

## ANTIDIABETIC ACTIVITY AND ANTI-OXIDANT ACTIVITY OF NIDDWIN, A POLYHERBAL FORMULATION IN ALLOXAN INDUCED DIABETIC RATS

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### ABSTRACT

**Objective:** The present study was focused to evaluate the antidiabetic activity and antioxidant activity of NIDDWIN, a polyherbal formulation in alloxan induced diabetic rats.

**Methods:** Alloxan induced diabetic rats were divided into four groups of five each. Group-I was given aqueous suspension of 2% gum acacia, Group-II and III was given aqueous suspension of NIDDWIN 50mg/kg and 100mg/kg, Group-IV was given aqueous suspension of Glibenclamide 10mg/kg were given orally for 10days. The blood samples were collected before and after administration of drugs at 0, 2, 4, 6, and 8hrs on 1<sup>st</sup>, 5<sup>th</sup>, and 10<sup>th</sup> day from retro-orbital sinus, serum was separated and estimated for glucose, cholesterol and triglycerides levels. On 10<sup>th</sup> day pancreas were isolated from all the groups and subjected for histopathological studies. Diabetic rats were evaluated for anti-oxidant activity by using NIDDWIN.

**Results:** NIDDWIN showed significant antidiabetic activity at 4<sup>th</sup> hr on 1<sup>st</sup>, 5<sup>th</sup> and 10<sup>th</sup> day was found to be effect in comparable with standard Glibenclamide 10mg/kg. Histopathological results of NIDDWIN showed positive results when compared with standard Glibenclamide 10mg/kg. NIDDWIN significantly showed the percentage reduction of lipid per-oxidation levels in diabetic rats.

**Conclusion:** NIDDWIN a polyherbal formulation concluded that it possesses antidiabetic activity and anti-oxidant activity in diabetic rats and further NIDDWIN should be evaluated to develop probable mechanism of action.

**Keywords:** NIDDWIN, Glibenclamide, Alloxan monohydrate, Glucose Kit, Cholesterol Kit, Triglycerides Kit, Alpha-tocopherol, Thiobarbituric acid, UV-spectrophotometer.

### INTRODUCTION

Diabetes is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism which causes hyperglycemia resulting from insufficient insulin secretion, insulin action or both [1, 2]. It is one of the refractory diseases identified by Indian council of medical research for which an alternative medicine is a need for the treatment. Diabetes mellitus has become a growing problem in the contemporary world [3]. Today India has become the diabetic capital of the world with over 20million diabetic patients and this number is likely to increase to 57million by 2025[4]. This astronomic increase in the prevalence of diabetes has made diabetes a major public health challenge for India and is become important human ailment afflicting many from various walks of life in different countries and once again the whole world being looked upon ayurvedic the oldest healing system of medicine for the treatment of diabetes [2]. Although there are many synthetic medicines developed for patients, but it is the fact that it has never been reported that someone had recovered that totally from diabetes [5]. The modern oral hypoglycemic agents showed undesirable side effects thus in the recent years considerable attention has been directed towards the antidiabetic potential of medicinal plants and their herbal formulation in the management of disease. The concept of polyherbalism is peculiar to ayurveda although it is difficult to explain in term of modern parameters. It is evident that there are many herbal formulations of varying potency since these preparation act by different mechanism, it is theoretically possible that different combination of these extract will do better job in reducing blood glucose. In the traditional system of plant medicine it is usual to use plant formulation and combined extract of plant are used as a drug of choice rather than individual ones [6] to get the benefit of synergism. Some of the polyherbal formulations which are in the market are: Diabet, Diasol, Diasulin, Dia-care, ESF/AY/500, EFPT/09, Karmin plus, Okudibet, 5EPHF.

NIDDWIN a polyherbal formulation which include 11 antidiabetic herbs and 1 mineral the 12 constituents of NIDDWIN were individually proved to have antidiabetic activity but the combination of these 12 constituents called NIDDWIN for its antidiabetic activity was not yet reported. Each 500mg of NIDDWIN consists of following formulation: *Tinospora*

*cordifolia* – 50mg, *Gymnema sylvestre* – 50mg, *Terminalia tomentosa* – 50mg, *Asphaltum* – 50mg, *Tribulus terrestris* – 50mg, *Emblica officinalis* – 58mg, *Mucuna pruriens* – 50mg, *Sida cordifolia* – 50mg, *Withania somnifera* – 25mg, *Terminalia belerica* – 8mg, *Terminalia chebula* – 8mg, *Momordica charantia* – 10mg.

Therefore, the present study was focused to evaluate the antidiabetic activity and antioxidant activity of NIDDWIN in alloxan induced diabetic rats.

### MATERIALS AND METHODS

#### Chemicals and standard drugs

Glibenclamide, Alloxan monohydrate, Gum acacia, Alpha-tocopherol (cipla), Thiobarbituric acid, sodium do decyl sulphate, potassium chloride, n-butanol, pyridine, glucose kit, cholesterol kit, triglycerides kit chemicals are purchased from SD Fine chemicals Ltd., India. All the chemicals used were of analytical grade.

#### Plant Material

NIDDWIN a polyherbal formulation containing 11antidiabetic herbs and 1mineral was manufactured by IMIS pharmaceuticals Pvt ltd., Vijayawada is evaluated for antidiabetic activity.

#### Animals

Male albino wistar rats weighing 180-200gms were obtained from authorized animal house (Albino research center, Hyderabad). Animals were housed at room temperature 25°C with a 12hrs light and 12hrs dark cycle.

The animals had free access to standard rat pellet diet and tap water.

After one week of acclimatization, the animals were considered for suitable study and the experiments were conducted according to CPCSEA guidelines no GNIP(TKR)/CPCSEA/IAEC/2013/11.

#### Acute toxicity study

The animals were divided into four groups each containing 5animals NIDDWIN a polyherbal formulation was given orally in logarithmic doses 30, 100, 300 and 1000mg/kg. The rats were observed

continuously for 2hrs for behavioral, neurological and autonomic profiles and after 24hours and 72hours for any lethality [7, 8, and 9].

**Experimental design**

**Experimental induction of diabetes mellitus**

The rats were injected alloxan monohydrate dissolved in sterile normal saline at a dose of 150mg/kg body weight, intraperitoneally. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 10% glucose solution (8-10ml) intraperitoneally after 6hrs. The rats were then kept for the next 24hrs on 5% glucose solution bottles in their cages to prevent hypoglycemia [10,11].

**Treatment**

Diabetic rats were randomly divided into four groups with 5rats in each group and all the drugs were given orally for 10days as follows.

- Group I was given aqueous suspension of 2% gum acacia as control
- Group II was given aqueous suspension of NIDDWIN 50mg/kg
- Group III was given aqueous suspension of NIDDWIN 100mg/kg
- Group IV was given Glibenclamide 10mg/kg

The blood samples were collected before and after administration of drugs at 2, 4, 6 and 8hrs on 1<sup>st</sup>, 5<sup>th</sup> and 10<sup>th</sup> day of from retro orbital sinus puncture. The serum was separated from the blood samples by centrifugation and was analyzed for glucose, cholesterol and triglycerides levels by analytical method [12]. The concentration of glucose, cholesterol, and triglycerides levels in the blood at each time interval was calculated and expressed as mg/dL. Percentage reduction of glucose, cholesterol, and triglycerides levels in blood [13, 14 and 15] from each group at different time [16] intervals was calculated and given in tables 1 – 3. On 10<sup>th</sup> day after blood samples collection the rats were sacrificed by cervical dislocation and the pancreas were isolated and taken into container containing formalin and subjected to histopathological studies were conducted in National Institute of Nutrition (NIN, Tarnaka, and Hyderabad).

**Anti-oxidant activity**

Diabetic rats were randomly divided into four groups with 5rats in each group and all the drugs were given orally as follows.

- Group I served as control received aqueous suspension of 2% gum acacia.
- Group II received aqueous suspension of NIDDWIN at a dose of 50mg/kg.
- Group III received aqueous suspension of NIDDWIN at a dose of 100mg/kg
- Group IV received aqueous suspension of 100mg/kg Alpha-tocopherol.

After 2hrs of treatment the rats were sacrificed by cervical dislocation and liver was isolated and washed with saline. About 300mg of liver was taken in a 3ml of 1.15%KCl and subjected to homogenization and centrifuged for 10minutes at 3000 rpm.

To the 0.2ml of tissue homogenate 0.2ml of 8.1% sodium laryl sulphate and 1.5ml of 20% acetic acid of pH 3.5 and 1.5ml of 0.8% thiobarbituric acid was added and incubated in a boiling water bath for 30minutes. Then the contents were cooled and 5ml of n-butanol-pyridine (15:1) mixture was added. Following vigorous shaking the tubes were centrifuged and absorbance of the upper organic layer was measured at 532nm.

The extent of absorbance depends upon extent of lipid per-oxidation in the liver tissue. The inhibition of lipid per-oxidation by NIDDWIN was determined by comparing the absorbance values of control and treated groups [17]. The results were tabulated in table - 4.

**Statistical Analysis**

Results were analyzed by applying unpaired student's t-test by using one way ANOVA using InStat3 software, followed by Dunnet's test. The percentage reduction values were expressed in Mean±SEM.

**RESULTS**

**Acute toxicity studies**

Acute toxicity studies indicated that there is mild toxicity with 1000mg/kg after 24hrs of treatment.

**Percentage reduction of glucose, cholesterol and triglycerides levels in blood with NIDDWIN 50mg/kg in diabetic rats.**

The percentage reduction of glucose, cholesterol and triglycerides levels in blood with 50mg/kg of NIDDWIN was found to be in (Day-1) 27.798, 21.388 and 20.46 (Day-5) 31.87, 27.42 and 21.98 (Day-10) 38.06, 30.402 and 24.818 after 4hrs of administration.

**Percentage reduction of glucose, cholesterol and triglycerides levels in blood with NIDDWIN 100mg/kg in diabetic rats.**

The percentage reduction of glucose, cholesterol and triglycerides levels in blood with 100mg/kg of NIDDWIN was found to be in (Day-1) 37.658, 27.358 and 23.274 (Day-5) 45.11, 34.95 and 27.06 (Day-10) 50.50, 40.90 and 32.74 after 4hours of administration.

**Percentage reduction of glucose, cholesterol and triglycerides levels in blood with Glibenclamide 10mg/kg in diabetic rats**

The percentage reduction of glucose, cholesterol and triglycerides levels in blood with 10mg/kg of Glibenclamide was found to be in (Day-1) 39.18, 21.20 and 18.65 (Day-5) 49.11, 23.81 and 20.87 (Day-10) 57.91, 29.35 and 24.06 after 4hrs of administration.

**Table 1: Consolidated table showing Mean ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with NIDDWIN 50mg/kg of diabetic rats after continuously for 10 days treatment.**

Group	NIDDWIN 50mg/kg (Day - 1)				NIDDWIN 50mg/kg (Day - 5)				NIDDWIN 50mg/kg (Day - 10)			
	2hrs	4hrs	6hrs	8hrs	2hrs	4hrs	6hrs	8hrs	2hrs	4hrs	6hrs	8hrs
% Reduction of Glucose	11.542 ± 0.560*	27.798 ± 1.304**	17.79 ± 0.506**	11.5 ± 0.683*	14.24 ± 1.573*	31.87 ± 2.495**	17.43 ± 2.240**	10.494 ± 0.849*	23.31 ± 0.692**	38.06 ± 1.591***	24.12 ± 1.729**	14.66 ± 1.718*
% Reduction of Cholesterol	7.334 ± 0.456*	21.388 ± 0.636**	15.51 ± 1.370*	9.24 ± 1.653*	16.69 ± 1.905*	27.42 ± 0.465**	19.94 ± 0.494*	11.854 ± 0.5749*	19.37 ± 0.833*	30.402 ± 0.5874**	22.41 ± 0.538**	13.16 ± 0.939*
%Reduction of Triglycerides	8.68 ± 0.897*	20.46 ± 0.654*	11.96 ± 0.745*	7.864 ± 1.224*	11.45 ± 0.572*	21.98 ± 0.760*	13.21 ± 0.687*	9.134 ± 0.982*	13.33 ± 0.441*	24.818 ± 0.881**	17.44 ± 0.629*	10.97 ± 0.555*

Each value is SEM of 5animals:\*\*\*P<0.0001, \*\*P<0.001, \*P<0.01. Comparison made between diabetic control rats and NIDDWIN 50mg/kg treated group rats

**Table 2: Consolidated table showing Mean ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with NIDDWIN 100mg/kg of diabetic rats after continuously for 10 days treatment.**

Group	NIDDWIN 100mg/kg (Day - 1)				NIDDWIN 100mg/kg (Day - 5)				NIDDWIN 100mg/kg (Day - 10)			
	2hrs	4hrs	6hrs	8hrs	2hrs	4hrs	6hrs	8hrs	2hrs	4hrs	6hrs	8hrs
% Reduction of Glucose	17.29 ± 1.659*	37.658 ± 1.075**	27.54 ± 1.477**	16.56 ± 0.440**	25.62 ± 0.822**	45.11 ± 2.155***	31.07 ± 2.902**	21.406 ± 1.755**	29.69 ± 0.699**	50.50 ± 1.906***	33.14 ± 1.849**	22.95 ± 1.042**
% Reduction of Cholesterol	13.81 ± 1.529*	27.358 ± 1.904**	19.02 ± 1.973**	16.50 ± 1.383*	18.45 ± 0.482*	34.95 ± 3.073**	25.92 ± 1.863**	17.50 ± 1.337*	21.79 ± 0.530*	40.904 ± 1.949**	28.02 ± 1.476**	18.32 ± 0.9316*
%Reduction of Triglycerides	10.64 ± 1.381*	23.274 ± 0.825**	17.37 ± 1.856**	10.03 ± 0.490*	15.52 ± 1.428*	27.06 ± 1.517**	18.42 ± 2.415**	12.704 ± 1.812*	18.11 ± 0.775*	32.742 ± 1.418**	21.15 ± 2.886*	14.41 ± 1.723*

Each value is SEM of 5animals:\*\*\*P<0.0001, \*\*P<0.001, \*P<0.01. Comparison made between diabetic control rats and NIDDWIN 100mg/kg treated group rats.

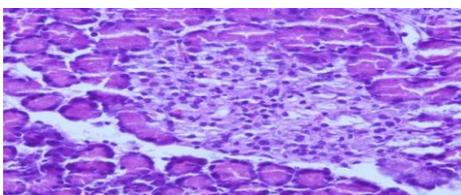
**Table - 3: Consolidated table showing Mean ±SEM values of percentage reduction of glucose, cholesterol and triglycerides levels in blood with Glibenclamide 10mg/kg of diabetic rats after continuously for 10 days treatment.**

Group	Glibenclamide 10mg/kg (Day - 1)				Glibenclamide 10mg/kg (Day - 5)				Glibenclamide 10mg/kg (Day - 10)			
	2hrs	4hrs	6hrs	8hrs	2hrs	4hrs	6hrs	8hrs	2hrs	4hrs	6hrs	8hrs
% Reduction of Glucose	19.09 ± 1.319*	39.18 ± 3.47**	23.78 ± 2.823**	18.39 ± 0.883**	23.08 ± 1.088**	49.11 ± 2.977***	34.33 ± 1.396**	22.156 ± 0.974**	34.79 ± 1.020**	57.91 ± 0.904***	41.98 ± 1.295**	27.00 ± 0.764**
% Reduction of Cholesterol	10.52 ± 0.347*	21.20 ± 0.739**	19.40 ± 2.317**	11.33 ± 1.215*	10.65 ± 0.526*	23.81 ± 0.875**	15.08 ± 0.626**	10.682 ± 0.518*	17.64 ± 0.611*	29.352 ± 1.117**	21.61 ± 0.973**	15.69 ± 0.947*
%Reduction of Triglycerides	9.482 ± 1.180*	18.65 ± 0.986**	11.75 ± 0.786**	10.21 ± 0.785*	10.356 ± 0.706*	20.87 ± 0.372**	13.21 ± 1.053**	9.656 ± 0.407*	14.99 ± 1.127*	24.068 ± 1.068**	16.17 ± 0.757**	10.74 ± 0.932*

Each value is SEM of 5animals:\*\*\*P<0.0001, \*\*P<0.001, \*P<0.01. Comparison made between diabetic control rats and Glibenclamide 10mg/kg treated group rats.

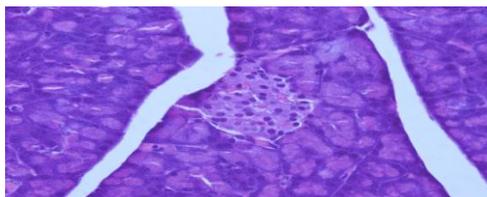
**Histopathological results**

1). Control Rat without diabetes: Shows normal pancreatic acini and ducts. Islets are also normal with round nuclei and fine abundant cytoplasm. (Fig 1)



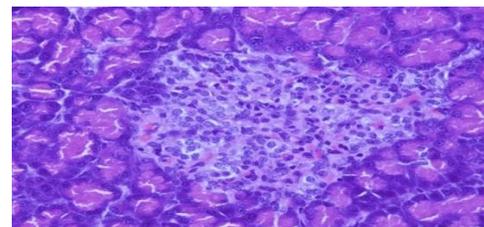
**Fig. 1: Microscopic features of control rat without diabetes**

2).Alloxan induced diabetic rat with control Rat-1 (2%gumacacia): Acini are normal. However the islets are decreased in number. There is increased vascular congestion seen with infiltration by lymphocytes. Many cells have pyknotic nuclei with vacuolated cytoplasm. Few cells have elongated nuclei. (Fig 2)



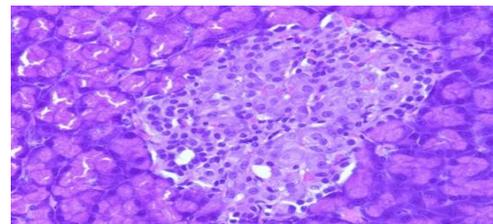
**Fig. 2: Microscopic features of alloxan induced diabetic rat with control (2% gum acacia)rat-1**

3).Alloxan induced diabetic rat with control Rat-2(2% gum acacia): Acini are normal. Number of islets cells is decreased in number. These cells have vacuolated cytoplasm and round to oval nuclei. The stroma shows congestion and mild lymphocytic infiltrate. (Fig 3)



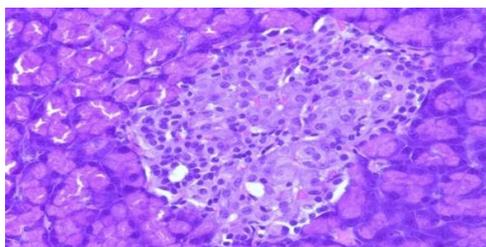
**Fig. 3: Microscopic features of alloxan induced diabetic rat with control (2% gum acacia)rat-2**

4).Alloxan induced diabetic rat with NIDDWIN (50mg/kg) in Rat-1: Islet cells are still decreased in number. The cells have round to oval nuclei with fine chromatin while few have dark nuclei. Cytoplasm of many cells is vacuolated. There is mild lymphocytic infiltrate seen. (Fig 4)



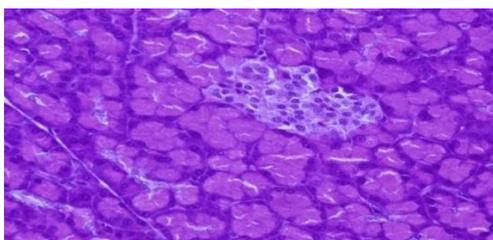
**Fig. 4: Microscopic features of alloxan induced diabetic rat with NIDDWIN (50mg/kg) rat-1**

5).Alloxan induced diabetic rat with NIDDWIN (50mg/kg) in Rat-2: Acini are normal. Islets are decreased in number. Their nuclei vary from round to oval with fine to dark chromatin. The cytoplasm of many cells is still vacuolated. The stroma shows few congested blood vessels or lymphocytic infiltrate. The findings are almost similar to position or Alloxan induced control rats. (Fig 5)



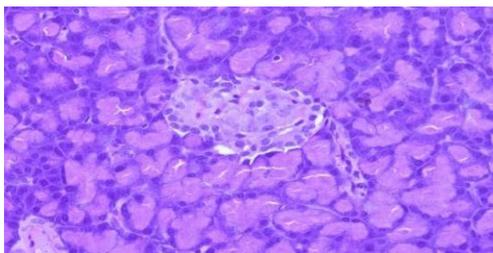
**Fig. 5: Microscopic features of alloxan induced diabetic rat with NIDDWIN (50mg/kg) rat-2**

6). Alloxan induced diabetic rat with NIDDWIN (100mg/kg) in Rat-1: Acini are normal. The islets are in process of regeneration with mild increase in numbers. Vacuolated cytoplasm is persisting in some cells. The remaining cells have moderate eosinophil cytoplasm. Number of inflammatory cells has decreased. Regeneration is seen and the changes are less severe when compared to positive control and standard. (Fig 6)



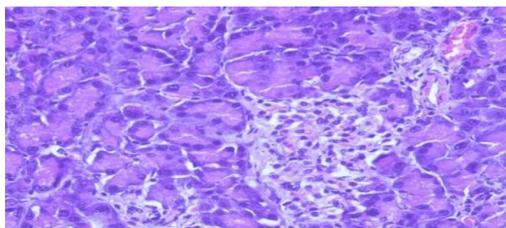
**Fig. 6: Microscopic features of alloxan induced diabetic rat with NIDDWIN (100mg/kg) rat-1**

7). Alloxan induced diabetic rat with NIDDWIN (100mg/kg) in Rat-2: Acini are normal. Regeneration is seen in islets and the histopathological changes are less in severity than the standard and positive control. (Fig 7)



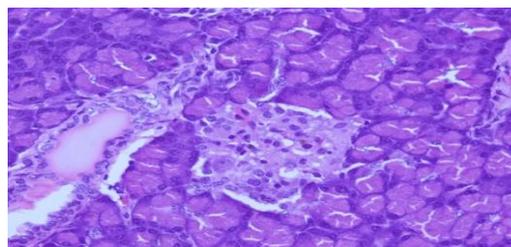
**Fig. 7: Microscopic features of alloxan induced diabetic rat with NIDDWIN (100mg/kg) rat-2**

8). Alloxan induced diabetic rat with Glibenclamide (10mg/kg) in Rat-1: Acini are normal. Islets are however slightly decreased in number with most show vacuolated cytoplasm. The nuclei are vesicular. The number of lymphocytes is also less. (Fig 8)



**Fig. 8: Microscopic features of alloxan induced diabetic rat with Glibenclamide (10mg/kg) rat-1**

9). Alloxan induced diabetic rat with Glibenclamide (10mg/kg) in group-2: Acini are normal. Islets are however slightly less in number with many having vacuolated cytoplasm. The nuclei are vesicular. (Fig 9)



**Fig. 9: Microscopic features of alloxan induced diabetic rat with Glibenclamide (10mg/kg) rat-2**

**Table 4: Effect of NIDDWIN on lipid per-oxidation**

Serial no	Percentage of lipid per oxidase inhibition in alloxan induced diabetic Rats		
1	Alpha tocopherol 100mg/kg	NIDDWIN 50mg/kg	NIDDWIN 100mg/kg
Mean±SEM	81.92±3.359	34.0374±3.543**	52.794±2.164***

Each Value is SEM of 5animals:\*\*P<0.01, \*\*\*P<0.001. Comparison between diabetic control rats and different treated group rats.

**DISCUSSION**

As NIDDWIN also showed reduction in the glucose levels at 4hours same as Glibenclamide. It indicates that may be NIDDWIN also as same mechanism of action as Glibenclamide. It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas [18] and these compounds are

active in mild alloxan induced diabetes where as they are inactive in intense alloxan induced diabetes nearly all beta cells have been destroyed. Since results showed that Glibenclamide reduced blood glucose levels hyperglycemic animals. The state of diabetes is not severe. Alloxan treated animals receiving the NIDDWIN showed rapid normalization of blood glucose levels in comparison to control and this be due to the possibility that some beta cells remaining still surviving to act upon by NIDDWIN to exert its insulin releasing effect. More over this suggests that the mode of action active ingredients of NIDDWIN is probably mediated by enhanced secretion of insulin, like sulphonylureas. The anti-hyperglycemic and hypoglycemic effects of NIDDWIN may be due to multiple effects of chemical constituents of different plants in NIDDWIN. Further study was conducted to establish the mechanism of action and antihyperlipidemic activity of NIDDWIN. Histopathological results showed the regeneration of pancreatic islets in NIDDWIN 100mg/kg when compared to standard Glibenclamide 10mg/kg.

Oxidative stress is reported to be increased in patients with diabetes mellitus. In case of diabetes mellitus elevated blood glucose levels was observed. Exposure of liver to elevated glucose levels results in the decreased activities of anti-oxidant enzymes such as SOD, CAT, GST and GSH which contributed to the increased [19,20] lipid per-oxidation in the liver [21]. It has been demonstrated that the polyunsaturated fatty acids of mammalian tissue and body fluids undergo lipid per-oxidation- Our results showed that the NIDDWIN reduced the lipid per-oxidation in alloxan induced diabetic rats. Reduction of lipid per-oxidation indicates anti-oxidant activity. Some of components of NIDDWIN were reported to possess anti-oxidant activity *Emblca officinalis* [22], *Withania somnifera* [23] and *Asphaltum* [24]. This shows the anti-oxidant activity of NIDDWIN.

**CONCLUSION**

The present study suggested that the polyherbal formulation NIDDWIN possess a potent antidiabetic activity as it significantly reduced blood glucose levels and also showed antioxidant activity in diabetic rats. In addition it also shown to reduce cholesterol, triglycerides levels in diabetic rats. As NIDDWIN is shown to have potent antidiabetic activity and antioxidant activity. Therefore further studies are planned to establish the probable mechanism of NIDDWIN.

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