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Annual Progress Report of Project (2011-13)

Ext. 2013-14

Section-A: Project Details

A1. Project Title: - Study the effect of medicinal plant aloe vera on hyperlipidemia and dysfunctional free radicals in pentamidine isethionate induced diabeteic rats

A2. Sanction Order No. & Date: 36 & 18-01-2011

A3. Name of Principal Investigator: Prof. U. K. Chauhan

A4. Institute: - Awadhesh Pratap Singh University

A5. Address (Landline & Mobile) & Email: School of Environmental Biology, Awadhesh Pratap Singh University, Rewa-486 001

A6. Total Cost: - Rs. 12, 62,000/-

A7. Duration: - 2 yrs

A8. Approved Objectives of the Project:

- To develop a natural drug which is not only lipid lowering agent but also has the property to restore the normal lipid values in diabetic patients.
- To establish *Aloe vera* as an standard antioxidative agent.
- To treat diabetes caused as a side effect of pentamidine isethionate medication through *Aloe Vera*.
- To study the natural drug (Aloe vera) and synthetic drug (pentamidine isethionate) interaction
- To measure the toxicity of pentamidine isethionate medication.
- To establish pentamidine isethionate as a standard synthetic drug in inducing diabetes in experimental rats for further researches.

A9. Specific Recommendations made by the Task Force (if any): Following recommendations were made by the PAC members (Project approval committee):

"Project has a novel approach but needs to be revised mentioning latest review of literature on the anti

Diabetic potential of *Aloe vera* and then to be used along with the treatment of infants with Pentamidine

Isothionate."

Research work completed so far:

B1. Progress made against the Approved Objectives:

Rates of diabetes are increasing worldwide. At least 171 million people currently have diabetes, and this figure is likely to more than double to 366 million by 2030. The top 10 countries, in numbers of people with diabetes, are currently India, China, the United States, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh. The greatest percentage increase in rates of diabetes will occur in India over the next 20 years. However, at least 80% of people in India with diabetes are undiagnosed, and many in their 30s to 60s will die from diabetes here. A number of treatments and therapies are available which are very expensive as well as have lot of side effects. Thus people are now shifting towards herbal and medicinal plants. Scientists and researchers are working in this field to develop such herbals drugs which are cheaper in comparison to synthetic drugs and also having lesser or no side effects. In-vitro and in- vivo studies have shown that aloe vera has anti-diabetic property. Still more evidences are required to confirm its efficacy. This Project is designed in such a way that not only the anti-diabetic property but also the anti-oxidant property of aloe vera can be studied. Pentamidine isethionate is taken as a synthetic drug for the induction of diabetes in experimental rats. Since pentamidine is widely used drug against pneumonia infection in patients, as a side effect it disturbs the glucose metabolism leading to diabetes. This study will help such patients to take aloe vera as a supplement drug during their treatment to prevent diabetes occurrence.

Project started in the month of January. A lot of material search and paper work was done before starting the experiments. Following progress in project has been done till date:

PLANT MATERIAL

The plant material of Aloe vera collected from the Jayantikunj campus (authorized botanical garden) and Identified by the botany division of APS University.

PREPARATION OF ALCOHOLIC EXTRACT

The *Aloe vera* leaves were cut into pieces and then dipped in alcohol for seven days and then the alcoholic extract was collected and the resultant alcoholic extract was concentrated using Rotatory Evaporator.

ANIMAL USED

Adult male rats of Charles Foster strain (125-150 gm) breed purchased from an authorized dealer of experimental rats were used. These animals were maintained on standard pellet diet and water ad- libitum. For experimental conditions, temp. (25-26^{o}C), relative humidity (60-80%) and 12/12 hour light/ dark cycle (light from 8:00am to 8:00 pm) were provided.

EXPERIMENT

Initially all the animals had free access to diet and water so that their body weight increase and hyperlipidemia is induced after injecting pentamidine isethionate drug. It is checked up by collecting blood from retro orbital plexus and calculating the cholesterol and TG level. If the TG and cholesterol is increased up to mark then the drug is administered to them orally (1gm/kg body weight as a suspension in gum acacia). The control rats were provided normal diet and some amount of gum acacia.

Control group, pentamidine isethionate fed group, pentamidine isethionate + drug (*Aloe vera*) and pentamidine isethionate + gemfibrozil. All groups except control were provided with the pentamidine isethionate (10 gm /animal daily)

COLLECTION OF BLOOD SAMPLES

Next day blood was collected after the 18hr fasting. Blood was withdrawn from the retro orbital plexus using 10µl (volume) of 20mm (length) x 0.8 mm (diameter) capillaries for the estimation of total cholesterol, triglycerides and phospholipids. Blood was collected in centrifuge tubes and was kept in ice for complete separation of blood plasma without any loss of enzyme activity and degradation of biomolecules. The tubes were centrifuged at 5000 rpm at 4˚C for 10 mins and supernatant (plasma) was separated for the estimation.

BIOCHEMICAL ESTIMATIONS (Completed in First Year)

1. ASSESSMENT OF DIABETOGENIC PARAMETERS IN DIABETIC RATS

a. **Glucose tolerance test**
b. **Estimation of plasma glucose level**
c. **Free fatty acid estimation**
d. **Protein estimation**

2. <u>ASSESSMENT OF DIABETIC HYPERLIPIDEMIA</u>:-

a. **Measurement of Phospholipids**
b. Measurement of cholesterols
c. Measurement of Triglycerides

3. <u>ASSESSMENT OF FREE RADICAL SCAVENGING ACTIVITY</u>

a. **Measurement of lipid peroxide levels**
b. **Measurement of Superoxide dismutase activity**

G. <u>STATISTICAL ANALYSIS</u>:-

Data will be expressed as mean ± standard error of the mean (S.E.M). Comparison of control and experimental groups according to the parameters will be performed using the test of one-way ANOVA in SPSS package (statistica version 5.0UK). Correlation coefficient will be calculated using spearman's procedure. P value less than 0.05 will be considered statistically significant.

% lipid lowering =

$$\frac{\text{Diabetes induced animal lipid value} - \text{drug \& diabetic animal lipid value}}{\text{Diabetes induced animal lipid value}} \times 100$$

Specific activity =

$$\frac{\text{A} \times \text{dilution factor} \times 1000}{1 \text{ unit} \times \text{vol. of enzyme} \times \text{mg/protein unit}}$$

Summary of the research work completed so far:

There have been several reports on the hypoglycemic activity of *Aloe*, which vary in regard to the plant species, the part of the plant used, and in the preparation of extracts as well as the animal models. The present work was undertaken to study the antidiabetic effect of *Aloe vera* extract in pentamidine isethionate induced diabetic rats.

In previous studies, it has been proposed that *Aloe vera* helps in decreasing the triglycerides level in diabetes patients. Many researches have been done in animal models to study the antidiabetic property of *Aloe vera*. Limited Synthetic drugs like streptozotocin, alloxan are available, used to induce diabetes in animal models and then study the effect of *Aloe vera*. So, there is a requirement of finding a new synthetic drug which can be used in future for studying diabetic animal models. This study was designed to establish a new standard diabetic rat model.

Pentamidine isethionate is a popular medicine taken against pneumonia since long time but due to its side effect that it increases the glucose level in the patients; its use has been restricted. Thus this study will evaluate the toxicity of pentamidine isethionate in terms of diabetes and also to cure the hyperglycemic condition with the help of herbal drug *Aloe vera*.

Three-month-old male Charles foster rats weighing 150–200 g were used. Rats were maintained under good laboratory conditions and were allowed free access of food and water *ad libitum*. Experiments were conducted according to the ethical norms approved by the Institutional Animal Ethics Committee guidelines. Pentamidine isethionate was purchased from Sigma (Bangalore). All other chemicals used were of analytical grade. After fasting, diabetes mellitus was induced by intraperitoneal injection of Pentamidine isethionate dissolved in 0.1 M cold sodium citrate buffer (pH 4.4) at a dose of (7.5mg/200gms/day). Pentamidine isethionate treated animals were considered as diabetic when the fasting plasma levels were observed above 250 mg/dl with glucosuria. After standardizing the dose of pentamidine isethionate, the experiments were conducted on animal groups to see the effect of *Aloe vera* on diabetic rats. In the experiments, six rats were used in each group (Control rats, Diabetic rats, *Aloe vera* treated diabetic rats, Gemfibrozil treated diabetic rats).

At the beginning of experiment, before the administration of *Aloe vera*, the body weight of rats was 221, 228, 230, and 223 gms of control, diabetic, *Aloe vera* treated and gemfibrozil treated group of rats. At the end of the experiment, the body weight of control, diabetic, *Aloe vera* treated and gemfibrozil treated group of rats changes to 402, 140, 231, and 223 gms. There was no significant difference between initial and final body weight levels in gemfibrozil treated group. A sudden increase in body weight was observed in pentamidine isethionate induced diabetic rats as *Aloe vera* was administered, from 213gms to 223gms.

We found that glucose increases in diabetic rats as compared to non-diabetic rats. It means glucose tolerance was poorer in pentamidine isethionate induced diabetic rats Treatment with *Aloe vera* decreases the glucose in diabetic rats as compared to control rats. Rats fed with gemfibrozil showed the decrement in blood glucose to the normal values. In control rats, blood glucose values remain same for every reading at different time interval. In *Aloe vera* treated diabetic rats, the glucose tolerance was 130mg/dl at 0min and it decreases to 90mg/dl on 10^{th} day. Similar results were obtained on 20^{th} and 30^{th} day of experiment.
We found that plasma glucose level was higher in diabetic rats whereas in gemfibrozil and *Aloe vera* given rats, the glucose level came down to normal value.
In our study, there was a significant increase in cholesterol, triglycerides, phospholipids and free fatty acids during pentamidine isethionate induced diabetes compared with those of corresponding control rats. Following the oral administration of *Aloe vera* extract and gemfibrozil, the levels were found to be similar to those in control rats. We found that the hyperlipidemia caused due to pentamidine isethionate was significantly lowered by the administration of *Aloe vera*.
The results obtained in our study were justified by the triton experiment model. By comparing the results of both the models, it is clearly verified that pentamidine isethionate induced diabetic rats showed a visible lowering of lipids after the administration of *Aloe vera*. Thus, from our study, it is established that *Aloe vera* is helpful in decreasing the lipids and can be given as an antidiabetic drug to the diabetic patients.
We also found that oxidative stress caused due to pentamidine isethionate induced diabetes was altered by the *Aloe vera*. The lipid peroxides level were elevated in the diabetic group ($p<0.05$). In *Aloe vera* treated group of diabetic rats 0.03 folds decrement in lipid peroxidase activity was observed. Similarly for super oxide dismutase activity, the diabetic rats showed 1.01 folds of increment, whereas in *Aloe vera* given diabetic rats, showed a decrement of 0.97 folds. These results indicated that *Aloe vera* activates the oxidative defense mechanism in pentamidine isehionae induced diabetic rats. Thus, *Aloe vera* acted as a free radical scavenger.

In conclusion, our initial findings of the present study shows that *Aloe vera* extract brings back the Fasting Plasma Glucose levels to normal in diabetes-induced rats, i.e. shows hypoglycemic activity. Although the exact chemical compound/s responsible for the hypoglycemic effects of *Aloe vera* extract still remain speculative, Experimental evidence obtained in the present laboratory animal study indicates that *Aloe vera* extract possesses antidiabetic property. More detailed studies on *Aloe vera* using different doses and covering longer periods of observation are needed before reaching a clear-cut conclusion. Future research to refine the extraction procedure of *Aloe vera* could lead to improved pharmaceutical products.

The conclusion from one year study was that aloe contains distinct anti-diabetic properties but its mode of action is unknown. Further study needs to be done to better understand the anti-diabetic activity of *Aloe vera*. However, *Aloe vera* along with other lifestyle and diet changes may be able to help control this dangerous disease. *Aloe vera* has potential in treating diabetes because it can lower not only blood sugar, but also triglyceride levels which are often high in diabetic patients.

The preponderance of evidence suggests a trend toward benefit from oral *Aloe vera* use in reducing fasting blood glucose concentration. Triglyceride levels also seem to be reduced,

Aloe vera contains many physiologically active substances that have effective antiinflammatory, immunomodulatory, and wound-healing effects. The active ingredients, whether acting alone or in concert, include glycoproteins, anthraquinones, polysaccharides, and low-molecular-weight species. Moreover, the fact that biologically active components in *Aloe vera* may be labile, varied, or modified explain some of the difficulties that investigators have reported in reproducing results using unfractionated materials from *Aloe vera*. In light of the many pharmacologic activities of the components of *Aloe vera*, each active component has several interacting factors, each of which may be affected by another substance(s). Thus, a further understanding of these individual components and of their effects is essential if *Aloe vera* is to be successfully developed for therapeutic purposes.

A number of species have been discovered which has same or different physiological, chemical, medicinal characteristics. The differences in their properties are due to variations in climatic and topographical conditions. Still, there is no any study done in Madhya Pradesh on *Aloe vera* plant which can confirms that the species found in the state, has such potential to treat diabetes patients. Evidences are not sufficient to prove the anti-hyperlipidemic and anti-oxidant efficacy of *Aloe vera* found in the state. Thus further study needs to be done mainly focusing on species of *Aloe vera* found in Madhya Pradesh having antidiabetic property and economical value of the plant for diabetes patients.

Result of one year research work

Body weights

In the present study, initially, changes in body weight on the treatment of diabetic and normal rats with plant extract were determined and these have been demonstrated in Table.01. There was no significant difference between initial and final body weight levels in gemfibrozil treated group. A sudden increase in body weight was observed in pentamidine isethionate induced diabetic rats as *Aloe vera* was administered, from 213gms to 223gms.

At the beginning of experiment, before the administration of *Aloe vera*, the body weight of rats was 221, 228, 240, and 213 gms of control, diabetic, *Aloe vera* treated and gemfibrozil treated group of rats. At the end of the experiment, the body weight of control, diabetic, *Aloe vera* treated and gemfibrozil treated group of rats changes to 402, 140, 231, and 223 gms.

Table.01.shows initial and final body weight of experimental rats

Group	**Initial body weight (0^{th} day)**	**Final body weight (30^{th} day)**
Control	221gms	402gms
Diabetic rats	228gms	140gms
Gemfibrozil treated diabetic rats	240gms	231gms
***Aloe vera* treated diabetic rats**	213gms	223gms

Data are the mean ±SD for five animals in each group. *P < 0.05 compared with control rats; † P < 0.05 compared with diabetic control rats.

Oral glucose tolerance test

Glucose tolerance test was measured according to Trinder et al. method. At 0 min blood glucose was measured for control rats, diabetic rats, diabetic rats treated with *Aloe vera* alcoholic extract and diabetic rats treated with gemfibrozil. After taking the values of blood glucose of each group, rats were fed a solution of glucose (3g/kg b.wt.). Then values of glucose at 30 mins, 60 mins, 90 mins and 120 mins were measured respectively. Results of oral glucose tolerance test as shown in Table. 02, revealed that glucose increases in diabetic rats as compared to non-diabetic rats. Treatment with *Aloe vera* decreases the glucose in diabetic rats as compared to control rats. Rats fed with gemfibrozil showed the decrement in blood glucose to the normal values. In control rats, blood glucose values remain same for every reading at different time interval.

On 10^{th} day, the glucose tolerance of control rats was 80mg/dl, 82mg/dl, 83mg/dl, 82mg/dl, and 80mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. The glucose tolerance test values of diabetic rats was 100mg/dl, 132mg/dl, 170mg/dl, 200mg/dl and 217mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. In *Aloe vera* treated diabetic rats, the glucose tolerance was 130mg/dl, 110mg/dl, 97mg/dl, 88mg/dl, and 80mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. Similarly, in gemfibrozil treated rats, glucose tolerance values was 140mg/dl, 95mg/dl, 80mg/dl, 78mg/dl and 75mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively.

Table.02.Blood glucose values of control, diabetic and drugs treated rats on 10th day

Experiment	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control	80mg/dl	82mg/dl	83mg/dl	82mg/dl	80mg/dl
Diabetic rats	100mg/dl	132mg/dl	170mg/dl	200mg/dl	217mg/dl
Diabetic + *Aloe vera* treated rats	130mg/dl	110mg/dl	97mg/dl	88mg/dl	80mg/dl

Diabetic + Gemfibrozil treated rats	140mg/dl	95mg/dl	80mg/dl	78mg/dl	75mg/dl

Data are the mean ±SD for five animals in each group. *P < 0.05 compared with control rats; † P < 0.05 compared with diabetic control rats.

Results of oral glucose tolerance test as shown in Table. 03 revealed that glucose increases in diabetic rats as compared to non-diabetic rats. Treatment with *Aloe vera* decreases the glucose in diabetic rats as compared to control rats. Rats fed with gemfibrozil showed the decrement in blood glucose to the normal values. In control rats, blood glucose values remain same for every reading at different time interval.

On 20th day, the glucose tolerance of control rats was 82mg/dl, 85mg/dl, 82mg/dl, 84mg/dl, and 80mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. The glucose tolerance test values of diabetic rats was 100mg/dl, 130mg/dl, 150mg/dl, 200mg/dl and 210mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. In *Aloe vera* treated diabetic rats, the glucose tolerance was 110mg/dl, 90mg/dl, 88mg/dl, 84mg/dl, and 80mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. Similarly, in gemfibrozil treated rats, glucose tolerance values was 130mg/dl, 85mg/dl, 80mg/dl, 78mg/dl and 75mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively.

Table.03.oral glucose tolerance test in control, diabetic and drug treated diabetic rats on 20th day

Experiment	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control	82mg/dl	85mg/dl	82mg/dl	84mg/dl	80mg/dl
Diabetic rats	100mg/dl	130mg/dl	150mg/dl	200mg/dl	210mg/dl
Diabetic + *Aloe vera* treated rats	110mg/dl	90mg/dl	88mg/dl	84mg/dl	80mg/dl

Diabetic + Gemfibrozil treated rats	130mg/dl	85mg/dl	80mg/dl	78mg/dl	75mg/dl

Data are the mean ±SD for five animals in each group. *P < 0.05 compared with control rats; † P < 0.05 compared with diabetic control rats.

On 30th day, the glucose tolerance of control rats was 88mg/dl, 84mg/dl, 80mg/dl, 81mg/dl, and 80mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. The glucose tolerance test values of diabetic rats was 125mg/dl, 140mg/dl, 152mg/dl, 208mg/dl and 220mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. In *Aloe vera* treated diabetic rats, the glucose tolerance was 130mg/dl, 95mg/dl, 87mg/dl, 83mg/dl, and 80mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. Similarly, in gemfibrozil treated rats, glucose tolerance values was 120mg/dl, 85mg/dl, 82mg/dl, 80mg/dl and 80mg/dl at 0 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively.

Table.04.oral glucose tolerance test in rats of different groups on 30th day

Experiment	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control	88mg/dl	84mg/dl	80mg/dl	81mg/dl	80mg/dl
Diabetic rats	125mg/dl	140mg/dl	152mg/dl	208mg/dl	220mg/dl
Diabetic + *Aloe vera* treated rats	130mg/dl	95mg/dl	87mg/dl	83mg/dl	80mg/dl
Diabetic + Gemfibrozil treated rats	120mg/dl	85mg/dl	82mg/dl	80mg/dl	80mg/dl

Data are the mean ±SD for five animals in each group. *P < 0.05 compared with control rats; † P < 0.05 compared with diabetic control rats.

Fasting Plasma Glucose

Table.05.gives the level of plasma glucose in control, diabetic, *Aloe vera* treated diabetic rats and gemfibrozil treated diabetic rats. Diabetic rats showed a significant increase in blood glucose as compared to control rats on 0 day. Level of glucose in experimental rats was also high. After the initiation of *Aloe vera* in diabetic rats, the level of glucose in blood decreases as shown in the graph of 10^{th} day readings.

The concentration of glucose in plasma prior to experiments was 100, 103.7, 104 and 99.1mg/dl in control, diabetic, *Aloe vera* treated and gemfibrozil treated group of rats respectively. On 10^{th} day, the concentration of glucose was 284.3, 300.3, 297.4, and 281.1 mg/dl in control, diabetic, *Aloe vera* treated and gemfibrozil treated group of rats respectively. On 20^{th} day of the experiment, glucose concentration in plasma of control, diabetic, *Aloe vera* treated and gemfibrozil treated group of rats was 104, 297.4, 104.7, and 105.8mg/dl respectively.

Table.05 shows plasma glucose value in experimental rats

Experiment	**0 day**	**10 days**	**20 days**	**30 days**
Control rats	100	103.7	104	99.1
Diabetic rats	284.3	300.3	297.4	281.1
Diabetic + *Aloe vera* treated rats	254.4	132.0	104.7	101.8
Diabetic + Gemfibrozil treated rats	262.1	141.3	105.8	102.4

Data are the mean ±SD for five animals in each group. *$P < 0.05$ compared with control rats;
† $P < 0.05$ compared with diabetic control rats.

Biochemical parameters (Pentamidine isethionate induced model)

Table 06 shows levels of cholesterol, triglycerides, phospholipids and free fatty acids in serum of the control and experimental groups of rats, respectively. There was a significant increase in cholesterol, triglycerides, phospholipids and free fatty acids during diabetes compared with levels in corresponding control rats. Following the oral administration of *Aloe vera* extract and gemfibrozil, the levels were found to be similar to those in control rats.

In control rats, the value of cholesterol was 92.6 whereas in diabetic rats, it was 228.3. In gemfibrozil treated rats, cholesterol level was 106.2. In *Aloe vera* treated rats, the cholesterol level was similar to that of control and gemfibrozil given rats, 98.3. Thus, in Diabetic rats, 15.1 folds increase was observed in cholesterol and in *Aloe vera* treated diabetic rats, 8.5 folds increase was seen.

The value of triglycerides in control rats as seen in table .06 is 73.5, whereas in diabetic rats, it is 229.3. In gemfibrozil treated rats, triglycerides level was 83.4. In *Aloe vera* treated rats, the triglyceride level was similar to that of control and gemfibrozil given rats, 79.2. Thus, in Diabetic rats, 16.1 folds increase was observed in triglycerides and in *Aloe vera* treated diabetic rats, 5.2 folds increase was seen.

In control rats, the value of free fatty acids was 58.3 whereas in diabetic rats, it was 145.2. In gemfibrozil treated rats, free fatty acids level was 66.1. In *Aloe vera* treated rats, the free fatty acids level was similar to that of control and gemfibrozil given rats, 64.7. Thus, in Diabetic rats, 10.5 folds increase was observed in free fatty acids and in *Aloe vera* treated diabetic rats, 4.1 folds increase was seen.

The value of phospholipids in control rats as seen in able .06 is 80.5, whereas in diabetic rats, it is 163.8. In gemfibrozil treated rats, phospholipids level was 88.8. In *Aloe vera* treated rats, the phospholipids level was similar to that of control and gemfibrozil given rats, 85.7. Thus, in Diabetic rats, 11.1 folds increase was observed in phospholipids and in *Aloe vera* treated diabetic rats, 5.8 folds increase was seen.

Table.06.Lipid lowering activity of *Aloe vera in* pentamidine isethionate induced diabetes model

Experiment	**Control rats**	**Diabetic rats**	**Diabetic + *Aloe vera***	**Diabetic + gemfibrozil**

Cholesterol	92.6 ± 5.7	228.3 ± 15.1*	98.3 ± 8.5	106.2 ± 7.0
Triglycerides	73.5 ± 5.2	229.3 ± 16.1*	79.2 ± 5.2	83.4 ± 5.8
Free fatty acids	58.3 ± 3.6	145.2 ± 10.5*	64.7 ± 4.1	66.1 ± 4.6
Phospholipids	80.5 ± 5.7	163.8 ± 11.1*	85.7 ± 5.8	88.8 ± 6.7

Data are the mean ±SD for five animals in each group. *P < 0.05 compared with control rats; † P < 0.05 compared with diabetic control rats.

Antioxidant Activity

Lipid peroxidase activity: Lipid peroxidation is one of the characteristic features of chronic diseases such as diabetes. Lipid peroxide mediated damage has been observed in both types of diabetes. It has been observed that insulin secretion is closely associated with lipoxigenase peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation leading to cellular damage.

In table.08, it has been observed that in control group of rats, the lipid peroxidase activity remains same throughout the experiment, 3.24, 3.30, and 3.87 on 10^{th}, 20^{th}, and 30^{th} day respectively. In diabetic rats, the lipid peroxidase activity increases during the experiment, 6.05, 7.36 and 7.65 on 10^{th}, 20^{th}, and 30^{th} day of the experiment respectively. In *Aloe vera* treated diabetic rats, the lipid peroxidase activity decreases from 3.90 to 3.68 and 3.59 on 10^{th}, 20^{th}, and 30^{th} day of the experiment respectively.

In Table 8 there was a significant increase in lipid peroxide levels ($p<0.05$) in streptozotocin induced diabetic rats with respect to normal controls. It also shows a statistically significant decrease in lipid peroxide levels in diabetic rats treated with extract of *Aloe vera* leaves.

Table.08. Lipid peroxidase activity in the serum of hyperlipidemic rats treated with *Aloe vera*

Experiment	**10 days**	**20 days**	**30 days**
Control	3.24±0.25	3.30±0.80	3.87±0.10

Diabetic rats	6.05±0.72	7.36±0.45	7.65±0.92
***Aloe vera* treated**	3.90±0.30	3.68±0.29	3.59±0.25

Data are the mean ±SD for five animals in each group. *P < 0.05 compared with control rats; † P < 0.05 compared with diabetic control rats.

Superoxide dismutase Activity: The activities of SOD were significantly higher in diabetic rats treated with extract of *Aloe vera* leaves compared to diabetic control rats ($p<0.05$). There was no significant difference among the treated diabetic and normal groups but there was significant difference between diabetic and normal controls.

In control group, on 10^{th} day, the value was 84.3 and on 20^{th} day the value was 84.0 . Finally on 30th days he value was 83.7. There was no significant increasement in the SOD activity was seen in control rats.

In *Aloe vera* treated rats, a significant rise in SOD activity was observed. On 10^{th} day, the value was 81.2, on 20^{th} day, it was 82.61 and at the end of the experiment, the value was 82.70.

Table.09. Superoxide dismutase activity in control and drug treated diabetic rats

Experiment	**10^{th} days**	**20^{th} days**	**30^{th} days**
Control rats	84.3 ± 2.14	84.0 ± 2.44	83.7 ± 2.74
Diabetic rats	46.42 ± 0.94	44.86 ± 2.50	43.42 ± 3.94
***Aloe vera* treated diabetic rats**	81.2 ± 2.24	82.61 ± 0.83	82.70± 0.74

Data are the mean ±SD for five animals in each group. *P < 0.05 compared with control rats; † P < 0.05 compared with diabetic control rats.

B4. Details of Publications & Patents, if any:

1. U. K. Chauhan, Sanjeev Dubey, Shilpa Mishra (2012). Diabetes mellitus and its treatment with the help of aloe vera. International journal of biotechnology and biochemistry. 8; 1, 35-45.

2. Shilpa Mishra, U. K. Chauhan, Sanjeev Dubey (2011). Role of medicinal plants in the treatment of diabetes mellitus. National conference on diabetes mellitus and cancer, (Dia-can '11'). Annamalai University.

3. Shilpa Mishra, U. K. Chauhan (2012). Oxidative stress and its treatment with aloe vera alcoholic extract. National conference on Biotechnology and biodiversity, Awadhesh Pratap Singh University.

4. Shilpa Mishra, U. K. Chauhan, Sanjeev Dubey (2012). Efficacy of aloe vera on oxidative stress during diabetes mellitus. National conference on Role of new technologies in conservation of environment, APSU.

Communicated publication

1. Shilpa Mishra, U. K. Chauhan, Sanjeev Dubey (2012). "Effect of aloe vera on hyperlipidemia in pentamidine isethionate induced diabetic albino rats", communicated o International journal of diabetes in developing countries.

Deliverables of the project:-

1. Present project is designed to study the anti-hyperlipidemic and anti-oxidant activity of *Aloe vera*. In previous studies, it has been proposed that *Aloe vera* helps in decreasing the triglycerides level in diabetes patients. A number of species have been discovered which has same or different physiological, chemical, medicinal characteristics. The differences in their properties are due to variations in climatic and topographical conditions. Still, there is no any study done in Madhya Pradesh on Aloe vera plant which can confirms that the species found in the state, has such potential to treat diabetes patients. Evidences are not sufficient to prove the anti-hyperlipidemic and anti-oxidant efficacy of aloe vera found in the state. Thus this project will focus on species of aloe vera found in Madhya Pradesh, to study the economical value of the plant for diabetes patients.
2. Diabetes is a fatal disease which causes many other serious complications like gangrene, cardiovascular diseases, kidney failure, blindness, stroke, disability. Diabetes is one of the leading cause of death not only in Madhya Pradesh but in whole India. Thus this project will help other researches to fight these risk factors associated with the disease- diabetes.
3. Many researches have been done in animal models to study the antidiabetic property of aloe vera. Limited Synthetic drugs like streptozotocin, alloxan are available, used to induce diabetes in

animal models and then study the effect of aloe vera. So, there is a requirement of finding a new synthetic drug which can be used in future for studying diabetic animal models. This project is designed to establish a new standard diabetic rat model.

4. Pentamidine isethionate is a popular medicine taken against pneumonia since long time but due to its side effect that it increases the glucose level in the patients; its use has been restricted. Thus this study will evaluate the toxicity of pentamidine isethionate in terms of diabetes and also to cure the hyperglycemic condition with the help of herbal drug aloe vera.

Benefits that will accrue to M.P. after completion of the research project

1. In Madhya Pradesh, it has become common to see Aloe Vera plants in homes - where it may be used, by cutting off a part of a leaf, to spread its cooling, soothing, healing gel on burns, scrapes and bruises. Today, Aloe Vera helps dealing with the effects of diabetes, asthma, epilepsy, osteoarthritis, different types of digestive problems, ulcers, gastritis, colitis, constipation, reduce the levels of cholesterol and triglycerides, helps the blood circulation, and cure skin and hair problems. Many in-vitro and in-vivo researches have shown that aloe vera has anti-diabetic property. Diabetes is a deficiency or absence of the hormone insulin, which is the main hormone responsible for the control of sugar in the blood. Research indicates that even moderately elevated blood sugar levels can increase the risk of cardiovascular disease, Research also shows that elevated blood sugar leads to increased oxidative stress and there is evidence that increased production of free radicals may be a contributing factor in the complications seen in diabetes. Aloe polysaccharides are thought to be the essential bioactive compound present in aloe. Many research have been done in the past which confirms the utility of Aloe Vera as an antidiabetic compound.
2. In today's world, the types of medicines available for diabetic patients are very expensive and not easily available for people below poverty line. Aloe vera can be used as a safe substitute of other costly synthetic drugs for diabetes treatment especially in states like Madhya Pradesh where 50-60% of population comes under BPL category.
3. Madhya Pradesh is gifted with a rich amount of this miraculous plant named 'aloe vera'. Thus, it's required to identify and quantify such components of aloe vera which are responsible for anti-hyperlipidemic and anti-oxidant activity of aloe vera plant. So that new herbal drug can be produced out of aloe vera, having medicinal as well as economical value also.

(For the year 2011-12)

Section-C: Details of Grant Utilization

Amount sanctioned by MP Biotechnology Council Rs. 12, 62,000

7. Amount released by MP Biotechnology Council Rs, 9, 06,000

8. Details of expenditure

NON-RECURRING

S. No	List of the approved equipment	List of the equipment procured	Date of purchase	Amount Sanctioned	Amount Utilized (Item wise)	Unspent Balance/Access Amount
1.	Cooling Centrifuge with stablizer	Cooling Centrifuge with stablizer		2,00,000	2,37,752	-37,752
2.	Clinical Analyzer (Glucometer)	Clinical Analyzer (Glucometer)		1,00,000	2,14,700	-1,14,700
3.	Rotatory evaporator	Rotatory evaporator		1,00,000	61,136	38,864
4.	Refrigerator			50,000	-	50,000
				4,50,000	5,13,588	Access amount=63,588.00

Justification- the amounts sanctioned for instrument were lesser then actual price therefore we utilized the amount from recurring grants in instruments purchasing and hereby declaring that the total budget will not exceed the sanctioned amount.

RECURRING

S. No	Activities undertaken	Period	Amount Sanctioned	Amount Utilized (Itemize)	Unspent Balance
1.	Manpower		96,000	96,000	Utilized
2.	Chemicals and Glasswares		3,00,000	2,25,432	74,568
3.	Contingencies		30,000	29,905	95/-

4.	Travel		30,000	8,067	21,933
			4,56,000	3,59,404	96,596
Amount adjusted for Nonrecurring items					63,588
Total Unspent balance					31,540

Certified that the grant has been utilized for the purpose for which it was sanctioned in accordance with the "Terms and Conditions" attached to the grant. If, as a result of check or audit objection some irregularity is sanctioned at a later stage, action will be taken to refund, adjust or regularize the amount objected to.

Principal Investigator

(For the year 2013-14)

To develop a natural drug which is not only lipid lowering agent but also has the property to restore the normal lipid values in diabetic patients.

Cardiovascular diseases with an incidence of approximately 50% are the main cause of death in most advanced countries. Most people would benefit from lowering their blood pressure and cholesterol level. The underlying primary cause of cardiovascular disease is believed to be arteriosclerosis, a progressive multifactorial disease of the arterial wall. Central to the pathogenesis of arteriosclerosis is deposition of cholesterol in the arterial wall. *Aloe vera* (L) has a long history of both as an ornamental plant and for herbal medicine. Previous studies reported that *Aloe vera* also have hypocholestremic effect. The available drugs like statins and nicotinic acid, though very effective, have a spectrum of adverse effects and are costly. The reason for interest in *Aloe vera* (L.) was its low toxicity and the hope that it might be additive in action with other cholesterol lowering regimes.

Phenolic compounds are principle active compounds of medicinal plants and exhibit pharmacological effects that contribute towards human health. The presence of polyphenols in *Aloe vera* extracts and their antioxidant and antimycoplasmic activities offer motivating results that suggest the potential feasibility of using *Aloe vera* leaf skin and flowers in the health food and general food industries, or as an ingredient in other products, as well as their possible applications in the pharmaceutical industry.

Anti-oxidants commonly present in plants, such as phenolic compounds and saponins, are known to reduce hyperlipidaemia in diabetes. Preliminary phytochemical screening revealed the presence of phenolic compounds and saponins in the gel extract of *A. vera*. Thus, the anti-oxidants present in the *A. vera* extract may be responsible, in part, for the antihyperlipidaemic effect of the gel extract. In addition to the anti-oxidant potential, the hypoglycaemic effect of the gel extract may be implicated as the major reason for the observed antihyperlipidaemic effect of the extract

(i) The level of glycaemic control is the major determinant of total cholesterol, VLDL-C and triglyceride levels.

(ii) Improved glycaemic control following sulphonyl urea therapy decreases the levels of serum VLDL-C and total triglycerides.

There is ongoing research to isolate and characterize the bioactive compound(s) responsible for the anti-hyperlipidemia action in this crude extract and to use the compound(s) in a bioassay directed experiment. Identification of this Phenolic compound was done through HPLC which are present in *Aloe vera* gel like Kaempferol, Apigenin and Quercetin. Previous studies reported that these compounds are responsible for anti hyperlipedemic activity.

Sample collection and preparation

The plant material of *Aloe vera* collected from the Jayantikunj campus (authorized botanical garden) and Identified by the botany division of APS University.

To prepare the samples for the HPLC quantification, the freeze-dried plant material (50 mg) and 2 mL of methanol were mixed and homogenized using a vortex for 30 s. The mixture was stirred in a rotator for 60 min at room temperature in darkness. After centrifugation at 7,000 ×*g* for 20 min at 4 °C, the supernatant was collected and evaporated. The dry residue and 0.5 mL of water were mixed and filtered through a 45 μm nylon syringe filter prior to injection.

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AT pump and Shimadzu SPD-20AV absorbance detector. Samples were injected through a Rheodyne 7725 injector valve. The separation was performed with a reverse-phase Sigma-Aldrich C18 (250 mm × 4.6 mm, 5 micrometers (μm)) column. A gradient system, involving two mobile phases, was used. Eluent A was water with 0.1% formic acid and eluent B, methanol. The flow rate was 1.0 mL/min, and the injection volume was 60 μL of crude extracts. The system operated at 27 °C. The elution conditions applied were: 0-4 min, linear gradient from 20% to 30% B; 4-10 min, 30% B isocratic; 10-13 min, linear gradient from 30% to 50% B; 13-15 min, linear gradient from 50% to 80% B and finally, washing and re-conditioning of the column. Monitoring was set at 254 nm for quantification.

Method-

To quantify the compounds quercitrin, kaempferol and apigenin in the extracts, five different concentrations of the analytes were injected in triplicate. The calibration curves were constructed by plotting the peak areas versus the concentration of each analyte. The linearity was assessed by linear regression analysis, which was calculated by the least squares method. Each point on the calibration plot was the mean of two area measurements. All correlation coefficients were over .9976 (Table 1). The wavelength was fixed at 254 nm for quantification. The selectivity of the method was determined by analysis of standard compounds and samples.

Results

The presence of polyphenols in the extracts was confirmed by comparing retention times (RT) and overlapping UV spectra with those of standard compounds. The phenolic compounds quercitrin (RT: 7.6 min), kaempferol (RT: 10.8 min) and apigenin (RT: 11.3 min) were well resolved. Limits of detection (LOD) and limits of quantification (LOQ) were estimated from signal-to noise-ratio of the individual peaks, assuming a minimum detectable signal-to-noise level of 3 and 10 respectively. The LODs were found to be in the range of 0.032-0.0977 $\mu g \cdot mL^{-1}$ and the LOQs were observed in the range of 0.106-

0.355 μg·mL^{-1} (Table 1). This indicated that the proposed method offers adequate sensitivity for the quantification of polyphenols. Reproducibility, expressed as the relative standard deviation (RSD), was obtained by analyzing six replicate samples containing 20 μg·mL^{-1} of each of the four compounds. The accuracy was expressed as the recovery of standard compounds added to the pre-analysed sample. The results are summarized in Table 1.

Table 1. Method validation data for the quantitative determination of three phenolic Compounds using RP-HPLC.

Compounds	Regression equation (r)	LOD [a] μg·mL−1	LOQ [a] μg·mL−1	Recovery [b] (%)	RSD [c] (%)
Quercitrin	y = 88302x − 20416 (0.9976)	0.0364	0.1235	116 ± 4	3.69
Kaempferol	y = 15436x − 28177 (0.9998)	0.09775	0.3258	105 ± 8	7.61
Apigenin	y = 20306x − 67494 (0.9993)	0.03196	0.1065	99 ± 3	2.66

[a] Detection limits are calculated as signal to noise ratio of ten times; [b] Means ± standard deviation of three measurements; [c] Reproducibility was obtained by analyzing six replicate samples containing 20 μg·mL−1 for every standard.

So, we identify three compounds (Quercitrin, Kaempferol and Apigenin) through Reverse-phase HPLC in compare to standard of these compounds.

Future prospects-We need to be the further study, for determination the comparatively effect of these components for anti hyperlipidaemia.

(For the year 2013-14)

- **Section-C: Details of Grant Utilization**
- Amount sanctioned by MP Biotechnology Council Rs. 12, 62,000
- Amount released by MP Biotechnology Council Rs, 3, 56,000
- Details of expenditure

RECURRING

S. No	Activities undertaken	Period	Amount Sanctioned	Amount Utilized (Itemize)	Unspent Balance
1.	Manpower		96,000	96,000	Utilized
2.	Chemicals and Glasswares		2,00,000	1,98,263	1737
3.	Contingencies		30,000	31,540	(-)1540
4.	Travel		30,000	-	30,000
			3,56,000	3,25,803	30,197
				Total Unspent balance	30,197

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Justification- Total unspent balance from the year 2011-12 (31,540/-) has been added to final year 2013-14 recurring expenditure. So, the excess amount of Rs. 1540/- adjusted to the total expenditure and the total unspent balance in the year 2013-14 is Rs. 30,197/-.

Certified that the grant has been utilized for the purpose for which it was sanctioned in accordance with the "Terms and Conditions" attached to the grant. If, as a result of check or audit objection some irregularity is sanctioned at a later stage, action will be taken to refund, adjust or regularize the amount objected to.

Principal Investigator