Streptozotocin (extrapure) was purchased from Sisco Research Laboratory (SRL), India. All the reagents and chemicals used in this study were of analytical grade.

2.2. Preparation of Vasant Kusumakar Ras formulation

Vasant Kusumakar Ras formulation was prepared as per the ayurvedic text *Yogratnakar –Prameha chikit sanadhaya* as follows:

1. Two parts of *Swarna bhasma* and *Rajat bhasma*
2. Three parts each of *Vangabhasma, Kanta Loha bhasma*
3. Four parts of *Parad bhasma, Abhrak bhasma, Praval and Mauktik*.

The above ingredients were processed and *Bhavana* (Trituration) was given 7 times separately with each of the following ingredients:

Cow milk, *Ikshu Ras* (*Saccharum officinarum*), *Vasa* (*Adhatoda vasica Nees.)*, *Kamal (Nelumbium speciosum Willd), Jalavetas*(*Salix tetrasperma Roxb*), *Haridra (Curcuma longa Linn), Kadalikanda(Musa sapientum* Linn) and one bhavana of Rose *(Rosa centifolia Linn)*, *Malati* (*Jasminum grandiflorum Linn*) and *Kastuti(*Musk*) (*either of these juices).

The formulation was developed in tablet dosage form.

2.3. Experimental animals

Animals (adult male Wistar rats, 200–250 g) were purchased from Bombay Veterinary College, Parel Mumbai, India. They were housed in the animal house of the institution. Animals were acclimatized for seven days prior to the study and maintained on a 12-h light–12 h–dark cycle with temperature 25±2 °C and relative humidity 50–70%. They were provided with commercial animal feed from Amrut laboratories, India and drinking water *ad libitum*. The experimental protocol (ICT/IAEC/2016/P08) was approved by Institutional Animal Ethics Committee (IAEC) registered under the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

2.4. Induction of experimental diabetes

Diabetes was induced in 12-h fasted male wistar rats by single intraperitoneal (*i.p*) injection of streptozotocin (45 mg/kg) in citrate buffer pH 4.5. The dose of streptozotocin was weighed individually for each rat in an amber coloured microcentrifuge tube which was placed on ice until used. Streptozotocin dose in each microcentrifuge tube was reconstituted with cold citrate buffer (pH 4.5) and immediately injected intraperitoneally within 10 s. Six hours after streptozotocin administration, drinking water was replaced with 10% glucose solution, for the next 24 h to prevent fatal hypoglycaemia due to massive insulin release from the pancreatic β-cells undergoing necrosis. Seven days after induction, fasting blood glucose was determined using commercially available kit (Accurex, Mumbai). Animals with blood glucose level higher than 13.88 mmol/L (>250 mg/dL) were termed diabetic and included in the study [6].

2.5. Experimental procedure

There are three groups in the study. Control group consisted of normal non-diabetic animals (n = 6); Diabetic control group (n = 10); Treatment group (n = 10) received *Vasant Kusumakar Ras*. Animals were kept without any treatment for period of 21 days (3 weeks) for induction of diabetic retinopathy. Treatment with was initiated from 4th week and continued till 8th week. A 10% mortality is generally observed in streptozotocin treated diabetic rats; thus, to account for this mortality, extra rats were included in diabetic control group and treatment group. Each *Vasant Kusumakar Ras* tablet was crushed and uniformly suspended in distilled water and dosed to animals. The daily human dose was extrapolated to yield 11.25 mg/kg dose for rats. The given dose accounts for excipients in the tablet.

The experimental groups were as follows:

1. Group I- Normal control group (n = 6) received distilled water *p.o*
2. Group II- Diabetic control group (n = 10)
3. Group III- Treatment group (n = 10) received *Vasant Kusumakar Ras* (11.25 mg/kg *p.o*)

2.6. Samples

At the end of the experimental period (8 weeks), rats were anesthetised under light isoflurane anaesthesia; and blood was withdrawn from retro-orbital plexus following 6 h fasting. Whole blood was used for estimation of HBA1c using standard commercial kit (Accurex diagnostics, Mumbai). Blood was centrifuged at 4000 g for 10 min, subsequently serum was separated and stored at −20 °C until further analysis. Serum was used for estimation of fasting blood glucose and serum lipid profile using standard commercial kits (Accurex diagnostics, Mumbai). Thereafter, rats were perfused with normal saline and both eyes were excised. Eyes were rinsed in ice-cold saline solution, blot dried. Left eye of each rat was fixed in 10% formalin solution for histopathological evaluation. Each right eye was cut into two equal parts using a scalpel, individually weighed and stored at −80 °C until further biochemical analysis. One half was used for estimation of oxidative stress and VEGF, whereas another half was used for determination of aldose reductase, in eye tissue homogenate as described in detail in following sections.

2.7. Biochemical assessment

2.7.1. HbA1c, fasting blood glucose, serum lipid profile

Whole blood was used for estimation of HbA1c using standard commercial kit from Accurex (Mumbai). Serum was used for analysis of fasting blood glucose and serum lipid profile (total cholesterol, triglycerides and high-density lipoprotein cholesterol) by enzymatic methods using standard commercial kits from Accurex (Mumbai).

2.7.2. Oxidative stress and vascular endothelial growth factor in eye tissue homogenate

Whole eye homogenate (10% w/v) was prepared by homogenising one half of each right eye in ice cold potassium phosphate buffer (pH 7.4). It was further centrifuged for 15 min at -4 °C using a cooling centrifuge (10,000 rpm; R-248M of CPR-24 plus Instrument, Remi, India). The supernatant thus obtained was used for assessment of endogenous antioxidant parameters according to Kanchan et al. [6]. Vascular endothelial growth factor (VEGF) level in the supernatant was determined by commercially available ELISA kit (Biotech, India) according to manufacturer's instructions. Protein content was estimated using Bradford method [7].

2.7.3. Aldose reductase activity in eye tissue homogenate

Aldose reductase activity in whole eye homogenate was measured as per the procedure of Hayman and Kinoshita with minor modifications [8]. One half of each right eye was homogenised in 3 volumes of cold 5 mM Tris–HCl buffer (pH 7.4) followed by centrifugation at -4 °C (10,000 rpm; R-248MofCPR-24 plus Instrument, Remi, India) for 20 min. Enzyme activity was measured spectrophotometrically at 340 nm with D-xylose as substrate. Protein content was estimated using Bradford method [7].

2.8. Histopathology of retina

Left eye of each rat was fixed in 10% formalin in potassium phosphate buffer (0.1 M, pH 7.4). The samples were then subjected to dehydration, through graded alcohol series and embedded in paraffin. The paraffin blocks thus obtained, were cut into 4 mm sections and stained with Hematoxylin &Eosin (H&E). The slides were examined at 400X magnification under light microscopy.

2.9. Statistical analysis

The results were analysed using one-way analysis of variance followed by Dunnett's test for statistical significance. Data is expressed as mean ± SEM, n = 6 rats in normal control group and n = 10 in diabetic control and treatment group.

3. Results

3.1. Fasting blood glucose level and HbA1c level

Fasting blood glucose level (FBG) and HbA1c level was significantly ($p < 0.001$) elevated in the diabetic control group (FBG: 511.4 ± 26.54 mg/dL, HbA1c: 8.66 ± 0.21%) as compared to normal control group (FBG: 94.67 ± 7.27 mg/dL; HbA1c: 5.20 ± 0.35%). However, treatment with *Vasant Kusumakar Ras* did not exhibit any changes in both; blood glucose level (488.60 ± 37.69 mg/dL) as well as HbA1c level (8.55 ± 0.16%) (Fig. 1).

3.2. Serum lipid profile

Diabetic control group exhibited increase in serum levels of cholesterol and triglycerides along with decrease in serum HDL-C as compared to normal control group. Treatment with *Vasant Kusumakar Ras*significantly decreased serum levels of cholesterol and triglycerides and increased serum HDL-C levels as compared to diabetic group (Table 1).

3.3. Oxidative stress parameters in eye tissue homogenate

Oxidative stress in eye tissue homogenate was increased in diabetic control group as compared to normal control group, as evidenced by decrease in levels of endogenous antioxidant

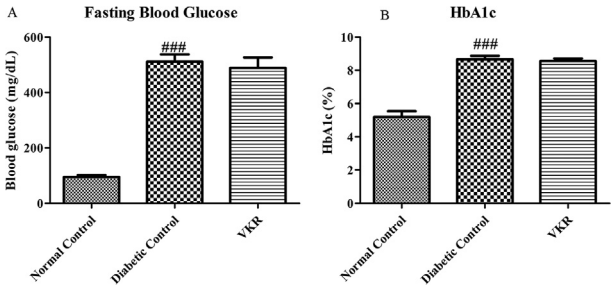

Fig. 1. a: Effect of *Vasant Kusumakar Ras* on fasting blood glucose level. b: Effect of *Vasant Kusumakar Ras* on HbA1c level. Data is expressed as mean ± SEM; ###significant difference ($p < 0.001$) with respect to the control, ***significant difference ($p < 0.001$) with respect to diabetic control, **significant difference ($p < 0.01$) with respect to diabetic control. VKR: *Vasant Kusumakar Ras.*

enzymes and increase in levels of lipid peroxidation. Levels of endogenous antioxidant enzymes namely catalase (CAT) and superoxide dismutase (SOD) was significantly ($^{###}p < 0.001$) increased in diabetic control group (CAT: 56.19 ± 1.21 Units/mg protein; SOD:6.12 ± 1.07 Units/mg protein) as compared to normal control group (CAT:78.69 ± 1.70 Units/mg protein; SOD:30.01 ± 1.18 Units/mg protein). Treatment with *Vasant Kusumakar Ras* significantly (**$p < 0.01$, ***$p < 0.001$) increased the levels of intracellular antioxidant enzymes (CAT: 63.00 ± 1.29 Units/mg protein; SOD: 26.60 ± 0.74 Units/mg protein) as compared to the diabetic control group (Fig. 2A, B). Diabetic rats displayed significantly ($^{###}p < 0.001$) low levels of reduced glutathione (GSH) in eye tissue homogenate (GSH: 7.88 ± 0.72 μg/mg protein) as compared to normal rats (GSH: 11.93 ± 0.59 μg/mg protein). *Vasant Kusumakar Ras* treatment significantly (***$p < 0.001$) restored the reduced glutathione content to normal level (GSH: 11.86 ± 0.60 μg/mg protein) (Fig. 2C). Lipid peroxidation (LPO), a marker of cellular injury was significantly increased in diabetic rats (LPO:376.04 ± 30.67nmols MDA/mg protein) as compared to normal rats (LPO: 185.88 ± 6.73nmols MDA/mg protein). Treatment with *Vasant Kusumakar Ras* significantly (***$p < 0.001$) decreased cellular injury as indicated by decrease in lipid peroxidation level (LPO: 219.18 ± 8.13nmols MDA/mg protein) compared to diabetic control group (Fig. 2D).

3.4. Aldose reductase activity in eye tissue homogenate

Aldose reductase (AR) activity was increased significantly ($^{###}p < 0.001$) in eye tissue of diabetic rats (AR activity: 1.13 ± 0.01 Units/mg protein) as compared to normal rats (AR activity: 0.21 ± 0.02 Units/mg protein). Treatment with *Vasant Kusumakar Ras* significantly (***$p < 0.001$) decreased Aldose reductase activity (0.79 ± 0.01 Units/mg protein) in eye tissue (Fig. 3).

3.5. Vascular endothelial growth factor (VEGF) in eye tissue homogenate

VEGF level was increased significantly ($^{###}p < 0.001$) in eye tissue of diabetic rats (7118.18 ± 16.07 pg/mg protein) as compared to normal rats (575.96 ± 47.59 pg/mg protein). Treatment with *Vasant Kusumakar Ras* significantly (***$p < 0.001$) decreased VEGF level (3030.77 ± 299.16 pg/mg protein) in eye tissue (Fig. 4).

3.6. Histopathological analysis of retina by hematoxylin & eosin staining

Histopathological analysis of retina by Hematoxylin and Eosin staining demonstrated that the cellularity in inner and outer

Table 1
Effect of *Vasant Kusumakar Ras* on serum lipid profile.

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)
Normal control	57.35 ± 4.07	84.33 ± 8.61	47.32 ± 5.16
Diabetic control	91.17 ± 0.53###	161.68 ± 8.05##	8.39 ± 0.92###
VKR	74.61 ± 3.54**	109.84 ± 23.39*	40.33 ± 1.77***

Data expressed as mean ± SEM. ###significant difference ($p < 0.001$), ## significant difference ($p < 0.01$), with respect to the control, ***significant difference ($p < 0.001$) with respect to diabetic control, **significant difference ($p < 0.01$) with respect to diabetic control, *significant difference ($p < 0.05$) with respect to diabetic control.. VKR: *Vasant Kusumakar Ras*.

nuclear layer was decreased in diabetic control group. However, treatment with *Vasant Kusumakar Ras* restored the cellularity similar to normal control group. Vascularity of ganglion cell layer was moderately increased in diabetic control group. White arrows indicate that new vessels appeared in the junction between ganglion cell layer and inner plexiform layer, and the junction between inner plexiform layer and inner nuclear layer. Treatment with *Vasant Kusumakar Ras* decreased the vascularity of ganglion cell layer and formation of new blood vessels (Fig. 5).

4. Discussion

Diabetic retinopathy is the most common complication of diabetes often resulting in visual impairment or blindness [9]. The current treatment for diabetes and its complications includes insulin, insulin sensitizers such as Metformin and/or oral hypoglycemic agents. However, this therapy focuses only on lowering of blood glucose levels and does not target the underlying pathophysiology of diabetic retinopathy. In addition, they do not prevent the eventual progression of Diabetic retinopathy. Therefore, it is essential to administer agents which specifically target the pathophysiological mechanisms involved in development of Diabetic retinopathy, in addition to treatment strategies involving glycemic control.

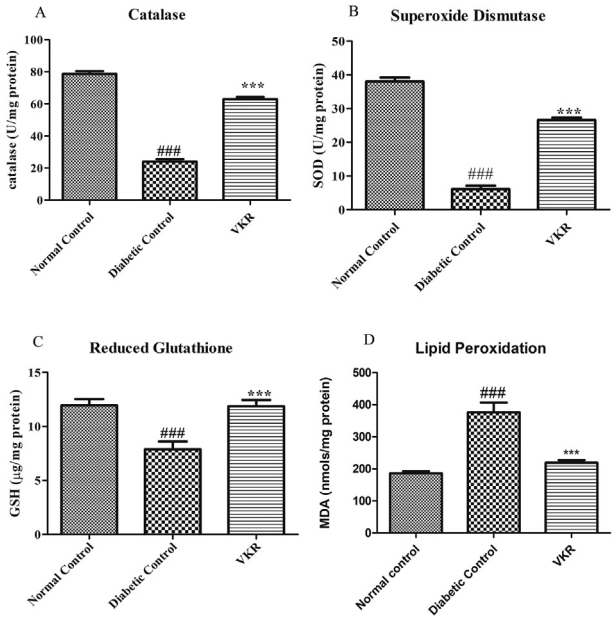

Fig. 2. Effect of *Vasant Kusumukar Ras* on Catalase activity (A), Superoxide dismutase activity (B), reduced glutathione content (C) and lipid peroxidation levels (D). Data is expressed as mean ± SEM; ###significant difference ($p < 0.001$) with respect to the control, ***significant difference ($p < 0.001$) with respect to diabetic control, **significant difference ($p < 0.01$) with respect to diabetic control.

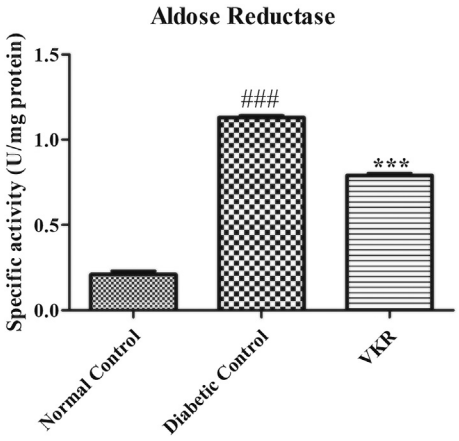

Fig. 3. Effect of Vasant Kusumukar Ras aldose reductase activity. Data is expressed as mean ± SEM; ###significant difference ($p < 0.001$) with respect to the control, ***significant difference ($p < 0.001$) with respect to diabetic control.

The biochemical changes associated with chronic hyperglycemic mileu of *Diabetes mellitus* contributes to the pathogenesis of Diabetic retinopathy. Chronic hyperglycemia induced generation of reactive oxygen species (ROS) has been identified as the central pathognomonic mechanism of diabetic retinopathy. ROS inhibits a key enzyme in glycolytic pathway namely glyceraldehyde 3-phosphate dehydrogenase (GAPDH) thus increasing the levels of all glycolytic intermediates upstream of glyceraldehyde 3-phosphate. Subsequently, the accumulated glycolytic intermediates enter one of these pathways Advanced glycation end products (AGE) formation, polyol pathway (aldose reductase), protein kinase C (PKC) pathway and hexosamine pathway, each of which are implicated in development of diabetic complications [3]. ROS cause retinal vasculature damage

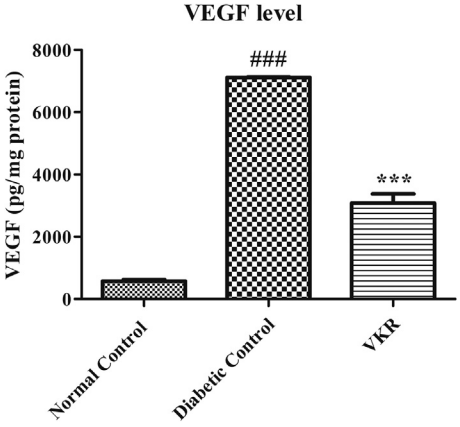

Fig. 4. Effect of *Vasant Kusumukar Ras* on level of vascular endothelial growth factor (VEGF) in eye tissue homogenate. Data is expressed as mean ± SEM; ###significant difference ($p < 0.001$) with respect to the control, ***significant difference ($p < 0.001$) with respect to diabetic control.

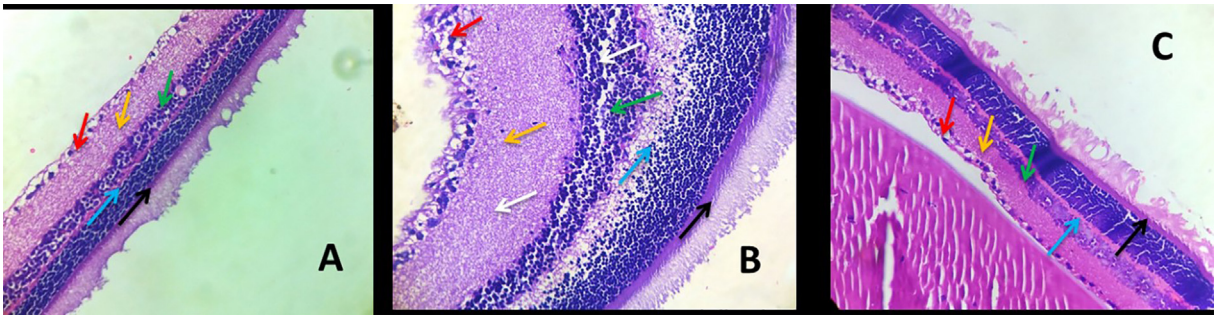

Fig. 5. Photomicrographs of histopathological analysis of retina by H&E staining; A: Normal Control, B: Diabetic Control, C: *Vasant Kusmumakar Ras*. Red arrow: ganglion cell layer, yellow arrow: inner plexiform layer, green arrow: inner nuclear layer, blue arrow: outer plexiform layer, black arrow: outer nuclear layer, white arrow: formation of new blood vessels.

as well as induce the production and release of proinflammatory cytokines, namely, interleukin 1β and Tumour necrosis factor-α (TNF-α) which further damage the endothelial cells and contribute to the pathogenesis of Diabetic retinopathy [10].

The present study aimed to evaluate the effect and mechanism of action of an Ayurvedic herbo-mineral formulation, *Vasant Kusumakar Ras* in the prevention of diabetic retinopathy using rodent model of *Diabetes mellitus*. *Vasant Kusumakar Ras* has been described in Ayurvedic texts for its use in the management of diabetic complications. It has been proposed that the ingredients of *Vasant Kusumakar Ras* comprise of antioxidants, micronutrients and immune-modulators which can help check the aetiology of *Diabetes mellitus* and have protective effects on eyes and nerves [5].

In the present study, *Vasant Kusumakar Ras* treatment did not significantly reduce the fasting blood glucose levels and HbA1c as compared to the diabetic rats. This indicates that *Vasant Kusumakar Ras* does not act on pancreas to stimulate insulin release, neither does it act as insulin sensitizer to increase peripheral glucose utilisation. However, it helps in correcting the metabolic imbalance caused due to *Diabetes Mellitus*. Diabetic rats depicted altered serum lipid profile which is a hallmark of diabetes induced metabolic dysfunction [11]. Diabetic rats showed elevated cholesterol and triglycerides levels along with decrease in HDL-C as compared to normal control rats. Treatment with *Vasant Kusumakar Ras* significantly decreased levels of cholesterol and triglycerides along with significant increase in HDL-C level as compared to diabetic rats. Thus, *Vasant Kusumakar Ras* treatment prevented diabetes induced metabolic dysfunction.

As mentioned earlier, hyperglycemia induced oxidative stress represents a 'unifying mechanism' linking all the deleterious biochemical pathways induced by hyperglycemia in the pathogenesis of Diabetic retinopathy [3]. Oxidative stress was measured in eye tissue homogenates by determining the levels of key intracellular antioxidant enzymes, namely superoxide dismutase and catalase, reduced glutathione (major intracellular antioxidant) and lipid peroxidation (marker of free radical mediated cellular injury). Eye tissue homogenates of diabetic rats displayed significantly lower levels of superoxide dismutase, catalase and reduced glutathione, whereas level of malondialdehyde, a marker for lipid peroxidation was significantly increased as compared to normal control group. However, treatment with *Vasant Kusumakar Ras* significantly increased the levels of superoxide dismutase, catalase and reduced glutathione along with significant decrease in level of lipid peroxidation in eye tissue homogenate as compared to the diabetic group. Thus, *Vasant Kusumakar Ras* treatment alleviated the oxidative stress induced by hyperglycaemia which can be attributed to the antioxidant principles present in the formulation.

Polyol pathway metabolises excess of glucose in diabetes producing sorbitol; this pathway has been widely explored in the pathogenesis of Diabetic retinopathy. Aldose reductase (AR) present in the retina reduces glucose into sorbitol using NADPH as a cofactor. Subsequently, sorbitol is converted to fructose by the enzyme sorbitol dehydrogenase. Under normal conditions (euglycemia), only 3% of glucose enters the polyol pathway to form sorbitol, however, in diabetic conditions (hyperglycaemia) about 30% of glucose enters this pathway resulting in formation and accumulation of excess of sorbitol. The accumulation of sorbitol induces osmotic damage being deleterious to retinal cells. Moreover, intracellular reduced glutathione is regenerated in the cells by the enzyme glutathione reductase which uses NADPH as a cofactor. The excess utilisation of NADPH in the polyol pathway diminishes the availability of cofactor for use by glutathione reductase which depletes intracellular reduced glutathione levels and further increases oxidative stress. In addition, the fructose undergoes phosphorylation to form fructose-3-phosphate, which degrades to 3-deoxyglucosone, both of which are potent glycating agents and can result in glycative stress and advanced glycation end product (AGE) formation [12,13]. Measurement of aldose reductase is considered to be an indicator of the progression of Diabetic retinopathy [14]. Diabetic rats had significantly elevated levels of aldose reductase in eye tissue homogenate as compared to normal control rats due to overactivation of the polyol pathway. Treatment with *Vasant Kusumakar Ras* decreased the levels of aldose reductase significantly as compared to the diabetic rats.

Chronic hyperglycemia causes tissue hypoxia resulting in upregulation of vascular endothelial growth factor (VEGF), an angiogenic growth factor. Tissue hypoxia leads to the production of a DNA binding protein, termed as hypoxia inducible factor-1 (HIF-1) which binds to VEGF gene and increases the transcription and translation of VEGF. VEGF regulates neovascularisation, increases the dilation and permeability of blood vessels contributing to the development of proliferative Diabetic retinopathy [15]. Diabetic rats had elevated VEGF levels in eye tissue homogenate which was significantly decreased by *Vasant Kusumakar Ras* treatment. This can be attributed to the anti-inflammatory and immunomodulatory principles present in the formulation.

To comprehend whether the above biochemical changes yielded structural variations at the microscopic level, histological analysis of the retinal tissue was carried out. Histological changes and retinal damage were investigated by Hematoxylin and Eosin staining under 400× magnification. The thickness and vascularity of ganglion cell layer was increased in diabetic control group indicating angiogenesis and progression to proliferative Diabetic retinopathy. This correlates with the increased levels of VEGF, a key mediator of angiogenesis, observed in eye tissue homogenates of diabetic rats. Treatment with *Vasant Kusumakar Ras* decreased the vascularity in ganglion cell layer which can be attributed to its ability to decrease VEGF level. Cellularity in the inner and outer

nuclear layer was decreased in diabetic group which indicates retinal damage and cellular injury. *Vasant Kusumakar Ras* treatment restored the cellularity as well vascularity to normal, indicating a protective effect. This protective effect can be attributed to the ability of *Vasant Kusumakar Ras* to ameliorate hyperglycemia induced oxidative stress and correlates with decreased lipid peroxidation and increase in antioxidant enzymes in the treatment group.

Vasant Kusumakar Ras is an Ayurvedic Bhasma which has been extensively used in Ayurveda for treatment of Diabetic complications. The protective effect of *Vasant Kusumakar Ras* can be attributed to pharmacological activities of each of the individual constituents which act synergistically to produce the desired effect. However, detailed reports indicating their mechanism of action is limited. *Swarna Bhasma* (gold ash), one of the components of *Vasant Kusumakar Ras* showed anti-oxidant and restorative effects against global and focal effects of ischaemia (stroke) [16]. In Ayurveda, it has been widely used for the treatment of rheumatoid arthritis and same effects have also been observed in arthritis rat model [17]. A clinical study by Strong et al. in 1973 demonstrated that gold compounds decreased levels of rheumatic factors and had immunomodulatory effect [18]. These data indicate that *Swarna Bhasma* possess anti-inflammatory and anti-oxidant effect which are also evident in the present study. *Loha Bhasma* is used for treatment of *Prameha* (Diabetes) and *Medoroga* (Hyperlipidemia), which may contribute to restoration of serum lipid profile by *Vasant Kusumakar Ras* treatment [19]. The *Bhavana dravyas* used in the formulation have their individual pharmacological effects on physiological pathways implicated in pathogenesis of diabetic retinopathy and amelioration of oxidative stress and inflammation. A few of these are reported in literature. *Vasant Kusmumakar Ras* treatment resulted in significant decrease in aldose reductase activity in eye tissue homogenate. This effect can be ascribed to the *Bhavana dravyas* present in the formulation. Aqueous, ethanol and chloroform extract of *A. vasica* possess rat lens aldose reductase inhibitory potential, anti-cataract and antioxidant activities [20]. Methanolic extract of stamens of *N. speciosum* demonstrated inhibitory effect on rat lens aldose reductase. Two flavonoids, namely kaempferol 3-O-α-L-rhamnopyranosyl-(1 → 6)-β -D-glucopyranoside and isorhamnetin 3-O-α-Lrhamnopyranosyl-(1 → 6)-β -D-glucopyranoside demonstrated highest inhibition of rat lens aldose reductase activity *in vitro* [21]. Aqueous extract of *C. longa* inhibited rat lens aldose reductase *in vitro* [22]. Methanolic extract of *S. tetrasperma* possess *in vitro* DPPH radical scavenging activity [23]. Extract of *R. centifolia* petals inhibited diacylglycerol acyltransferase, the enzyme catalysing the ultimate step of triacylglycerol synthesis, in a dose-dependent manner and thereby suppressed triacylglycerol synthesis in cultured cells. This activity was due to ellagitannins namely rugosin B, rugosin D and eusupinin. The extract also suppressed post-prandial increase of plasma triacylglycerol in oral fast load test in mice [24]. This suggests that rose extract can have beneficial effect in controlling lipid dysfunction which accompanies *diabetes mellitus*. In present study, *Vasant Kusumakar Ras* treatment ameliorated lipid dysfunction in diabetic rats which could be attributed to this principle of the formulation. A study conducted by Shukla, 2018 has shown that *Vasant Kusumakar Ras* treatment provided good symptomatic relief in patients with diabetic neuropathy. Although, *Vasant Kusumakar Ras* has been used extensively used as a therapy for treatment of diabetic complications, including diabetic retinopathy; detailed studies determining its mechanism of action are relatively scarce. This provided the premise for our study to determine the mechanism of action of *Vasant Kusumakar Ras* in the treatment of diabetic retinopathy.

5. Conclusion

The present study investigated the protective effect of an Ayurvedic herbo-mineral formulation, *Vasant Kusumkar Ras* in diabetic retinopathy. *Vasant Kusmukar Ras* demonstrated a protective effect and prevented the progression and development of Diabetic retinopathy. The protective effect can be attributed to its ability to act on multiple biochemical pathways implicated in pathogenesis of Diabetic retinopathy including, amelioration of oxidative stress, effect on polyol pathway and VEGF level. Thus, the study endorses the therapeutic utility of *Vasant Kusumakar Ras* as an adjuvant therapy for prevention and treatment of diabetic retinopathy, in addition to the conventional therapy aimed at controlling blood glucose level. Although the experimental findings of the present study demonstrating the protective effects of *Vasant Kusumakar Ras* on amelioration of diabetic retinopathy are promising, further studies are warranted. These could include studying the effect of *Vasant Kusumakar Ras* on additional parameters such as inflammatory cytokines (e.g. interleukin-1β) implicated in pathogenesis of diabetic retinopathy. In addition, lenticular and fundoscopic examination of rat pupils can be performed to score the severity of retinal damage. These studies would further reinforce the protective effects and therapeutic potential of *Vasant Kusumakar Ras* for treatment of diabetic retinopathy.

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Conflicts of interest

None.

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