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Antidiabetic Effect of Siddha Polyherbal Decoction, Nilavembu Kudineer, in Rats with Fructose-Induced Insulin Resistance

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ABSTRACT: Diabetes is a metabolic disorder characterized by chronic hyperglycemia and alternations in carbohydrate, protein and lipid metabolism with absolute or relative deficiencies in insulin secretion or insulin production. Nilavembu Kudineer (NVK) is a Siddha polyherbal concoction containing nine ingredients. There are very few studies to explore the antidiabetic potential of the NVK formulation and lack of scientific documentation to validate the efficacy of this formulation. Our study aimed to investigate the anti-diabetic activity of NVK in rats with fructose-induced insulin resistance. The total duration of the study was 90 days, and during the induction period (*i.e.*, 60 days/8 weeks) 20% fructose was given to all animals through drinking water and weekly blood glucose and body weight were measured. After 8 weeks animals with blood glucose level more than 135mg/dl were taken for the study and the animals were randomly divided into four groups. The study continued for a period of 4 weeks. Body weight, blood glucose was measured weekly and after experimental period all the animals were sacrificed for biochemical, hematological and histopathological studies. Standard and NVK treated group were found to be reducing the blood glucose, creatinine, WBC and there was increase in body weight, HDL levels, RBC, Hemoglobin, Platelet count, red cell distribution width when compared to negative control group. In silico analysis also reveal sotetsuflavone and hesperidin from NVK possess antidiabetic activity.

Keywords: Anti-diabetic, Nilavembu Kudineer, Fructose, Docking, insulin.

INTRODUCTION

The term "Diabetes" is a metabolic disorder characterized by chronic hyperglycemia and alternations in carbohydrate, protein and lipid metabolism with absolute or relative deficiencies in insulin secretion or insulin production. Etiology is heterogenous and includes heredity, diet, obesity, lifestyle and stress (Kumar *et al*., 2021). Symptoms of high blood sugar include frequent urination, polydipsia, hyperphagia. If the diabetes is left untreated it can cause various complications such as diabetic ketoacidosis, heart disease, stroke, chronic kidney failure, damage to eyes (Bai *et al*., 2021).

Epidemiology of Diabetes Mellitus. The epidemiology to the study of diabetes mellitus has provided valuable information on several aspects of the disease such as its natural history, prevalence, incidence, morbidity and mortality in diverse populations around the world (Roy *et al*., 2022). According to World Health Organization (WHO) an estimated 422 million people were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5%. Over the past decade, diabetes prevalence has risen faster in low- and middle-income countries and also caused 1.5 million deaths in 2012. The High prevalence of Type 2 Diabetes continues to rise globally and there are no signs of stabilizing it (Omara *et al*., 2010).

In the past years, Diabetes has achieved the status of potential epidemic in India with over 62 million diabetic individuals diagnosed with the disease currently (Zheng *et al*., 2018). India has 41 million diabetics and is expected to increase by 2025. The increased number of diabetics in India is likely to be due to significant increase in the incidence of type-II diabetes, caused by unprecedented rates of urbanization, which results in environmental and life style changes (Patel *et al*., 2012).

Pathophysiology of Diabetes Mellitus. Pathophysiology of the diabetes is between insulin action and insulin secretion which results in abnormal high glucose level in the blood. In β-cell dysfunction, insulin secretion is decreased limiting the body's capacity to maintain physiological glucose level. IR contributes to increased production in the liver and reduced glucose uptake both in the muscles, liver and adipose tissue. When both β-cell dysfunction and IR is present, hyperglycemia arises leading to progression of Type II Diabetes Mellitus (Singh *et al*., 2013). Fructose consumption model has been dramatically increasing in the developed and industrialized nations. Fructose is rapidly absorbed from the intestine and transported to

liver through portal vein. Long term consumption of fructose causes deleterious effects on body weight, glucose, lipid profile which impair insulin sensitivity and leads to IR (Shafi *et al*., 2012).

Siddha Formulation in Diabetes. Traditionally, Siddha System of Medicine are one the more primitive medical system and is increasing now a days, Nilavembu Kudineer (NVK) popularly called the Neem of grounds, Bile Earth and King of bitters is native to India and Sri lanka. It is a polyherbal concoction with *Andrographis paniculata* as the chief ingredient that controls all types of fever associated with body ache. Other components include Vettiver (*Vetiveria zizanioides*), Vilamiccam ver (*Coleus vettiveroides*), Cantanam (*Santalum album*), Peyputtal (*Trichosanthes cucumerina*), Koraik kilanku (*Cyperus rotandus*), Cukku (*Zingeber officinale*), Milaku (*Piper nigrum*) and Parpatakam (*Mollugo cerviana*) (Mekala and Murthy 2020).

The ingredients of Nilavembu Kudineer have been traditionally used for Madhumeha which can be correlated with Diabetes Mellitus. Siddha concept of management of diabetes is still recognized specifically due to its potential, ready availability and lack of toxicity and side effects. However, the formulation of NVK has not been studied for antidiabetic activity though it has been prescribed off-label by Siddha medical practitioners for diabetes.

Thus, aim of the study was to investigate the Anti-diabetic activity of Nilavembu Kudineer in rats with fructose-induced insulin resistance.

MATERIALS AND METHODS

Materials: NVK, a siddha formulation was obtained from Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL), Chennai. Fructose, Metformin was purchased and used with their analytical grade.

METHODOLOGY

Preparation of decoction: 10g of Nilavembu Kudineer was boiled in 100ml of water until it gets reduced to half (*i.e.,* 50ml). The residue was filtered and cooled. The filtrate was prepared daily before administration to the test animals at two different dose levels (200mg/kg, 400mg/kg) (Srivatsava *et al*., 2021).

PHARMACOLOGICAL STUDIES

Experimental Animals

Animal approval: Adult male rats of Sprague Dawley strain were used for the study and all animals were procured from Mass Bio Tech Pvt Ltd, Tamil Nadu. The study was approved by Institutional Animal Ethical Committee (IAEC) in K.K College of Pharmacy with the approval no: 8552/KKCP/ 2020 and the work were carried out as per CPCSEA guidelines, New Delhi.

Preparation of animal: In the study adult male Sprague Dawley rats weighing 180-250g were used. Animals were acclimatized to the study environment for a week prior to the drug treatment. Temperature of the study room will be maintained between 22±3ºC and relative humidity between 50-70% during the experimental period. The experimental room was provided with 12h light and 12h dark lighting cycle. Animals were housed in Standard polypropylene rat cages with stainless steel grill and provided with standard pellets ad libitum and free access to drinking water. Paddy husk was used as the bedding material and was changed every day and washed thoroughly with water along with disinfectant.

Induction of Diabetes. The total duration of study period was 90 days. Animals were fasted for 12 hours and on the following day, Base line glucose and body weight were measured and 20% fructose(w/v) was given through drinking water for 60days (*i.e.,* 8 weeks), which is the induction period. Weekly blood glucose and body weight was measured. After 8 weeks animals with the blood glucose level more than 135mg/dl were taken for the study. The animals were randomly allocated into 4 groups (n=6) and housed as Control group, Negative control, Standard group, Test group (Low dose and High Dose). Negative Control (Group I) diabetic rats that fulfilled the above-mentioned criteria. Standard group (Group II) Diabetic treated with metformin (250mg/kg, p. o), Test group (Group III, IV) Diabetic treated with Nilavembu Kudineer at two different dose levels (200mg/kg, p. o and 400mg/kg, p. o) (Table 1). The study was continued for a period of 4 weeks which is the experimental period. Blood glucose level & Body weight was measured weekly. After the experimental period, all the animals were sacrificed for biochemical, hematological and histopathological studies (Furman, 2021).

In the study adult male Sprague Dawley rats weighing 180-250g will be used and after the induction period animal were grouped as follows:

Table 1: Experimental design.

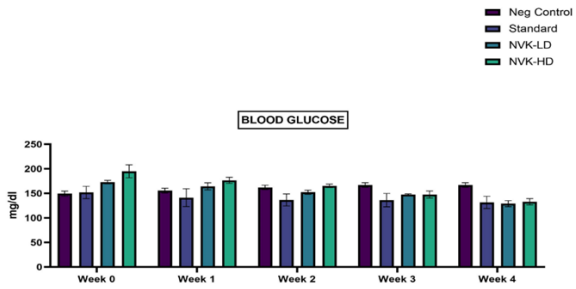
Groups	Description	Treatment	No. of Animals
I	Negative Control	20% fructose	6
II	Standard	20%fructose+metformin (250mg/kg, p. o)	6
III	NK-Low dose	20%fructose + NVK (200mg/kg, p. o)	6
IV	NK-High dose	20% fructose + NVK (400mg/kg, p.o)	6

Statistical Analysis: Values obtained from blood glucose, hematological, biochemical, organ weight were expressed as mean ± SEM. The data obtained was statistically analyzed using one-way analysis of variance (ANOVA) with multiple comparisons. Tukey's test was used to compare the level of significance using Graph pad prism version 9.3.1.

RESULTS

In Vivo Analysis

Effect on Blood Glucose Level

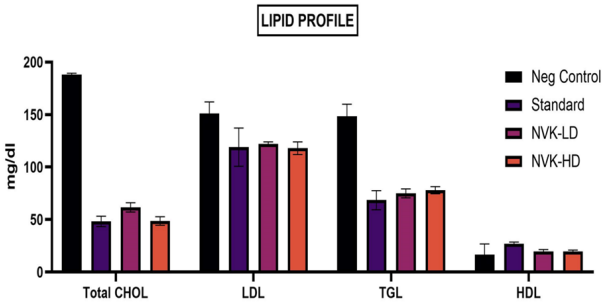


Each bar represents blood glucose level at Week 0,1,2,3 and 4 respectively.

Graphical representation of the results of the effect on the blood glucose level in fructose induced diabetic rats

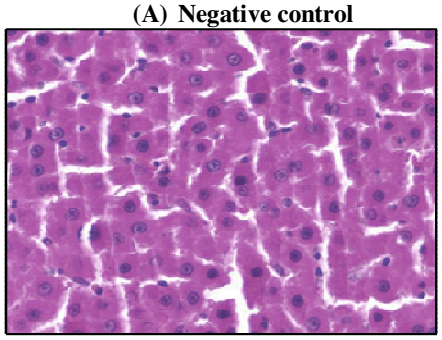
BIOCHEMICAL CHANGES

Graphical representation of the results of the effect on lipid profile in fructose induced diabetic rats

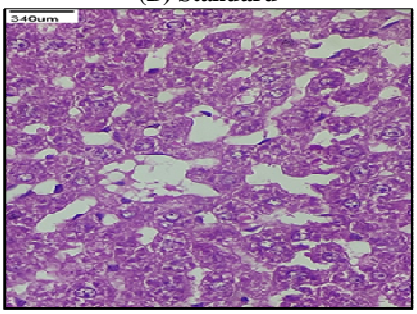


HISTOPATHOLOGICAL STUDIES LIVER

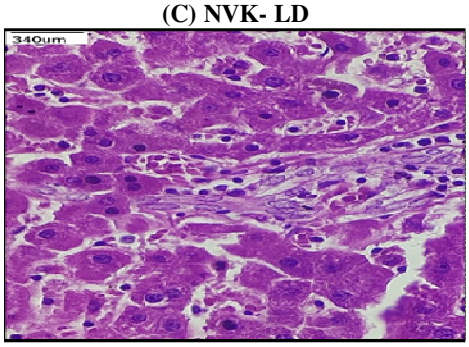
(A) Negative control



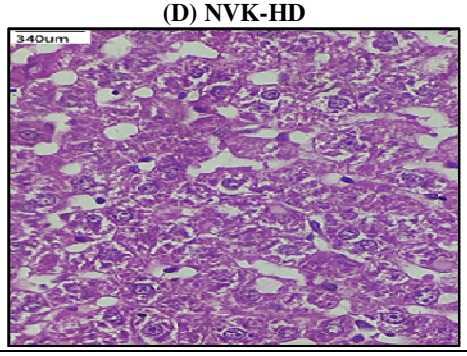
(B) Standard



(C) NVK- LD

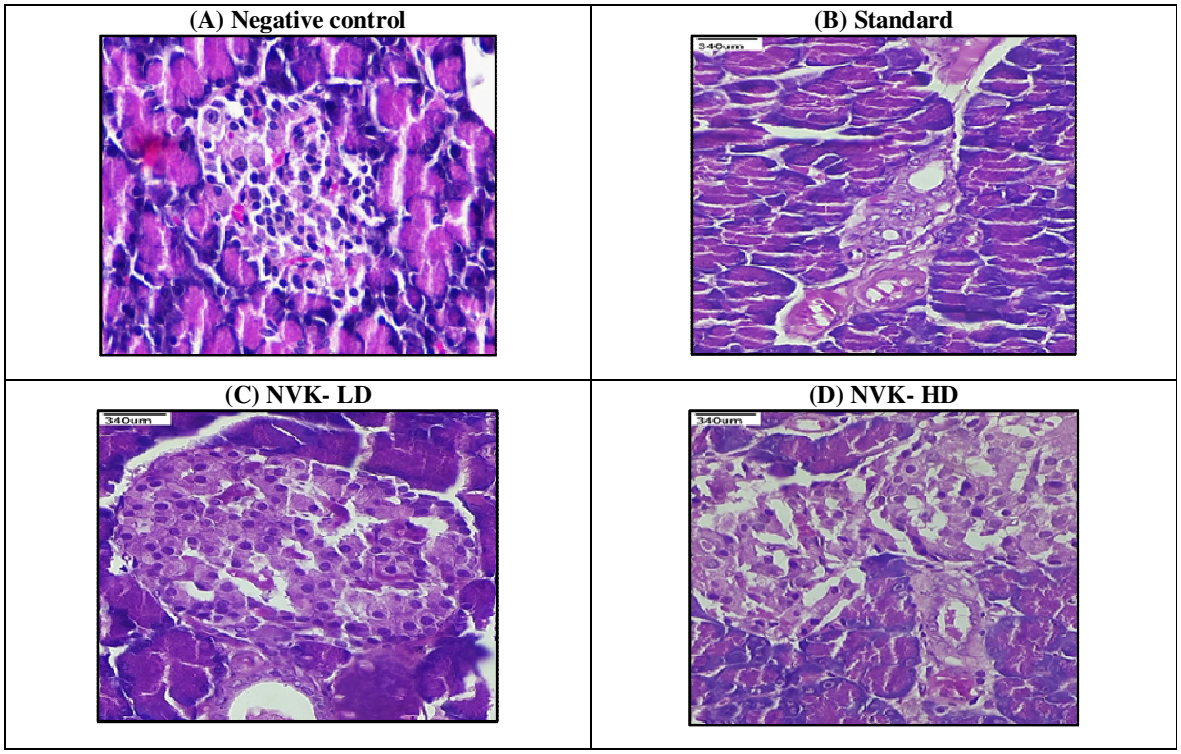


(D) NVK-HD



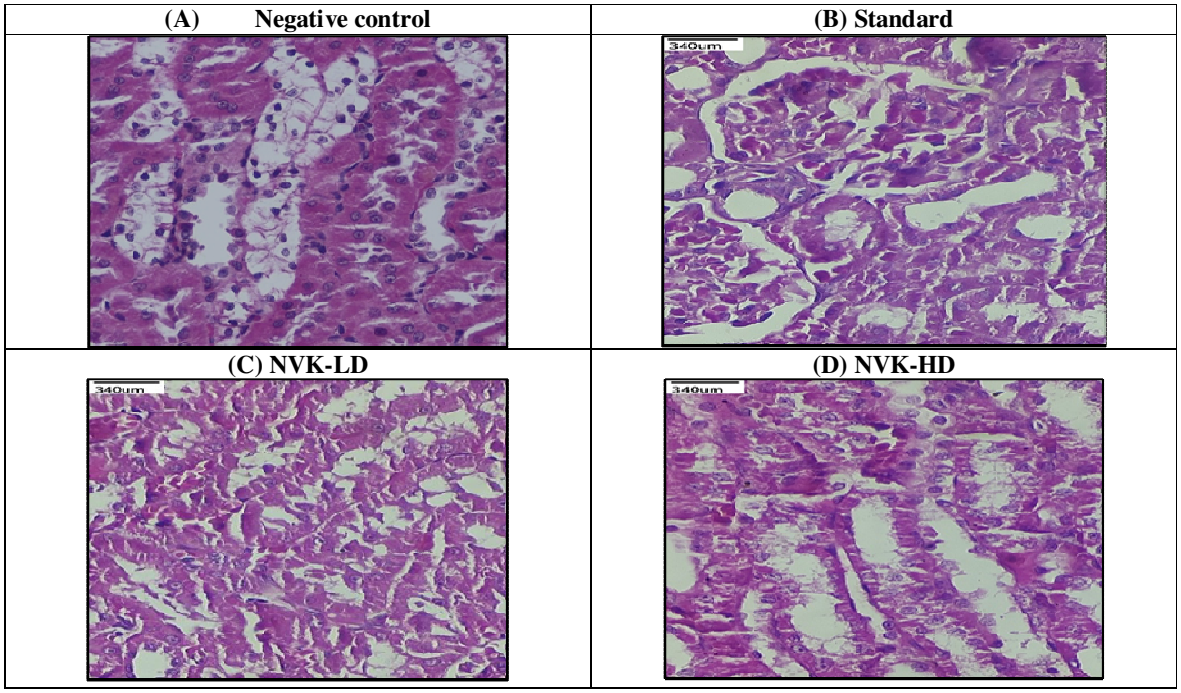
Mild infiltrates, mononuclear, minimum microgranuloma, minimum necrosis was observed in the liver of negative control rats (A). Few central veins were dilated, portal tracts showed dilated vessels and very minimal lymphocytic infiltrates, few hepatocytes showed feathery degeneration, hepatic steatosis and fibrosis was nil showed by standard group (B). Portal tracts showed moderate lymphoplasmacytic infiltration and dilated and congested portal vessels, sinusoids were congested and showed Kupffer cell hyperplasia, Focal areas showed spotty necrosis of hepatocytes (Karyolysis), Few central veins were dilated and congested, which were observed in low dose group (C). Portal tracts and cords of hepatocytes showed normal morphology, few portal tracts showed very minimal lymphoplasmacytic infiltration, Occasional sinusoids show Kupffer cell hyperplasia, no fatty change or necrosis noted in High Dose group (D).

PANCREAS



Degeneration, Islet cells, diffuse, marked was observed in negative control rats (A). Islets of Langerhans appear adequate in number and showed normal morphology and seen dispersed among the pancreatic acini, no inflammation or fibrosis noted, Pancreatic ducts appeared normal which were showed by standard group (B). Few dilated ducts filled with eosinophilic secretions seen, few congested and dilated blood vessels and no inflammation were observed in low dose group (C). Pancreatic acini showed normal morphology lined by acinar cells with basal nuclei and acidophilic cytoplasm, Atrophic, Degenerative and necrotic changes of islets was nil which was observed in High dose group (D).

KIDNEY



Mild degeneration, tubular, mononuclear, interstitial was observed in negative control rats (A). Glomeruli showing normal architecture with few congested capillaries, tubules showing normal morphology with occasional dilated tubules, focal congested peritubular capillaries, no inflammation infiltrates or necrosis were observed in standard group (B). Congested peritubular capillaries and no necrosis were observed in low dose group (C). Tubules showing normal morphology, few tubules are dilated filled with eosinophilic fluid material, occasional congested period capillaries and no inflammation were observed in high dose group (D).

DISCUSSION

Diabetes Mellitus is a metabolic disorder in which glucose metabolism is impaired due to the loss of insulin after the destruction of pancreatic β-cells or insensitivity to target insulin. The basic mechanism involved excessive hepatic gluconeogenesis and glycogenolysis and decrease utilization of glucose by the tissue. Allopathy medications have various side effects such as dry mouth, flatulence, myalgia, Heartburn and dyspepsia.

To overcome the adverse effect exhibited by the allopathic drugs there is need for traditional system of medicine. Traditional system of medicines is playing a key role in global health care needs. Among these Siddha and Ayurveda are most popular. In the modern era, Siddha formulation have gained greater importance mainly due to their efficacy and easy availability as well as less side effects. Nilavembu Kudineer is one of the known polyherbal siddha formulation containing nine ingredients, which is prescribed twice a day to get rid of dengue and corona virus infection. Many clinical investigations have shown that ethanol extract of NVK has shown anti-inflammatory, analgesic, immunomodulatory activities.

Our study was supported by research study conducted with the aqueous extract of Nilavembu Kudineer against Streptozotocin model and found that NVK can be used in the management of type II Diabetes mellitus and its related complications. Streptozotocin has been used to induce diabetes in experimental animals and research has proved the selective cytotoxic effect of STZ on β-cells (Hassan *et al*., 2021). To overcome the disadvantage of streptozotocin, an alternative model fructose induced model was selected for our study. Fructose consumption model is intensely increasing globally, due to its rapid absorption from the intestine and metabolization in the liver where it gets converted to pyruvate, finally entering into TCA cycle and FA synthesis there by leading to type II diabetes (Bagheri *et al*., 2021).

In our study male rats were employed because female rats fail to develop insulin resistance with fructose due to protective effects of the sex hormones in female rat. The feed and water consumption of the animals was recorded at regular intervals. Feed and water consumption of the rats of individual group were compared with other groups. In the present study, a gradual increase in body weight was observed in all group.

Hyperglycemia and insulin resistance together play an important role in the pathogenesis of diabetes mellitus. A steady increase in blood glucose levels was observed in negative control group. Nilavembu Kudineer treated groups i.e., Low dose and high dose, showed a gradual decrease in blood glucose levels, a similar situation was seen in in standard group (metformin) and it confirms the presence of anti-diabetic activity. This finding was same from another study where there was increase in serum glucose level in experimental animals by drinking 10% fructose solution (Salau *et al*., 2021).

Biochemical parameters such as Liver Function test, Renal Function Test and Lipid profile have been performed and the values for each group was recorded. In Liver Function test diabetic group showed significant elevation in Bilirubin (direct), SGOT, SGPT compared to control group. Standard and NVK treated showed increase in SGOT, SGPT when compared to negative control. This finding was same from another study (Wardhana *et al*., 2021). Bilirubin (Total), Total protein, Albumin, Globulin, A-G ratio, Gamma Glutamyle Transferase (GGT) were found have no significant variation. In Renal Function test diabetic group showed significant increase in creatinine when compared to control group. Standard and treatment group showed significant decrease in creatinine level when compared to diabetic group. There was no significant variation observed in Blood Urea Nitrogen (BUN). Hypertriglyceridemia and hypercholesterolemia are the significant abnormalities observed in diabetes. Cholesterol is one of the important blocks in the biological membranes and used in the synthesis of steroid hormones. Increase in cholesterol concentration can lead to atherosclerosis and cardiovascular complications. LDL takes the cholesterol from liver to tissues, whereas HDL facilitates the translocation of cholesterol from peripheral tissues to liver. LDL and HDL has a useful effect in increase and decrease of serum cholesterol is suggested. The CH0/HDL ratio is important predictor of coronary artery disease. In our study, there was an increase in Total Cholesterol, LDL and decrease in HDL levels when observed in diabetic group compared to control group. NVK treated and standard groups showed increase in HDL levels when compared to diabetic group. This finding was same from another study reported in the literature were there was an increase in HDL levels in standard and treatment groups. Triglycerides, VLDL, CHO/HDL ratio were found to have no significant variation (Azhagarasi *et al*., 2022).

Histopathological studies of heart, liver, pancreas and kidney also supported our findings. Histopathological examination of heart, liver, pancreas and kidney showed normal architecture in control groups. There was degeneration, mononuclear, interstitial observed in negative control groups when compared to control group. Standard group (metformin), Low dose and High dose group (NVK) showed recovery of damaged tissue, with no inflammation or necrosis were seen when compared to negative control group.

In our study, hematological parameters showed that diabetic group exhibited significant increase in WBC,

Eosinophils when compared to control group. This finding was different from another study where reduction in WBC, eosinophils was observed (Padugupati *et al*., 2022). Metformin and NVK treated group showed decrease in WBC and significant increase in lymphocytes, Eosinophils, RBC, HB, PCV, PC, RCDW when compared to diabetic group. These parameters serve as the predictors of endothelial dysfunction and inflammation in type II diabetes. Platelets play a vital role in the integrity of normal homeostasis; Activation of platelets play a key role in inflammation and atherothrombosis process which contributes to the development of coronary vascular disease in the patients with type II DM. There was a significant increase in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) when compared to diabetic group. No significant variation was observed in Neutrophils, Monocytes and Mean Platelet Volume (MPV). Mean platelet volume reflects the changes either in platelet stimulation or rate of platelet production has been observed in diabetes and its related complications. Hence, hematological parameters of Nilavembu Kudineer proved to have anti-diabetic activity against fructose induced insulin resistance (Li *et al.*, 2022).

CONCLUSIONS

The present study is an attempt to investigate the effect of NVK on fructose induced diabetes in rats. The serum glucose, lipid profile, SGOT and SGPT shown to be increased in NVK treated diabetic animals. In the present study the bodyweight, HDL shown to be increased in NVK treated diabetic animals when compared to negation control. The findings of the present investigation suggest that NVK has the potential for its evaluation as an anti-diabetic agent against fructose induced diabetes. Assessment of NVK for its underlying mechanisms will be an interesting topic and requires further study.

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