



## **Evaluation of Antidiabetic Activity of Polyherbal Formulation “*Vasant Kusumakar Ras*” on Alloxan-induced and Dexamethasone-induced Diabetic Rats**

**Talha Jawaid <sup>a</sup>, Kumari Nishu <sup>b</sup>, Mehnaz Kamal <sup>c\*</sup> and Saud M. Alsanad <sup>a</sup>**

<sup>a</sup> Department of Pharmacology, College of Medicine, Al Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 13317, Saudi Arabia.

<sup>b</sup> Hygia Institute of Pharmaceutical Education and Research, Lucknow, Uttar Pradesh, India.

<sup>c</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al Kharj 11942, Saudi Arabia.

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors TJ and MK conceptualized and supervised the study, conducted a literature search and helped in drafting the final manuscript. Author KN conducted the literature search, performed all the experiments, statistical analysis and drafted the initial manuscript. Author SMA helped in drafting the final manuscript. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JPRI/2021/v33i51A33462

#### Editor(s):

(1) Dr. Wenbin Zeng, Central South University, China.

(2) Dr. Alyautdin Renad N, Scientific Centre for Expert Evaluation of Medicinal Products, Russia.

#### Reviewers:

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(4) Said Sani Said, Federal University Dutsin-MA, Nigeria.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/76389>

**Original Research Article**

**Received 05 September 2021**

**Accepted 11 November 2021**

**Published 20 November 2021**

### **ABSTRACT**

**Aim:** The current study observed the antidiabetic effect of *Vasant Kusumakar Ras*, an Ayurvedic polyherbal formulation, in alloxan-induced and dexamethasone-induced diabetic rats.

**Materials and Methods:** Alloxan (120 mg/kg, i.p.) and dexamethasone sodium phosphate (5 mg/kg, i.p.) were used to induce diabetes in rats. The oral antidiabetic activity of *Vasant Kusumakar Ras* was evaluated by single doses of *Vasant Kusumakar Ras* (400 and 600 mg/kg, p.o.) in albino rats during a 10-day treatment period, with the effect of the *Vasant Kusumakar Ras* on blood glucose levels and serum lipid parameters measured on 0, 7<sup>th</sup>, and 11<sup>th</sup> day. Glibenclamide (5 mg/kg, p.o.) was used as the reference drug.

\*Corresponding author: E-mail: [mailtomehnaz@gmail.com](mailto:mailtomehnaz@gmail.com), [m.uddin@psau.edu.sa](mailto:m.uddin@psau.edu.sa);

**Results:** In alloxan-induced diabetic rats, the elevated levels of blood glucose significantly ( $p < 0.05$ ) decreased after oral administration of *Vasant Kusumakar Ras* (400 mg/kg and 600 mg/kg), and Glibenclamide (5 mg/kg). When compared to the diabetic control group, treatment with *Vasant Kusumakar Ras* and Glibenclamide for 10 days reduced total cholesterol (TC) significantly ( $p < 0.001$ ). Treatment with *Vasant Kusumakar Ras* and Glibenclamide for 10 days, significantly ( $p < 0.001$ ) decreased low-density lipoprotein (LDL) level when compared to the diabetic control group. In dexamethasone-induced diabetic rats, all rats given with dexamethasone and *Vasant Kusumakar Ras* (400 mg/kg and 600 mg/kg) showed a significant ( $p < 0.05$ ) decrease in the level of blood glucose when compared with diabetic control rats. The rats treated with dexamethasone and Glibenclamide showed a significant ( $p < 0.05$ ) decrease in blood glucose level when compared to diabetic control rats. When compared to the diabetic control group, treatment with *Vasant Kusumakar Ras* and Glibenclamide (5 mg/kg) for 10 days reduced TC significantly ( $p < 0.001$ ). Treatment with *Vasant Kusumakar Ras* and Glibenclamide for 10 days, significantly ( $p < 0.001$ ) decreased LDL level when compared to the diabetic control group.

**Conclusion:** *Vasant Kusumakar Ras* was shown to have significant antidiabetic activity comparable to that of glibenclamide and it also improves the lipid metabolism in both alloxan-induced and dexamethasone-induced diabetic rats.

**Keywords:** Polyherbal formulation; vasant kusumakar ras; alloxan; dexamethasone; antidiabetic.

## 1. INTRODUCTION

Diabetes mellitus is one of the most common diseases, affecting over 6% of the global population, and the diabetes dynamics are changing speedily in low- and middle-income nations [1]. According to International Diabetes Federation's (IDF) estimates, 80% of the world's diabetes population would come from low- and middle-income nations in 2030 [2]. Diabetes is one of the six leading causes of death worldwide, as well as a source of numerous systemic problems. Insulin therapy or glucose-lowering medicines such as alpha-glucosidase inhibitors, biguanides, sulfonylureas, and thiazolidinediones are used to treat diabetes mellitus. One of the challenges in the treatment of any systemic condition is the development of an adverse event; as a result, numerous research institutes and pharmaceutical companies are active in drug development to uncover molecules with strong therapeutic potential and fewer adverse events [3-4]. Despite the fact that several synthetic treatments have been developed for patients, no one has ever been reported to have completely recovered from diabetes [5]. The adverse effects of existing oral hypoglycemic medications are unfavorable. As a result, in recent years, much attention has been paid to the anti-diabetic potential of medicinal plants and their herbal formulations in disease treatment [6]. The World Health Organization expert committee on Diabetes has recommended that traditional systems of medicine should be supported for the prevention and management of diabetic complications. This is especially true when

mainstream pharmacotherapy is ineffective [7]. *Vasant Kusumakar Ras* is described by Yogaratnakar in Prameha Chikitsa Adhyaya [8]. It is a powerful anti-diabetic medicine [9] that works by combining the effects of each of the drug's ingredients. It pacifies tridosha i.e. vata, pitta and kapha as it contains Praval Pishti, Ras Sindoor, Mukta Pishti, Abhrak Bhasma, Swarna Bhasma, Rajat Bhasma, Loha Bhasma, Naga Bhasma, Vanga Bhasma, Vasa, Haldi, Ikshu, Kadali, Kamal, Chameli, Shatavari and Chandan. *Vasant Kusumakar Ras* is a well-known polyherbal formulation used to treat diabetes and its symptoms around the world [10]. According to the Ayurvedic literature, *Vasant Kusumakar Ras* possess *Pramehgana* (anti-diabetic), *Ojovardhak*, and *Rasayana* (improves immunity, rejuvenator) characteristics and so may play a protective role in diabetes [11]. *Vasant Kusumakar Ras* polyherbal formulation is manufactured by Dabur India Ltd. Company, and it is easily accessible to use for everyone. The goal of this study was to confirm the antidiabetic activity of *Vasant Kusumakar Ras* in alloxan and dexamethasone-induced diabetic rats.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

Glibenclamide tablet (Aventis Pharma Ltd., Mumbai, India); dexamethasone sodium phosphate (Tridoss Laboratories Ltd., Mumbai, India); alloxan (Sigma-Aldrich, USA); glucose estimation kit. (Span diagnostic Ltd., Surat, India), cholesterol and LDL estimation kits

(Agappe Diagnostics, Kerala) were used in this study. All the other reagents and chemicals used in this study were of analytical grade.

## 2.2 Preparation of Vasant Kusumakar Ras Formulation

*Vasant Kusumakar Ras* polyherbal formulation is manufactured by DABUR INDIA LTD. Company, a manufacturer and exporter of herbal ayurvedic goods. *Vasant Kusumakar Ras* formulation was made as per the ayurvedic text *Yogratnakar – Prameha chikit sanadhaya* as follows:

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

The above ingredients were processed and 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 the tablet dosage form.

## 2.3 Animals

Adult Wistar rats (170 ± 20 g) of either sex were obtained from Central Drug Research Institute, Lucknow, India. The animals were housed in huge, spacious polypropylene cages at an ambient room temperature with a 12-h light/12-h dark cycle. Rats had free access to water and a standard normal pellet diet.

## 2.4 Induction of Experimental Diabetes in Alloxan-induced Model and Experimental Design

Diabetes was induced in 24-h fasted Wistar rats by a single intraperitoneal (*i.p.*) injection of alloxan (120 mg/kg) in 0.9% saline. Four hours after alloxan administration, drinking water was replaced with a 10% glucose solution, for the next 24 hours to prevent catastrophic

hypoglycemia caused by enormous insulin release from the necrosing pancreatic  $\beta$ -cells. On the third day, the diabetes was confirmed by a blood glucose level (BGL) assessment. Diabetic animals were defined as those with blood glucose levels greater than 13.88 mmol/L (>250 mg/dL) and were included in the study [12]. The animals were divided into five groups of six animals in each group. Groups 1 received only 0.9% saline. Groups 2 received only alloxan (120 mg/kg, *i.p.*). Group 3 received Glibenclamide (5 mg/kg, *p.o.*) which served as the reference standard group. Polyherbal formulation at the doses, 400 and 600 mg/kg was administered orally to the rats of groups 4 and 5, respectively (Table 1). The blood glucose concentrations of the animals were measured at the beginning of the study on day 0 and measurements were repeated on the 7<sup>th</sup> and 11<sup>th</sup> days.

## 2.5 Induction of Experimental Diabetes in Dexamethasone-induced Model and Experimental Design

The animals were divided into five groups of six animals in each group. Groups 1 received only 0.9% saline. Groups 2 received only dexamethasone sodium phosphate (5 mg/kg, *i.p.*). Group 3 received Glibenclamide (5 mg/kg, *p.o.*) which served as the reference standard group. Polyherbal formulation at the doses, 400 and 600 mg/kg was administered orally to the rats of groups 4 and 5, respectively (Table 2). All the treatments were made for 10 days. One hour after the test drug administration all the rats received dexamethasone (5 mg/kg, *p.o.*) daily for 10 days except group 1 which served as the normal control group [13]. The blood glucose concentrations of the animals were measured at the beginning of the study on day 0 and measurements were repeated on the 7<sup>th</sup> and 11<sup>th</sup> day after 1 h post dexamethasone treatment.

## 2.6 Biochemical Analysis

At the end of the experimental period (on the 11<sup>th</sup> day), rats were anesthetized under mild ether anesthesia; and blood was withdrawn from the retro-orbital plexus. Glucose levels were measured in blood samples. Blood was centrifuged at 4000 g for 10 min., subsequently, serum was separated and kept at -20 °C until further investigation. Standard commercial kits (Agappe Diagnostics, Kerala) were used to estimate TC and LDL in serum.

**Table 1. Administration of Vasant Kusumakar Ras and Glibenclamide in the alloxan-induced model in rats**

S. No.	Group	Treatment
1	Group 1	Control (0.9% Normal saline)
2	Group 2	Kept as a negative control, i.e., treated with alloxan (120 mg/kg, i.p.)
3	Group 3	Treated with standard oral hypoglycemic drug, i.e., Glibenclamide (5 mg/kg, p.o.) + alloxan (120 mg/kg, i.p.)
4	Group 4	Treated orally with 400 mg/kg <i>Vasant Kusumakar Ras</i> + alloxan (120 mg/kg, i.p.)
5	Group 5	Treated orally with 600 mg/kg <i>Vasant Kusumakar Ras</i> + alloxan (120 mg/kg, i.p.)

**Table 2. Administration of Vasant Kusumakar Ras and Glibenclamide in the dexamethasone-induced model in rats**

S. No.	Group	Treatment
1	Group 1	Control (0.9% Normal saline)
2	Group 2	Kept as a negative control, i.e., treated with dexamethasone sodium phosphate (5 mg/kg, i.p.)
3	Group 3	Treated with standard oral hypoglycemic drug, i.e., Glibenclamide (5 mg/kg, p.o.) + Dexamethasone sodium phosphate (5 mg/kg, i.p.)
4	Group 4	Treated orally with 400 mg/kg <i>Vasant Kusumakar Ras</i> + Dexamethasone sodium phosphate (5 mg/kg, i.p.)
5	Group 5	Treated orally with 600 mg/kg <i>Vasant Kusumakar Ras</i> + Dexamethasone sodium phosphate (5 mg/kg, i.p.)

## 2.7 Statistical Analysis

Data were expressed as Mean  $\pm$  S.E.M. Statistical significance between the groups was tested using one-way ANOVA, followed by Dunnett's Multiple Comparison Test. Results were considered significant, when p values were less than 0.05 (\*p < 0.05).

## 3. RESULTS

### 3.1 Effect of *Vasant Kusumakar Ras* on Blood Glucose Level in Alloxan-induced Diabetic Rats

Alloxan administration at a dose of 120 mg/kg body weight generates a strong diabetogenic response in albino rats, according to the results of this study (Fig. 1). Blood glucose levels were assessed randomly on the 0, 7<sup>th</sup>, and 11<sup>th</sup> days of study. The elevated levels of blood glucose significantly ( $p < 0.05$ ) decreased after oral administration of *Vasant Kusumakar Ras* (400 mg/kg and 600 mg/kg), and Glibenclamide (5 mg/kg). If blood glucose levels are the main metric, *Vasant Kusumakar Ras* and Glibenclamide were both highly effective in inducing a strong antihyperglycemic response in rats. Though all two doses of formulations

produced equivalent antihyperglycemic activity to Glibenclamide in terms of relative efficacy, *Vasant Kusumakar Ras* 400 mg/kg was shown to have stronger antihyperglycemic activity than *Vasant Kusumakar Ras* 600 mg/kg.

### 3.2 Effect of *Vasant Kusumakar Ras* on TC Level in alloxan-induced Diabetic Rats

The serum TC of diabetic control group rats increased significantly on the 11<sup>th</sup> day as compared to normal control group rats. When compared to the diabetic control group, treatment with *Vasant Kusumakar Ras* and Glibenclamide for 10 days reduced TC significantly ( $p < 0.001$ ) (Fig. 2). Though all two doses of formulations demonstrated equivalent serum cholesterol-lowering activity to Glibenclamide (5 mg/kg), *Vasant Kusumakar Ras* 400 mg/kg has been proven to have higher serum cholesterol-lowering activity than *Vasant Kusumakar Ras* 600 mg/kg in terms of relative efficacy.

### 3.3 Effect of *Vasant Kusumakar Ras* on LDL Level in Alloxan-induced Diabetic Rats

On the 11<sup>th</sup> day, there was a significant increase in serum LDL level of diabetic control group rats,

as compared to normal control group rats. Treatment with *Vasant Kusumakar Ras* and Glibenclamide for 10 days, significantly ( $p < 0.001$ ) decreased LDL level when compared to the diabetic control group (Fig. 3). Though all two doses of formulations demonstrated equivalent serum LDL lowering activity to Glibenclamide (5 mg/kg), *Vasant Kusumakar Ras* 400 mg/kg has been proven to have stronger serum LDL lowering activity than *Vasant Kusumakar Ras* 600 mg/kg in terms of relative efficacy.

### 3.4 Effect of *Vasant Kusumakar Ras* on Blood Glucose Level in Dexamethasone-induced Diabetic Rats

Blood glucose levels were assessed randomly on the 0, 7<sup>th</sup>, and 11<sup>th</sup> days of study. When comparing the diabetic control group to the normal control group, there was a significant increase in blood glucose levels. All rats given with dexamethasone and *Vasant Kusumakar Ras* (400 mg/kg and 600 mg/kg) showed a significant decrease ( $p < 0.05$ ) in the level of blood glucose when compared with diabetic control rats (Fig. 4). The rats treated with dexamethasone and Glibenclamide showed a significant ( $p < 0.05$ ) decrease in blood glucose

level when compared to diabetic control rats. On the 11<sup>th</sup> day, *Vasant Kusumakar Ras* 400 mg/kg was found to have stronger antihyperglycemic activity than *Vasant Kusumakar Ras* 600 mg/kg, even though all two doses of formulations produced comparable antihyperglycemic activity to Glibenclamide (5 mg/kg). The *Vasant Kusumakar Ras* has a considerable effect on dexamethasone-induced insulin resistance, according to the findings.

### 3.5 Effect of *Vasant Kusumakar Ras* on TC Level in Dexamethasone-induced Diabetic Rats

The serum TC of diabetic control group rats increased significantly on the 11<sup>th</sup> day as compared to normal control group rats. When compared to the diabetic control group, treatment with *Vasant Kusumakar Ras* and Glibenclamide (5 mg/kg) for 10 days reduced TC significantly ( $p < 0.001$ ) (Fig. 5). Though all two doses of formulations generated equivalent serum cholesterol-lowering activity to Glibenclamide in terms of relative efficacy, *Vasant Kusumakar Ras* 600 mg/kg was shown to have higher serum cholesterol-lowering action than *Vasant Kusumakar Ras* 400 mg/kg.

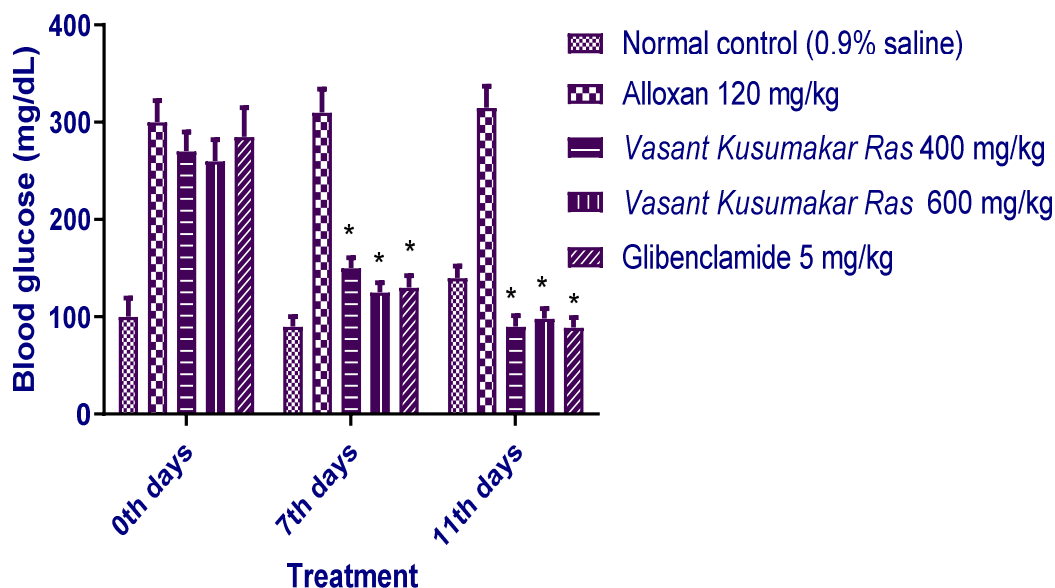
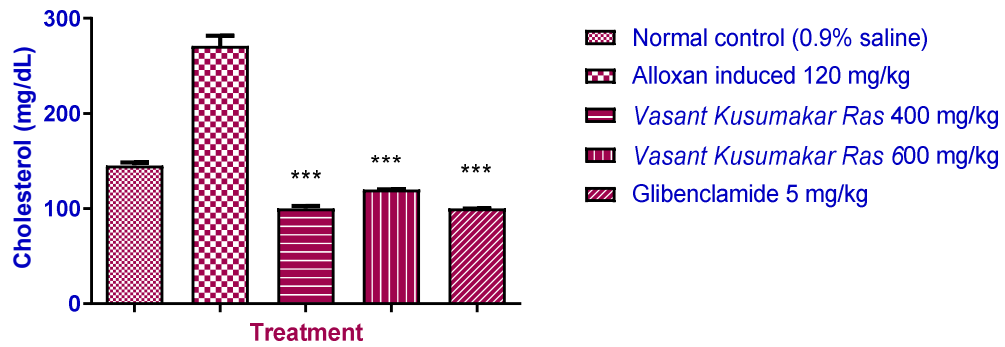
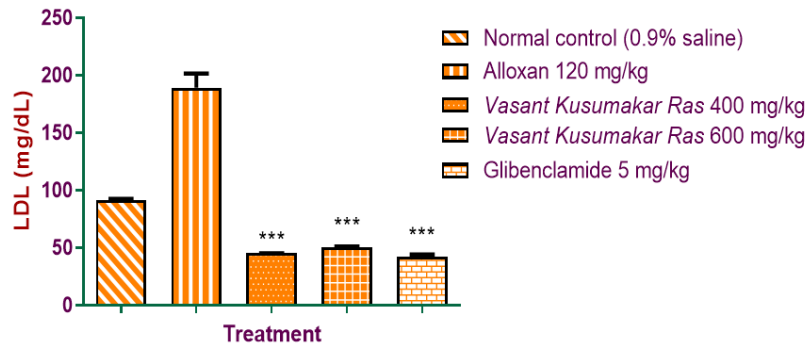


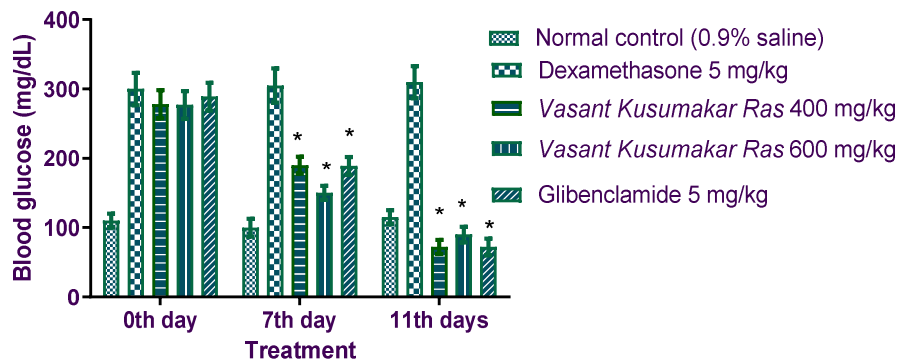
Fig. 1. Effect of *Vasant Kusumakar Ras* and Glibenclamide on blood glucose level in alloxan-induced diabetic rats. Data are expressed as Mean  $\pm$  S.E.M.; \*significant difference ( $p < 0.05$ ) with respect to diabetic control



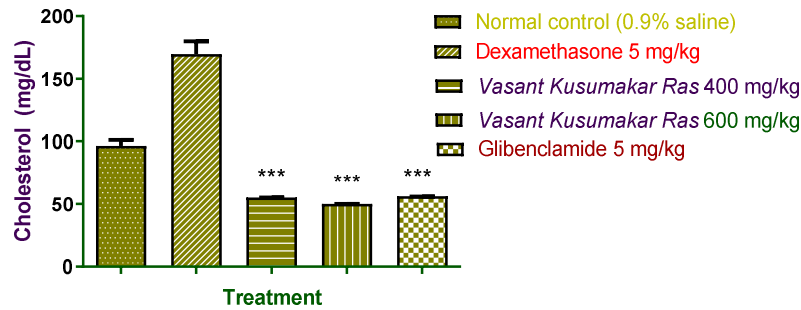
**Fig. 2.** Effect of *Vasant Kusumakar Ras* and Glibenclamide on TC level in alloxan-induced diabetic rats. Data are expressed as Mean  $\pm$  S.E.M.; \*\*\*significant difference ( $p < 0.001$ ) with respect to diabetic control



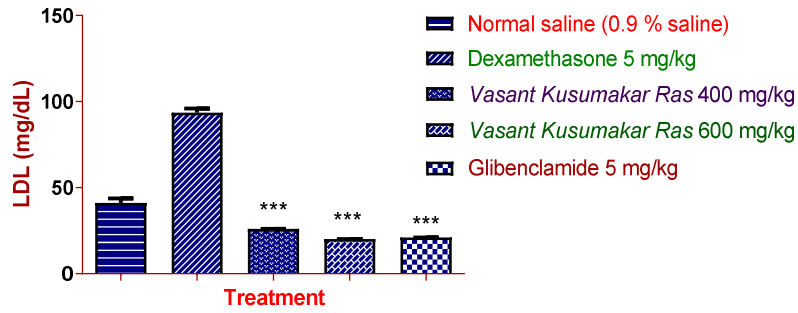
**Fig. 3.** Effect of *Vasant Kusumakar Ras* and Glibenclamide on LDL level in alloxan-induced diabetic rats. Data are expressed as Mean  $\pm$  S.E.M.; \*\*\*significant difference ( $p < 0.001$ ) with respect to diabetic control



**Fig. 4.** Effect of *Vasant Kusumakar Ras* and Glibenclamide on blood glucose level in dexamethasone-induced diabetic rats. Data are expressed as Mean  $\pm$  S.E.M.; \*significant difference ( $p < 0.05$ ) with respect to diabetic control



**Fig. 5. Effect of *Vasant Kusumakar Ras* and Glibenclamide on TC level in dexamethasone-induced diabetic rats. Data are expressed as Mean  $\pm$  S.E.M.; \*\*\*significant difference ( $p < 0.001$ ) with respect to diabetic control**



**Fig. 6. Effect of *Vasant Kusumakar Ras* and Glibenclamide on LDL level in dexamethasone-induced diabetic rats. Data are expressed as Mean  $\pm$  S.E.M.; \*\*\*significant difference ( $p < 0.001$ ) with respect to diabetic control**

### 3.6 Effect of *Vasant Kusumakar Ras* on LDL Level in Dexamethasone-induced Diabetic Rats

On the 11<sup>th</sup> day, there was a significant increase in serum LDL level of diabetic control group rats, as compared to normal control group rats. Treatment with *Vasant Kusumakar Ras* and Glibenclamide for 10 days, significantly ( $p < 0.001$ ) decreased LDL level when compared to the diabetic control group (Fig. 6). As far as the relative efficacy is concerned, though all the two doses of formulations produced comparable serum LDL lowering activity to Glibenclamide (5 mg/kg), *Vasant Kusumakar Ras* 600 mg/kg has been proven the higher serum LDL lowering activity than *Vasant Kusumakar Ras* 400 mg/kg.

## 4. DISCUSSION

Diabetes mellitus is a metabolic condition characterized by a lack of endogenous insulin secretion or activity, hyperglycemia, and an

unknown cause [14]. Diabetes mellitus is a multifactorial disease characterized by flaws in reactive oxygen species (ROS) scavenging enzymes [15], hyperglycemia [16], lipoprotein abnormalities [17], a high basal metabolic rate [18], and a high level of oxidative stress-induced damage [19]. In rodents, diabetes is caused by injecting alloxan, which induces diabetes while also producing ROS, resulting in pancreatic  $\beta$ -cell destruction [20]. Chronic hyperglycemia is the leading cause of a variety of long-term diabetic problems.

The polyherbal formulation was formulated and evaluated for its antidiabetic effect in alloxan-induced and dexamethasone-induced diabetic rats. Alloxan is a beta-cytotoxin that has been demonstrated to cause "chemical diabetes" (alloxan diabetes) in a variety of animal species by causing damage to the pancreas' insulin-secreting beta-cells [21]. This damages a high number of beta-cells, resulting in a reduction in endogenous insulin production. As a result, rats given alloxan become hyperglycemic in a short



time, followed by hepatic glucose overproduction [22]. After a single dosage of 120 mg/kg body weight alloxan injection, we noticed a significant ( $P < 0.05$ ) increase in the rats' blood glucose levels. The rise in glucose levels was due to alloxan-induced reactive oxygen species, as well as a large rise in cytosolic calcium concentrations, which resulted in the rapid death of pancreatic islet cells and a reduction in insulin synthesis and release [23-24]. Our findings of an increase in blood glucose in alloxan-administered rats are consistent with those of Isitua et al., who found hyperglycemia in rats following alloxan administration [22]. Increased blood glucose levels were also reported in rats after alloxan administration by Agarwal et al. and Emordi et al. [25-26].

After diabetes induction, the diabetic control group's blood glucose levels increased significantly until the experiment's last day. Treatment of diabetic rats with various doses of *Vasant Kusumakar Ras*, on the other hand, led in significant ( $P < 0.05$ ) decreases in blood glucose levels in each group (Fig. 1). The ability of *Vasant Kusumakar Ras* to arrest and reverse oxidative stress-induced destruction of pancreatic beta-islet cells, resulting in beta-islet cell regeneration, insulin secretion, and enhanced transport of blood glucose to peripheral tissues, could explain the reduction in blood glucose levels in diabetic rats treated with the polyherbal formulation. Our finding agrees with other reports on the antidiabetic activities of *Vasant Kusumakar Ras* on streptozotocin-induced diabetic rats [10].

Our study has shown that TC and LDL levels increased significantly, in the diabetic control group compared to that of the normal control group (Fig. 2-3). Treatment of diabetic groups with different doses of *Vasant Kusumakar Ras* resulted in significant ( $P < 0.001$ ) reductions in the serum TC and LDL. Insulin deficiency produces elevated blood glucose levels (hyperglycemia) and hypercholesterolemia in diabetics, as observed in our diabetic control group. This could be owing to the ongoing effects of lipolytic hormones on adipose tissues and hormone-sensitive lipases on fats, resulting in an increase in free fatty acid mobilization. Extra fatty acids are transferred to the liver and transformed to phospholipids and cholesterol. Dyslipidemia observed in the diabetic control group was restored to near-normal levels in the *Vasant Kusumakar Ras* treated groups. Other studies on dyslipidemia in diabetes mellitus [27-

29] corroborate our findings. The antilipidemic effect of *Vasant Kusumakar Ras* on streptozotocin induced diabetic rats was also previously reported [10].

Steroids are medications that have been widely utilized to treat a range of ailments. Glucocorticoids, despite being frequently recommended for their anti-inflammatory and immunosuppressive characteristics, have several adverse effects, with hyperglycemia being one of the most common and prominent. Increased hepatic glucose synthesis and insulin resistance in peripheral tissues contribute to glucocorticoid-induced hyperglycemia [30]. Insulin secretion has also been demonstrated to be suppressed by glucocorticoids. However, treatment of the diabetic rats with different doses of *Vasant Kusumakar Ras* resulted in significant ( $P < 0.05$ ) reductions in the blood glucose levels in each (Fig. 4). Other investigations have found that dexamethasone-induced hyperglycemia causes an increase in TC and LDL levels (Fig. 5-6). *Vasant Kusumakar Ras*, a polyherbal preparation, has a therapeutic benefit by significantly lowering the TC and LDL levels. Glibenclamide was chosen as the positive control to evaluate the effectiveness of the model in our study because it is thought to decrease insulin resistance in the presence of insulin [13].

## 5. CONCLUSION

The present findings showed that *Vasant Kusumakar Ras*, a polyherbal preparation has anti-diabetic potential. *Vasant Kusumakar Ras* showed a significant antidiabetic effect in albino rats at 400 and 600 mg/kg, and this effect was comparable to that of glibenclamide. The biochemical analysis supports the anti-diabetic effectiveness of the polyherbal formulation. *Vasant Kusumakar Ras* treatment improved the serum lipid profile which was altered in diabetic rats.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country (India). There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.



## CONSENT

It is not applicable.

## ETHICAL APPROVAL

The study was approved by the Institutional Animal Ethics Committee (CPCSEA1087/07) of the Hygia Institute of Pharmaceutical Education and Research, Lucknow (Hygia/M. Pharm/24/2012-13), and all the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Ministry of Environment and Forests, Government of India.

## ACKNOWLEDGMENTS

This Publication was supported by the Deanship of Scientific Research at Prince Sattam Bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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