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ORIGINAL ARTICLE

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# Formulation and Pharmacological Evaluation of Bark Extract of Albizia Odoratissima (L.F) Benth

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Albizia Odoratissima has been used in folk medicines for the treatment of Diabetes. To substantiate this claim the present studies have been undertaken in order to evaluate the Antihyperglycemic activity of Alcoholic extract of bark of Albizia odoratissima (AlEAO) and its Aqueous extract of bark of Albizia Odoratissima (AqEAO). These two fractions was assessed for hypoglycaemic activity on alloxan induced (150mg/kg) swiss albino rats. The $LD_{50}$ of the extracts were found to be 1000mg/kg.The extract powder are formulated as conventional dosage form by direct compression technique using polymer HPMC K4M and CMC Sodium in the concentration range of 5%. The tablets are evaluated with alloxan induced diabetic rats. The antidiabetic activity was compared with that of the reference drug glibenclamide (5mg/kg). The AlEAO fraction produces the significant reduction ($P<0.001$) in BGL (Blood Glucose Level) after singe administration (Dose: 100mg/kg, Route: Oral) for 10 days prolonged treatment. The BGL was measured using Glucometer on the $0^{th}$, $3^{rd}$ and $10^{th}$ day. The AlEAO shows the significant hypoglycaemic activity compared to that of the standard drug.

*Keyword:* Albizia odoratissima, Direct compression, Glibenclamide, Alloxan.

**INTRODUCTION:** In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. Diabetes mellitus is caused due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. It is a global problems and number of those affected is increasing day by day. The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes. The main objective of this study was to focus on the anti-diabetic activity of Albizia odoratissima with special reference to its putative protective role in alloxan-induced diabetes animal model. Herbal drugs play an important role in the treatment of diseases. Numerous medicinal plants and their formulations are used for various disorders in

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ethno-medicinal practices as well as traditional systems of medicines in India. Since pre-historic days attempts have been made to find out suitable drugs from natural sources for treatment of different diseases. The rational approach on the experience of folk medicines provides a valuable approach in the search for the development of new and useful therapeutic agents. Diabetes mellitus ranks among top ten disorders causing mortality throughout the world. With the rapid advancement of medicine, a treatment without side effects for the long-term control of this disorder has become important. Alternative therapies have also received attention recently. A growing public interest in herbal medication for diabetes has been in the raise around the world. Application of medicinal plants in the control of diabetes has renewed and the WHO expert committee on diabetes recommended such as alternative treatment. During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries a large proportion of the population relies heavily on traditional practices and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complimentary therapies, including medicinal herbs. Although there are numerous traditional medicinal plants reported to have hypoglycemic and antidiabetic properties, many of them proved to be not very effective in lowering glucose levels in severe diabetes. Further, most of the hypoglycemic agents used in allopathic medicines are reported to have side effects in long term. Therefore, there is a need to search effective and safe drugs for diabetes. Herbal drugs are prescribed widely even when their biologically active compounds are unknown, because of their effectiveness, less side effects and relatively low costs. The objective of the present study was to develop the herbal formulation of the plant Albizia Odoratissima which has anti diabetic activity. The herbal formulation was more reliable with the less risk of side effects than compared to allopathic system of medicine on continued therapy. The reason for selecting Albizia Odoratissima for this work was that no such activity has been reported individually in this plant. So based on the literatures collected this plant was selected for the formulation of conventional dosage of herbal tablet used for the treatment of Diabetes mellitus.

## EXTRACTION OF PLANT

The collected and powdered plant bark is subjected to extraction and the results of the extraction is with different solvent system and their percentage yield has been listed below

## FORMULATION OF TABLETS

### Selection of tablet punching method

While selecting the tableting methodology, compressible characteristics of the drug are to be considered. For drugs, which are poorly compressible and have moderate to high dose the most obvious and direct approach would be to follow wet granulation methodology.

For drugs with low to moderate doses, direct compression technique offers various advantages to the pharmaceutical formulator in terms of

- Economy, because less number of processing step, persons and time is required.
- Stability, because product is not required to expose to a moisture and heat.

- Performance, since tablets will directly disintegrate gives higher dissolution.

In the present study, the direct compression technique was employed to prepare conventional dosage form of both alcoholic and aqueous extract tablets.

## Selection of Excipients

Excipients are critical to the design of any drug delivery system and play a major role in determining its quality and performance.

The following recipient was selected for the formulation of alcoholic and aqueous extract tablets.

**Diluents**

Insoluble diluent was rejected because they result into unacceptable grittiness. Soluble diluents, preferably sugars, were selected so as to give pleasant feel while the tablets disperse.

Sugars used in the present work is

1) Micro crystalline cellulose (MCC)

**Table No 1 Extraction of Plant**

| Solvents used for extraction | Temperature maintained | Method of extraction | Percentage yield |
|---|---|---|---|
| Petroleum ether | 60-70 ºC | Continuous hot percolation process | 1.68% |
| Chloroform | 55-60 ºC | " | 1.52% |
| Acetone | 55-60 ºC | " | 5.74% |
| Alcohol | 75-80ºC | " | 19.03% |
| Aqueous | Room temperature | cold maceration process | 11.25% |

**Disintegrants:**

To produce better disintergration of drug from the solid dosage form to produce the onset of action quick and faster rate the disintegrants are to be used. Disintegration time with good dispersibility is the most important characteristics of a conventional dosage form.

In the present study the sodium carboxy methyl cellulose (CMC Sodium) grade was used as disintegranting agent at the concentration range of 5%.

## Binders

Binders are used to produce the mechanically strong tablets, having good integrity and low

friability. In the present study the hydroy propyl methyl cellulose (HPMC) grade K4 M was used as binding agent at the concentration range of 5%.

### Lubricants/Glidants

Lubricants are intended to reduce the friction during compression and ejection of tablets. In the present study, aerosol was used as lubricants/glidants.

## FORMULATION OF CRUDE ALCOHOLIC AND AQUEOUS EXTRACTS TABLETS.

### Formulation of crude alcoholic and aqueous tablets using direct compression method.

Weighed amount of HPMC K4 M, Microcrystalline cellulose, CMC Sodium, bronopol, and vanilla (flavoring agent) was added separately to weighed amoumt of crude alcoholic and aqueous extracts accurately. All the materials were passed through 40 # screen prior to mixing. Then add the remaining excipient aerosil and mix well and pass through 80 # screen. The resulting bulk was compressed into tablets using rotary tablet machine.

**Table no: 2 FORMULAS**

| **INGREDIENTS** | **FORMULATIOMS** | |
|---|---|---|
| | **F1** | **F2** |
| Alcoholic extract | 100 | -------- |
| Aqueous extract | --------- | 100 |
| HPMC K4M | 12.5 | 12.5 |
| Carboxy methyl cellulose sodium | 12.5 | 12.5 |
| Bronopol | 0.125 | 0.125 |
| Micro crystalline cellulose | 120.125 | 120.125 |
| Aerosil | 1 | 1 |
| Vannila | 3.75 | 3.75 |
| TOTAL WEIGHT | 250 mg | 250 mg |

Where,

F1 – Alcoholic Extract tablet

F2 – Aqueous Extract tablet

**Table No 3. Preformulation studies of crude extracts and polymers**

| Name of the powder | Bulk Density in (g/ml) | Tapped Density in (g/ml) | Angle of Repose (in °) | % Compressibility | Hausner's ratio |
|---|---|---|---|---|---|
| Pure Alcoholic extract | 0.6004±0.012 | 0.6825±0.015 | 23.68±0.042 | 11.86±0.33 | 1.136±0.003 |
| Pure Aqueous extract | 0.5173±0.007 | 0.6127±0.012 | 25.38±0.97 | 15.54±0.27 | 1.183±0.003 |
| HPMC K4M | 0.2970±0.002 | 0.3409±0.003 | 29.14±0.36 | 12.83±1.87 | 1.148±0.024 |
| MCC | 0.2803±0.003 | 0.3335±0.006 | 30.31±0.35 | 15.90±1.01 | 1.189±0.014 |
| CMC SODIUM | 0.2326±0.001 | 0.2703±0.004 | 31.34±0.32 | 14.73±0.77 | 1.161±0.002 |

**Table No 4. Preformulation studies of blend powder**

| Name of the parameters | Name of the Blend | |
|---|---|---|
| | Alcoholic Extract | Aqueous Extract |
| Bulk density (g/ml) | 0.4004±0.014 | 0.4126±0.015 |
| Tapped density (g/ml) | 0.4760±0.018 | 0.4810±0.020 |
| Angle of Repose (in °) | 24.42±0.45 | 25.01±0.54 |
| % Compressibility | 15.96±0.38 | 14.76±0.41 |
| Hausner's ratio | 0.4768±0.007 | 1.194±0.009 |

## PREFORMULATION

### Preformulation studies of crude extracts and polymers

Preformulation study was done initially and results directed for the further course of formulation. The preformulation studies include the test like Bulk density, Tapped density, Compressibility index, Hausner's ratio and Angle of repose.

### Preformulation studies of blend powder

Based on the Preformulation studies both alcoholic and aqueous tablet batches were prepared using selected excipients. Blend were prepared and evaluated for tests like Bulk density, Tapped density, Compressibility index, Hausner's ratio, Angle of repose before punching of tablets

## EVALUATION OF FORMULATED TABLETS

The prepared or compressed conventional tablet of both alcoholic extract and aqueous extract are subjected to physio-chemical evaluations like weight variation, hardness, thickness, friability, uniformity of weight and disintegration. The results of these studies are listed below in the table.

**Table No 5 Evaluation of Formulated Tablets**

| S/NO: | Parameters | Formulations | |
|---|---|---|---|
| | | Alcoholic extract tablet (F1) | Aqueous ext tablet (F2) |
| 1. | Weight variation | Passes | Passes |
| 2. | Hardness $Kg/Cm^2$ | 4.43 ± 0.03 | 4.21 ± 0.03 |
| 3. | Thickness mm | 4.4 ± 0.06 | 4.35 ± 0.06 |
| 4. | Friability (in %) | -0.44 | -0.42 |
| 5. | Uniformity of weight | +ve = 1.1 mg<br>-ve = 1.278 mg | +ve = 1.28 mg<br>-ve = 1.18 mg |
| 6. | Disintegration time (in mins) | 3.16 ± 0.032 | 3.16 ± 0.035 |

**Table No. 6 Anti-hyperglycemic effect of alcoholic extract and aqueous extract tablets of Albizia odoratissima on alloxan induced diabetic rats**

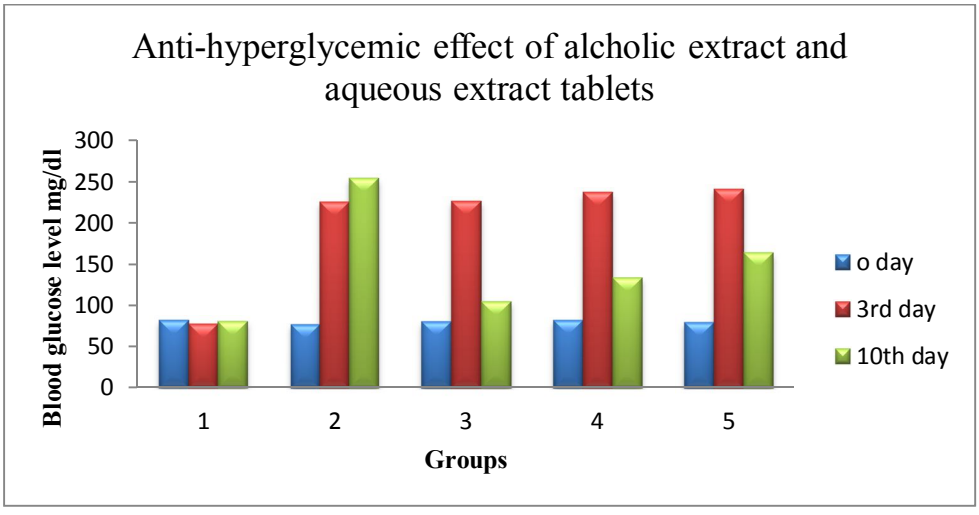
| S/No | Groups | Blood glucose level mg/dl | | |
|---|---|---|---|---|
| | | 0 day | 3rd day | 10th day |
| 1 | 1-normal saline | 81.16±0.91 | 76.80±0.90 | 80.00±1.81 |
| 2 | 2- toxic control alloxan(150mg/kg) | 77.30±1.33 | 225.2±4.05 | 253.80±4.9 |
| 3 | 3- standard glibenclamide(5mg/kg) | 80.60±1.76 | 227.0±4.04 | 105.16±4.37 |
| 4 | 4- alcoholic extract tablet(100mg/kg) | 82.50±1.89 | 237.0±6.63 | 133.83±3.92 |
| 5 | 5- aqueous extract tablet(100mg/kg) | 80.00±1.23 | 242.0±5.05 | 164.33±3.36 |

Values are expressed as Mean ± SEM of 6 rats in each group.

$^{z}p < 0.001$, as compared to Normal group.

$^{c}P < 0.001$, as compared to diabetic control group.

**Figure no: 1. Anti-hyperglycemic effect of alcoholic extract and aqueous extract tablets**



## PHARMACOLOGICAL ACTIVITY

### Invivo antidiabetic activity

The invivo anti diabetic activity was performed and the alcoholic extract shows more activity compared to aqueous extract tablet of bark of albizia odoratissima.

### Invitro anti-diabetic activity

### α- Amylase inhibition activity of alcoholic and aqueous extract tablets of bark of Albizia Odoratissima

There are many enzymes in the human digestive system that help in the digestion of food. α-Amylase catalyses the breakdown of polysaccharide (Starch) in to monosaccharide (glucose) and only monosaccharide form of food only can absorbed in the stomach. It is known that the degradation of starch to glucose in the alimentary canal proceeds rapidly. A few minutes after the ingestion of starch a marked hyperglycemia leading to hyperinsulinaemia is observed. Both phenomena are undesirable in patient part of GIT. The α- Amylase enzyme which is present in different part of GIT is responsible for the metabolism or digestion of starch and carbohydrate into glucose molecule.

As the concentration of α- Amylase increases the rate of reaction is also increases but the time of reaction decreases because of high concentration of α- Amylase will digest the starch rapidly. Glibenclamide is a α- Amylase inhibitory agent as the concentration of glibenclamide increase the time of reaction is also increase because the number on enzyme molecule required for digestion of starch in not in sufficient.

The present study deals with the inhibition of α-Amylase by alcoholic and aqueous extract tablets of bark of Albizia Odoratissima. Both the extract tablet of bark having α- Amylase inhibition activity which is shown by increase in reaction time i.e the time taken by α- Amylase to digest the starch. From the observation it was found that as the concentration of extract increase the time of reaction is also increase but as compare to standard drug they have little activity, have been presented in table no 20-23

**Table No: 7 Control tube of amylase solution**.

| TUBE | AMYLASE SOLUTION | BUFFER SOLUTION Ph 6.8 | TIME UNTIL STARCH DISAPPERAR (in min) |
|---|---|---|---|
| 1 | 1 ml tube 1 + 0.5 ml starch solution+2% amylase solution | 20 drops | 7.5 |
| 2 | 1 ml tube 1 + 0.5 ml starch solution+1% amylase solution | 20 drops | 11.5 |
| 3 | 1 ml tube 1 + 0.5 ml starch solution+0.5% amylase solution | 20 drops | 12.5 |
| 4 | 1 ml tube 1 + 0.5 ml starch solution+0.25 amylase solution | 20 drops | 14.5 |

**Fig No. 2: Control tube of amylase solution.**

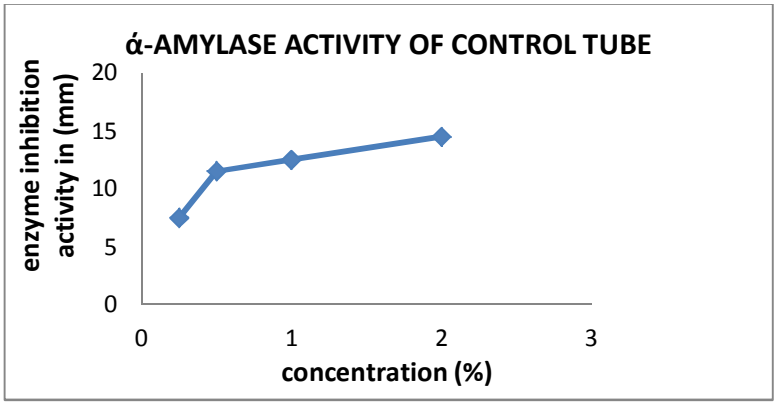



**Table No 8. Observation of standard drug (Glibenclamide) on α-amylase inhibition**

| TUBE | AMYLSE SOLUTION | BUFFER SOLUTION PH 6.8 | TIME UNTIL STARCH DISAPPEAR (in min) |
|---|---|---|---|
| 1 | 1 ml tube 1 + 0.5 ml starch solution+2% amylase solution+2% standard drug solution | 20 drops | 24.75 |
| 2 | 1 ml tube 1 + 0.5 ml starch solution+1% amylase solution+1% standard drug solution | 20 drops | 19.75 |
| 3 | 1 ml tube 1 + 0.5 ml starch solution+0.5% amylase solution+0.5% standard drug solution | 20 drops | 17.50 |
| 4 | 1 ml tube 1 + 0.5 ml starch solution+0.25 amylase solution+0.25% standard drug solution | 20 drops | 14.25 |

As the concentration of α-amylase increase the rate of reaction is also increase but the time of reaction decrease because of high concentration of α-amylase will digest the starch rapidly

Glibenclamide is an antidiabetic drug which has α-amylase inhibition activity. As the concentration of Glibenclamide increase the time of reaction is also increase because the number of enzyme required for digest for starch is not sufficient

**Fig no. 2: Observation of standard drug (Glibenclamide) on α-amylase inhibition**

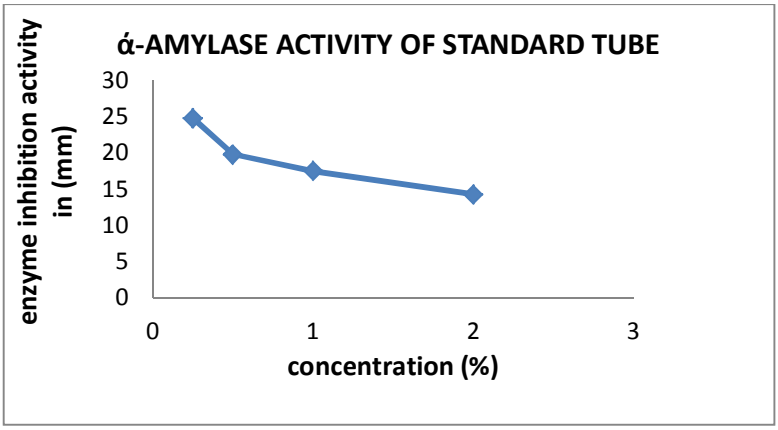



**Table No: 9 Observation of aqueous extract of bark of Albizia Odoratissima on α-amylase inhibition activity**.

| TUBE | AMYLASE SOLUTION | BUFFER SOLUTION Ph 6.8 | TIME UNTIL STARCH DISAPPERAR (in min) |
|---|---|---|---|
| 1 | 1 ml tube 1 + 0.5 ml starch solution+2% amylase solution+2% AQU.EAO solution | 20 drops | 17.25 |
| 2 | 1 ml tube 1 + 0.5 ml starch solution+1% amylase solution+1% AQU.EAO solution | 20 drops | 13.60 |
| 3 | 1 ml tube 1 + 0.5 ml starch solution+0.5% amylase solution+ 0.5% AQU.EAO solution | 20 drops | 9.45 |
| 4 | 1 ml tube 1 + 0.5 ml starch solution+0.25 amylase solution+0.25% AQU.EAO solution | 20 drops | 6.75 |

**Fig no.3: Observation of aqueous extract of bark of Albizia Odoratissima on α-amylase inhibition activity.**

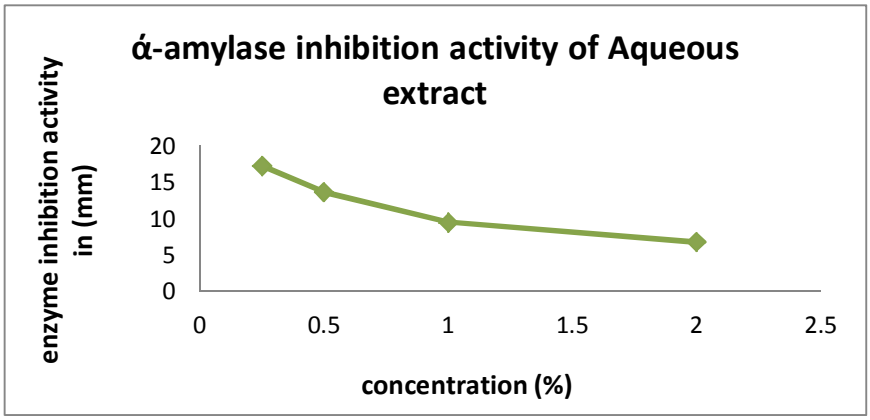



**Table No: 10 Observation of alcoholic extract tablet of bark of Albizia Odoratissima on α-amylase inhibition activity**

| TUBE | AMYLASE SOLUTION | BUFFER SOLUTION Ph 6.8 | TIME UNTIL STARCH DISAPPERAR (in min) |
|---|---|---|---|
| 5 | 1 ml tube 1 + 0.5 ml starch solution+0.25 amylase solution + 0.25% ALC.EAO solution | 20 drops | 7.75 |
| 6 | 1 ml tube 1 + 0.5 ml starch solution+0.5% amylase solution+ 0.5% ALC.EAO solution | 20 drops | 12.25 |
| 7 | 1 ml tube 1 + 0.5 ml starch solution+1% amylase solution+1% ALC.EAO solution | 20 drops | 16.50 |
| 8 | 1 ml tube 1 + 0.5 ml starch solution+2% amylase solution+2% ALC.EAO solution | 20 drops | 2O.80 |

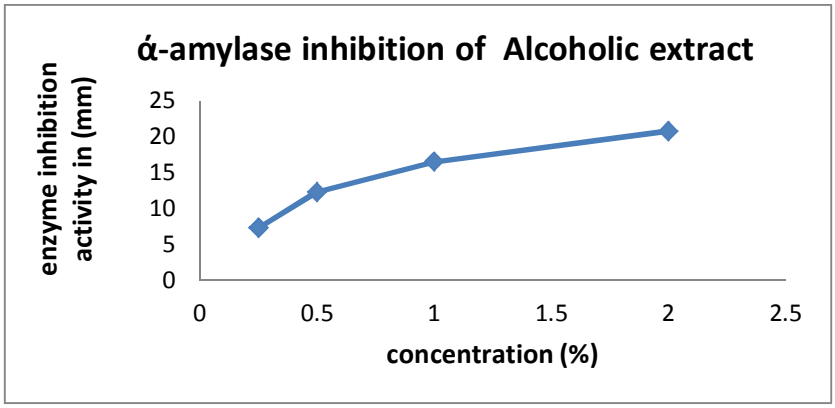
Aqueous extract tablet of bark of Albizia Odoratissima having α-amylase inhibition activity. From observation it was found that as the concentration of extract increases the time of reaction is also increases but as compared to standard drug they have some less activity.

From the observation it was found that the Alcoholic extract tablet of dried bark of Albizia

Odoratissima having the α-amylase inhibition activity. But as compare to standard drug is less activity but compare to Aqueous extract is having more activity.

It was clearly evident of amylase inhibition action of both alcoholic ether and aqueous extract given a clue of antidiabetic action in this plants bark extract of Albizia Odoratissima. This evident was the basic clue for these extracts for further research in-vivo experimental use on animals.

**Fig no: 4 Observation of alcoholic extract tablet of bark of Albizia Odoratissima on α-amylase inhibition activity**



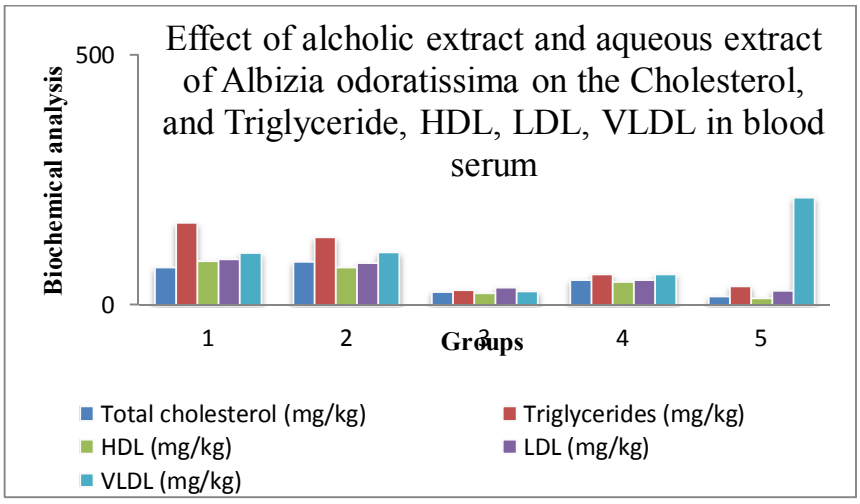

## BIOCHEMICAL ANALYSIS

**Table No 10: Effect of alcoholic extract and aqueous extract of Albizia odoratissima on the Cholesterol, and Triglyceride, HDL, LDL, VLDL in blood serum**

| S/No | Groups | Total cholesterol (mg/kg) | Triglycerides (mg/kg) | HDL (mg/kg) | LDL (mg/kg) | VLDL (mg/kg) |
|---|---|---|---|---|---|---|
| 1 | 1-normal saline | 75.5±0.22 | 87.33±0.33 | 27.66±0.21 | 50.33±0.21 | 18±0 |
| 2 | 2- toxic control alloxan(150mg/kg) | 164.83±0.30 | 135.5±0.22 | 30.67±0.33 | 61.8±0.17 | 38±0.25 |
| 3 | 3- standard glibenclamide(5mg/kg) | 87.83±0.16 | 75.33±0.21 | 24.83±0.16 | 47.5±0.22 | 15±0 |
| 4 | 4- alcoholic extract tablet(100mg/kg) | 92.66±0.21 | 84.33±0.21 | 35.83±0.16 | 51.33±0.33 | 30.16±0.16 |
| 5 | 5- aqueous extract tablet(100mg/kg) | 104.33±0.21 | 105.33±0.33 | 28.33±0.21 | 62.66±0.21 | 215. ±0.22 |

Values are expressed as Mean ± SEM of 6 rats in each groups control group.
$^{z}p < 0.001$, as compared to Normal group.
$^{c}P < 0.001$, as compared to diabetic control group.

**Fig no 5: Effect of alcholic extract and aqueous extract of Albizia odoratissima on the Cholesterol, and Triglyceride, HDL, LDL, VLDL in blood serum**




## HISTOPATHOLOGY STUDIES

**Figure – pancreas**

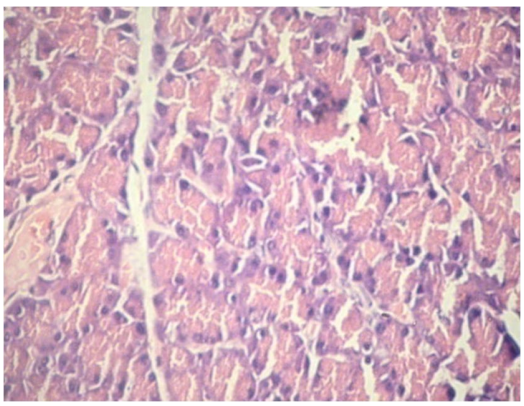


**Fig. No 6: Normal Control**

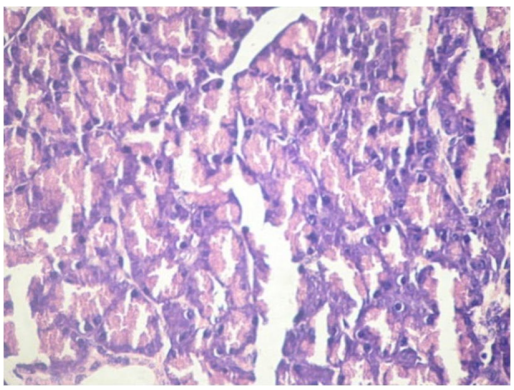


**Fig No 7: Diabetic Control**

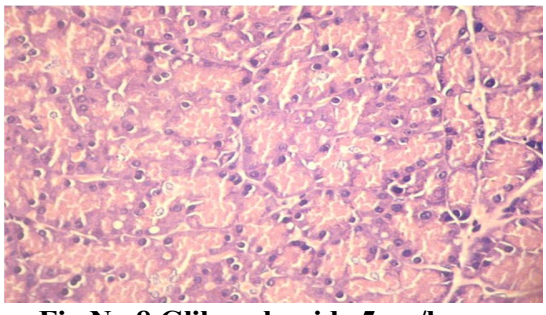


**Fig.No 8 Glibenclamide 5mg/kg**

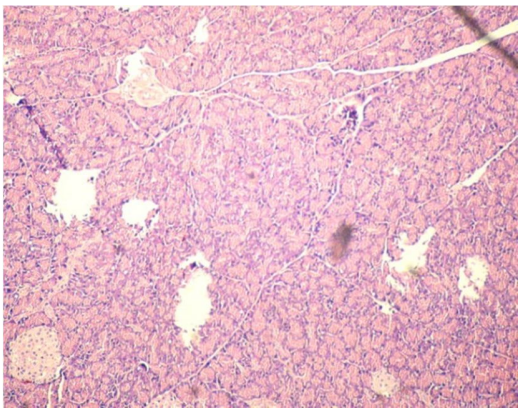


**Fig.No 9 100 mg/kg alcoholic extract tablet Albizia odoratissima**

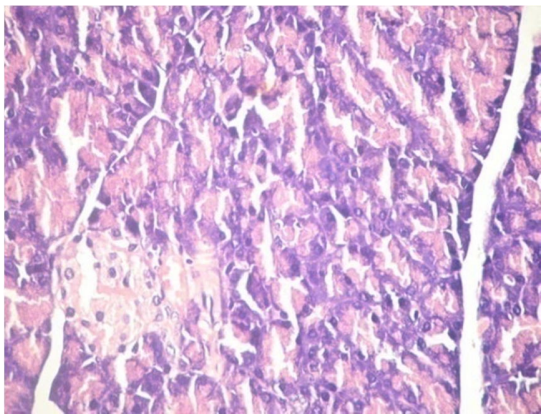


**Fig.No 10 100 mg/kg aqueous extract tablet Albizia odoratissima**

## SUMMARY & CONCLUSION

Traditional medicines that are derived from medicinal plants are used by about 60% of the world's population. Many Indian herbal drugs are used in the treatment of diabetes. In India it is exposing as to be major health problem especially in the urban areas, one of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence an anti-diabetic compound with antioxidant properties would be more beneficial. Despite the great strides that have been made in understanding and management in this disorder, serious problems like diabetic retinopathy diabetic nephropathy and lower extremity amputation continue to discomfort the patients and the physicians. The present study was undertaken with an aim to formulate and evaluation of conventional dosage tablet of the herbal medicine *Albizia odoratissima* for the treatment of diabetes mellitus.After the extraction of the plant *Albizia odoratissima* with different solvent system and the phyochemical screening was performed and the alcoholic and the aqueous extraction product was selected for the further process of formulation and evaluation as the both have the properties for the treatment for diabetes mellitus. The aqueous extract and alcoholic extract product was subjected to acute oral toxicity studies as per OECD guidelines and the effective dose was fixed as 100mg.Preformulation study was done initially before formulation of conventional dosage form of both the extracts. Based on preformulation studies the alcoholic and aqueous extract tablets were prepared. The powder blends was evaluated for tests such as Angle of Repose, Bulk density, Tapped density, Compressibility ratio, and hausner's ratio before punching of tablets. The formulated and evaluated alcoholic and aqueous extract tablets was subjected to pharmacological evaluation such as invitro antidiabetic activity, invivo antidiabetic activity, histopathological studies of pancreas and serum analysis for estimation of Total Cholesterol, Triglycerides, HDL, LDL, and VLDL. Effect of alcoholic extract tablet of *Albizia odoratissima* on glucose, triglyceride, cholesterol profile status in alloxan induced diabetic rats was studied based on this potentiation to treat disorder like Diabetes mellitus may shows a ray for better protocol for future treatment. The efficacy of *Albizia odoratissima* in experiment showed significant

decrease in the blood glucose level, increase the antioxidant efficacy in alloxan induced diabetes. It was demonstrated that the oral administration of alcoholic extract tablet of *Albizia odoratissima* to alloxan induced diabetic rats is useful in controlling diabetes because there were significant positive changes in the biochemical and physiological parameter related to protein, carbohydrates and lipid metabolism. The strong antihyperglycemic effect observed in alloxan induced diabetic rats justified the use of *Albizia odoratissima* for the treatment of diabetic related complications is better compared to that of the aqueous extract tablet of *Albizia odoratissima* which possessing the same anti diabetic activity.From the results and discussion it was concluded that the formulated conventional tablet of Alcoholic extract tablet of *Albizia odoratissima* was an ideal or optimized formulation for the treatment of Diabetes Mellitus compared to that of Aqueous extract tablet of *Albizia odoratissima* . The alcoholic extract tablet produces the desired hypoglycemic effect same as that the standard Glibenclamide used for the treatment of Diabetes Mellitus.

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