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Polyherbal effects of Berberius lycium and Hedra helix on Alloxan monohydrate induced diabetes

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Abstract:

Diabetes mellitus is endocrinological disorder caused by insulin deficiency. This disease was present over million years ago. Diabetes main reason is the disturbance in release of the insulin by pancrease. Many scientific drugs are used now days like metformin etc. But these medications on the other hand had side effects too. So for this polyherbal drugs should be used having no side effects. When metformin is compared to the herb *Berberis lycium* it was observed that the alkaloid berberine was more effective than metformin. Hypoglycemic effects are assessed on normal rabbits and in those rabbits in which diabetes is induced by alloxan monohydrate. Then the combine effects of the extracts of the *Berberis lyceum* and *Hedera helix* on alloxan monohydrate induced rabbits was observed. After one week treatment blood glucose level was checked significant results observed. Previous data from different experimentation revealed that aqueous extract of polyherbal drug is more effective in reducing blood glucose level than methanolic herbal extracts or scientific medications. The main centre of attention of this review is to elaborate the polyherbal drug development for diabetes instead of medications like metformin.

Introduction:

An endocrinological disorder called as diabetes is caused by insulin deficiency. It effects how body responds to glucose a kind of sugar. In this disease pancrease is unable to produce insulin or is caused by improper use of insulin by the body. Different bits of DNA which affect insulin formation process in our body now found by the scientists.(Abdul Mannan *et al.*, 2014).

History:

Over three million years ago medical authors focused on a diseased condition having continuous urination, excessive thirst and weight loss. A fatal disease called as diabetes mellitus now a day was described earlier by the physicians of ancient time period. First person who investigated this disease along with other ailments was Ebers Papyrus. He wrote about this ailment around 1500 BC., He exhumed in graveyard of Egypt, Thebes in 1862 AD and in 1874 Egyptologist Georg Ebers published this. He

investigated many diseases especially reference for diabetes mellitus a diseased state of "too great emptying of the urine". Physician of the Egypt that time used fruit, wheat, grains to treat this condition. (Jacek Zajac *et al.*, 2010), (N. H. Aboelsoud. 2011).

Then at same time Indian physicians started observing that ants and flies were attracted towards the urine of the diabetic patient. Terms used for this condition were"madhumeha" or "honey urine. Patients suffering with "madhumeha" were experiencing extreme thirst and stinking breath. Clinicians of that time failed to distinguish polyuria and diabetes. The term diabetes was used firstly by Memphis inhabitant named Appolonius having Greek meaning "to pass through" (dia – through, betes – to go) around 230 BC. He and other his coworkers thought that this was kidney disease. (Papaspyros NS. 1964).

Diabetes mellitus and diabetes insipidus were differentiated first time by the Greek physician Aretaeus. Aretaeus mentioned about symptoms showed by the diabetic patient at initial stage and wrote about it in *On The Causes and Indications of Acute and Chronic Diseases*. Diabetes was considered as a rare disease by Roman physician. (Sanders LJ. 2002). According to Galen opinion diabetes was basically started by disturbance in the kidney and called it as diarrhea urinosa. (Medvei VC 1993).

Two of the Indian physicians, was formed to differentiate between the two types of diabetes mellitus by studying the lean individual suffering from diabetes at younger age. Sushruta and Charaka started focusing on diabetes in fifth century AD. Chinese physician Li Hsuan in seventh centuary AD stated that diabetic patients were suffered from boils and pulmonary infections. His opinion was that averting of sex and plum(wine) were the only cure for the diabetes. From 980- 1037AD Avicenna, or Ibn-Sina, Caliphs of Baghdad, wrote about diabetes in detail.. All the clinical symptoms like sweet urine ,increased appetite, were described by him. He also wrote about complications related to diabetes. (Medvei VC. 1993).

Renewal of Diabetes:

Some new aspects of diabetes was observed in Europe between sixteenth and eighteenth centuries. Paracelsus a Swiss physician observed white residues when urine was allowed to evaporate in the urine of the diabetic patient. He claimed that these white residues were salts and responsible for the excessive thirst as well as its deposition in kidneys of the diabetic patient. Then later in Oxford sweet taste of urine of diabetic patient was noticed by the Thomas Willis in 1670. Then later in eighteenth century Thomas Cawley suggested that pancrease is also playing its role in development of the diabetes. (Medvei VC. 1993).

The sweet taste of the urine of the diabetic patient was because of the presence of sugar. This was investigated by the Matthew Dobson (1713–1784) in his book *Experiments and Observations on the Urine in Diabetics.* The sweetness in the serum was also noticed by him. Dobson was the first one who gave the theory that the diabetes was a universal disease, somewhat different from the kidneys. (Medvei VC. 1993).

Discovery of Insulin:

With the modern scientific disciplines like physiology, biochemistry, molecular biology new aspects related to diabetes mellitus was seen in nineteenth century. Paris physician in 1815 Eugene Chevreul also observed that sweetness in urine of the diabetic patient was because of the sugar or glucose. Next to it quantitative test for urine was developed for glucose by in 1848 by Von Fehling. European Sorbonne University professors of physiology also worked on diabetes. One of the known physiologist was Claude

Bernard who worked in 1813–1878. Bernard showed great interest towards this disease and worked on physiology of gastrointestinal tract. He experimentally observed lots of things related to diabetes like ligation of pancreatic duct, degeneration of pancreas. Further investigations by him revealed pancreas is responsible for controlling glucose level. He observed and suggested that liver is the organ storing glycogen and then secreted sweet and sticky substance in the blood. He gave theory that over secretion of sugar leads to emergence of the disease called as diabetes.

In 1785-1850 a term diabetic coma was described by the William Prout. In 1857 Wilhelm Petters studied about the presence of acetone in diabetic patient's urine. John Rollo used term mellitus to differentiate diabetes from inspidus. (Schullian DM. 1965).

Main contributions towards this diabetes was of Banting and Best, who worked hard and finally found what is called as diabetes now. They experimented on laboratory model animals and also on a 14 years old boy too at Toronto General Hospital. Nobel prize was awarded to them on their hard work. New antidiabetic substance was found by Banting called as "isletin." MacLeod change this name later as "insulin". (Bliss M. Triumph. 1982) . Banting and Best was called as Dawn of insulin era. At the discovery of insulin strong enthusiasm was found around the world. Patients suffering from this disease now had a satisfaction for the cure of this disease. Firstly it was experimented on four childs. Ted Ryder was the one from the initial four children experimented in 1922 in Toronto with insulin. In 1993 he died at the age of 76. With the passage of the time new methods to prepare and purify insulin was discovered. In 1930s a long-acting insulin called as Protamine-zinc, was found. In 1940s Neutral Protamine Hagedorn (NPH) and in 1950s Lente series of insulin was discovered. (MacCracken J. 1997).

First witness for insulin introduction in clinical use was given by Portugese Ernest Roma, a physician. The world's first union for people with diabetes on returning was founded in Portugal – the *Portuguese Association for Protection of Poor Diabetics*. The main contribution of this was free of charge insulin delivery to poor.

Types of Diabetes:

It is of three types.

One is insulin dependant called as Type 1 diabetes , second one is non insulin dependant called as Type 2 diabetes, and third one called as Gestational diabetes GDM.

Type 1 Insulin Dependant (IDDM):

The first subclass of diabetes, type I or insulin-dependent diabetes mellitus (IDDM), is caused by the damage of pancreatic cells. Function of pancrease is unbalanced by destruction of cells which leads to the failure of insulin production. Increased or decreased frequency of specific histocompatibility antigens (HLA) on chromosome 6 is also due to the genetic effects. (Nerup, J. *et al.*, 1976). (Cudworth, A. G. *et al.*, 1976). This can be cured by insulin induction.

Type 2 Non Insulin Dependant (NIDDM):

NIDDM patients are not dependent on insulin and are not prone to ketosis. Due to the imbalancement of carbohydrate, fat, and lipid metabolism insulin secretion is decreased or declined in insulin action leads to hyperglycemia. Another disturbance caused by this is hyperlipidemia. (Mohammad Rahimi Madiseh. *et al.*, 2014).

NIDDM is also related to genes, which are stronger than in IDDM. This is concluded by the pattern of occurrence. High caloric diet is one of the cause of this type of diabetes. Tablets are used mainly to cure this type of diabetes like metformin, pioglitazone etc.

Gestational diabetes (GDM):

This type is present only in pregnant women. But the women diabetic before marriage or conceiving is not considered in this category. Newly diagnosed diabetes in pregnancy is considered as GDM. Fetal loss or miscarriages is the result of this type of diabetes. (O'Sullivan, J. M. *et al.*, 1971)

Prevalence:

Lipid and protein metabolism or break down is mainly affected in diabetes. Etiology of diabetes mellitus comprises of factors like mental tensions, inherited diseases, age, chubbiness, diet, gender, desk bound life style, hypertension.(Alam K and Mahpara S. 2003). In the present century the disease from which most of the population is suffering is the diabetes mellitus. Many number of people affected by diabetes is increasing day by day. According to an estimation it is to be 366 million by year 2030 worldwide. (Veeramani C. *et al* 2008). Diabetes mainly results due to the complications of cardiovascular diseases. (Carmena R. 2005).

Diabetes mellitus type 2 is considered as one of the risk factor affecting heart and blood vessels. High lipid profile and increase in triglycerides considered as important causative agents of diabetes mellitus. Sudden increase in free fatty acids (FFA) in the body leads to insulin resistance and lipid triad of diabetes. Reduction in triglycerides and free fatty acids is best to avoid from this disease. (Defronzo RA and Ferrannini E. 2005).

Excess of lipids in the blood stream leads to a condition called as hyperlipidemia. Atherosclerosis is caused by the high lipid profile in blood which eventually results in other cardiac disorders and diabetes too. (Goldstein JL. *Et al.,* 1973). According to recent consensus atherosclerosis leads to a state of sharp oxidative stress caused by oxidation of lipid and protein in the vascular wall. (Uemura S. *et al.,* 2001). Infact diabetes affect whole body systems.

Treatment:

All Type 2 diabetes medication have main focus on how to reduce the glucose level in body. Each drug acts and finally achieve target in a unique way. Each medication for diabetes mainly targets on a particular organ. Sometimes combination of these medicine is used to control the blood glucose level. These combinations targeted different organs of the body. Doctors prescribe a drug called as metformin usually. But if this primary medication is not working properly then doctors prescribe combination of medicines. Usually Sulfonylureas may be prescribed with metformin in such cases. Or other alternative medications like DPP-4 inhibitors or Actos is prescribed or given to patient.

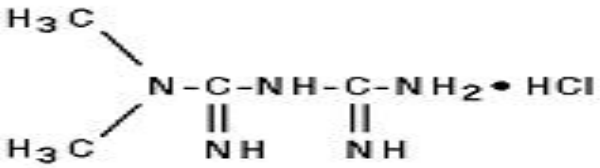
Classes of Diabetes Type 2 Medication:

Drugs that increase insulin sensitivity:

Insulin resistance leads to a disease called hyperglycemia. Hyperglycemia made the cell sensitive and less resistant to insulin,so by treating directly the blood glucose levels should be normalized or treated.

Glucophage (metformin):

Main drug used to treat diabetes mellitus is metformin.it has been in use since 1920s. It suppresses glucose in the blood. Metformin also lowers the fats level in the body.



Actos (pioglitazone): From the thiazolidinediones this drug is taken., Thiazolidinediones causes many cardiovascular problems. New researches revealed this drug also causes bladder cancer and heart stroke. Pioglitazone makes the muscle cells, liver cells and fat cells more sensitive to insulin.

Drugs Stimulating Production of Insulin by the Pancreas:

Pancreas produces maximum amount of insulin by these drugs.

Sulfonylureas and non-sulfonylurea secretagogues:

These include glipizide or Glucotrol, glyburide or Diabeta and many others. By the beta cells of the pancrease insulin is released which lowers the glucose level of the blood.

Drugs that Slow Digestion of Carbohydrates:

In hyperglycemia more amount of glucose entered in the blood. These drugs slows down the rate of sugar addition in blood is decreased.

Alpha-glucosidase inhibitors and amylin analogues;

Digestion of sugar is slowed by this drug.Side effects caused was nausea and flatulence.

Alpha-glucosidase inhibitors:

Least effective medications for lowering blood sugar are also present. Pramlintide is used for this purpose. Sometimes diabetes medications are combined for better efficacy like metformin with Non-Sulfonylureas, Sulfonylureas , Alpha-glucosidase inhibitors , Thiazolidinediones , Insulin. All these drugs affects our body's other organs so we have to focus on herbal medications.

Herbal Treatment for Diabetes:

People were using plants to cure diseases in ancient times. Popularity of these medicinal plants in tribal as well as urban areas are increasing day by day. As there are clear evidences that theses medicinal plant are unlimited in numbers and are present everywhere in the world. (Uemura S. *et al*., 2009).

These herbal medications considered as healthy source of life. Promising effects seen in different experimental and clinical researches for different diseases like atherosclerosis, diabetes, cancer, infection, gastrointestinal and central nervous system disorders. (Tyler VE. 1994).

These herbal medications are used to treat hyperlipidemia. (Farnsworth NR. 1998).

For treatment of different cardiovascular disorders and insulin resistance alternative therapies like functional foods and nutraceuticals are used. Low cardiovascular problems and lipid profile can be treated with Barberry extract in different concentrations and efficacy can be enhanced by changing concentrations. (Derosa G. *et al.,* 2013).

There are so many herbs used as home remedies to control diabetes for example *Acacia Arabica* (babhul) , *Allium sepa* (onion), *Helix hedra, Aloe vera*, *Vinca rosea* (sadabahar), *Capparis deciduas* (karir or pinju), *Allium sativum* (garlic*), Azadirachta indica* (neem), *Berberis lyceum* (Indian barberry), *Cinannomum zeylanicum* (cinnamon) etc. (Baby Joseph and D. Jini. 2011).

Berberis lyceum:

One of the natural vital source to cure human ailment is herbal treatment or the use of the herbs. In Pakistan many herbs are present which are used for healing purposes and their extracts as herbal medicines. Broad spectrum biological and pharmacological activities are shown by these flora, and then used for inflammation as well as bactericidal and fungicidal agents. (Cowan MM. 1999).

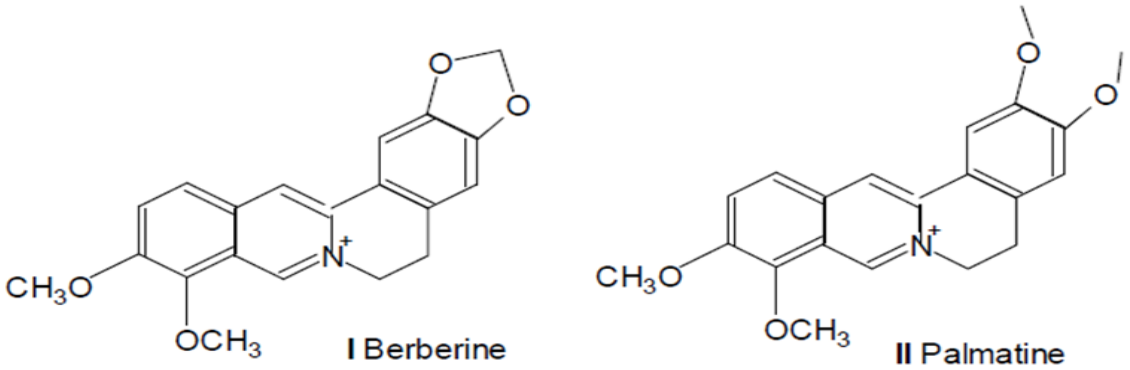
The organs of different plants like root, seeds, leaves, flowers are used to prepare different syrups and extracts. (Imtiaz UH and Manzoor H. 2003). A medicinal plant (*Berberis lyceum*) Ambaribis was named by Al Biruni. In Persian he used a term Zirkash for this herb. In 1837 John Forbes Royle commonly called this as Indian barberry in English and Kashmal or Ishkeen in Urdu. (Sabir S. *et al*., 2013). *Berberis lyceum* is a spiky plant .It belongs to Berberis genera and Berberidaceae family. It is distributed in the Asia, Europe and America. Majorly in semitropical and moderate areas. In Pakistan it is found at the elevation of 900 to 2900m in Northern areas like Azad Kashmir, Gilgit, Swat, KPK, Baluchistan. (Ali MN and Khan AA. 1978). (Ahmad M. *et al*., 2009).

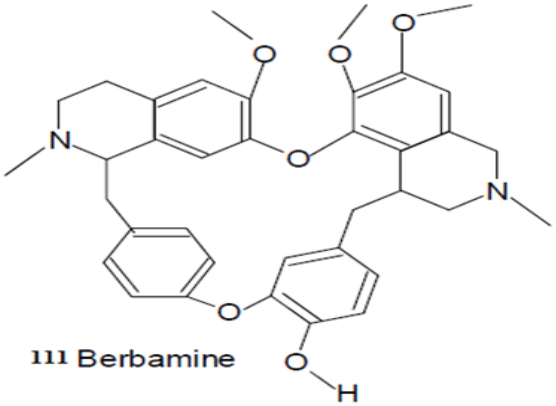
Berberis lycium is 3-4 meters in length and is vertical thorny plant. *Berberis lyceum* is surrounded by a fragile bark and having a solid stem. This plant is hermaphtodite. Pollination in this plant is self and also pollinated by the help of the insects. (Jafri SMH). (Irshad AH., *et al*., 2013). The branches of *Berberis lyceum* are slightly white to grayish having alternate thorns pattern on them. Leaves are in vibrant color. (Ahmad M and Alamgeer Sharif T. 2009). The blooming duration is from May to June. The flower is beautifully cupped shape in raceme pattern. Flower showing pale yellow color, and are larger in size than the leaves.(Malik TA., *et al*., 2013).

Fruit of this plant is oval in shape which show purplish or bright red color on maturation. The size of the fruit is 7 mm in length having 4 mm width and weighing 227 mg. 2-5 seeds are present in its fruit's purplish juice. Root is yellow in color and is stiff showing branched pattern. Root is smooth inside and outside rough and having width of 3mm.36.It is used against many diseases like arthritis, eye infections, PCO, common cold, tooth disease, ulcer, jaundice, loose bowels , UTI etc.

Components of Berberis lyceum:

Berberis lyceum comprises of many biologically significant alkaloids which are berberine, palmatine, berbamine, aromoline, oxyacanithine, umbellatine, ß-sitosterole, punjabine, balochistanamine, oxyberberine, berberine chloroform and palmatine chloroform. The major biological activitity is shown by an alkaloid named as berberine.





Berberine is used against different conditions and act as different agents like as anti-inflammatory agent, antidiabetic agent and is one of the main anti depressant. (Gulfraz M., *et al.,* 2008).

Antidiabetic activity of Berberine:

Its antidiabetic acivity reduces the blood glucose level in the model organisms. Effects of *Berberis lyceum* roots extract was studied in an alloxan induced diabetic rabbits. The glucose levels in blood of both diabetic and normal rabbits are decreased by powder of *Berberis lyceum*. Different herbal extracts was prepared in water, methanol, n-hexane and chloroform and antidiabetic activity is seen in alloxanized rabbits. Conclusion of these extract was that the aqueos extract of this herb show better efficacy as compared to other extracts. Other extracts like methanolic, aqueous methanolic and n-hexane extract also causes decline in glucose level but the time duration is small as compared to aqueous extract. No significant change was observed by the chloroform extract.(Ahmad M., *et al*., 2009). All this was helpful in determination of the insulin efficacy. (Gulfraz M., *et al*., 2007).

Berberine V/S Metformin::

Scientific drugs used nowadays for diabetes have side effects. To cope this herbal medication is preferable. Like pharmacologists carried out experimentation on active alkaloids of berberine and used instead of the guanidine in the metformin.

Guanidine was extracted from *Galgea officinalis* and used against diabetes. This plant is enriched in guanidine which is hypoglycemic component. But guanidine is toxic for medical use. To reduce this toxicity pharmacologists have to focus on berberine alkaloid. Hypoglycemic effects of berberine are same as that of metformin but later researches revealed that berberine is effective as compared to metformin.

Mechanism of Action of Berberine:

1.**Mimicking insulin action**[i]

Berberine enhances glucose uptake capability of 3T3-L1 adipocytes (fat cells) and L6 myocytes (muscle cells) thus mimicking the insulin. Berberine mimicking also inhibits activity of an important agent to depress insulin and leptin signaling. As mentioned earlier protein tyrosine kinase phosphatase 1B activity is also inhibited by this mimicking action of berberine on the other side increases phosphorylation in 3T3-L1 adipocytes. It improves impaired glucose tolerance in diabetic rats, but showing no effect on insulin release and synthesis. So berberine showed different kind of effects responsible for reduction of blood glucose level.

2.Improving insulin action by Activation of AMPK:

One of the enzyme playing important role in cellular energy or liveliness homeostasis is AMPK. The functions of this enzyme is to stimulate hepatic fatty acid oxidation , ketogenesis and also inhibits synthesis of cholesterol. This enzyme also inhibits lipogenesis , triglyceride synthesis. AMPK stimulates fatty acid oxidation in skeletal muscle cells, enhances glucose uptake by muscles. It also effects secretion of insulin by beta-cells of pancreas. (Winder WW and Hardie DG 1999).

AMPK acts as metabolic master switch and is present in brain tissues, liver tissues and skeletal muscles. AMPK acts regulates intracellular systems like absorption of glucose by the cells, the beta-oxidation of fats or fatty acids, and the glucose transporter 4 (GLUT4) biogenesis. (Viollet B. *et al.,* 2007).

3. Reducing insulin resistance through protein kinase C (PKC)-dependent up-regulation of insulin receptor (InsR) expression:

Berberine leads to induction of InsR gene expression. Berberine lowers fasting glucose level and serum insulin in animal models. Berberine elevated InsR mRNA in the animal model on ther hand also effecting PKC activity in the liver.(Zhang H and Wei J, *et al.*, 2009). (Kong WJ and Zhang H, *et al.*, 2009).

4. Inducing glycolysis:

Berberine inhibits the glucose oxidation in mitochondria by this increases the glucose metabolism. As it leads to the induction of the special type of enzyme AMPK this is also induced by mitochondrial inhibition eventually increasing the AMP/ATP ratio. (Yin J, Gao Z, *et al.*, 2008).

5. Enhancing GLP-1 secretion and modulating its release:

Intestinal L cells releases insulinotropic gut hormone called as GLP-1. After having meal beta cells of the pancrease secreted insulin this is also because of incretin tha is GLP-1. There are many drugs for this incretin but those drugs are toxic one. It is observed that berberine is one of the factor causing increase in GLP-1 in rats having diabetes induced by streptozotocin. Berberine shows its effects both in vitro and in vivo experiments respectively. Proglucagon mRNA appearance or expression and prohormone convertase 3 is promoted by berberine. So it is concluded that by enhancing GLP-1 secretion and biosynthesis of GLP-1 berberine changes GLP-1. (Yin J, *et al.,* 2008). (Lu SS, *et al.,* 2009).

6. Inhibition of DPP-4:

Berberine also shows anti-hyperglycemic activities. Docking experiments are performed to fit berberine in the DPP IV binding pocket. While it readily fits in the binding pocket of DPP IV in a low energy direction.DPP IV have electrostatic attractive interactions linking the nitrogen atom having positive charge in berberine (isoquinolinium) and the DPP IV negatively charge acidic residue of glutamic acid-205 (GLU205).

Berberine inhibits human recombinant DPP IV in vitro or in lab with Ic(50)=13.3micr M.So by this berberine also exhibits its anti hyperglycemic effect by DPP IV inhibition.(Al-masri IM *et al.,*2009).

7. Inhibition of hepatic gluconeogenesis:

Huanglian (*Coptis chinensis* French) a Chinese herbal medicine contains original natural berberine components. Berberine speed up the glucose breakdown in Type 2 diabetic patients. Liver shows reaction against berberine in the rats in which diabetes is induced by high fat diet. Berberine also causes decline in fasting glucose significantly. In liver berberine causes significant decrease in Gluconeogenic genes, Phosphoenolpyruvate carboxykinase (PEPCK) and Glucose-6-phosphatase (G6Pase). Berberine also lessen the chances of hepatic steatosis.
Forkhead transcription factor O1 (FoxO1), sterol regulatory element-binding protein 1c (SREBP1) and another protein called as carbohydrate responsive element-binding protein (ChREBP) was also effected by berberine. Berberine cause decrease in activity of these proteins. Berberine stops the oxygen consumption and causes reduction in adenosine triphosphate (ATP) in cellular level in the cultured liver cells. By the direct hang up of gluconeogenesis in liver it improves fasting blood glucose level and is independent of insulin action. Mitochondrial inhibition by berberine leads to inhibition in hepatic gluconeogenesis. By insulin-independent pathway berberine improves metabolism of the glucose. (Xia X *et al.,* 2011).

Polyherbal formulation of Drug for Diabetes:

Materials and Methods:

Plant Material:

Root of *Berberis lyceum* and leaves of *Hedera helix* washed with water to remove dirt. Then shade dried. By the help of grinder both root and leaves are grinded separately.

Preparation of aqueous and methanolic extracts:

For aqueous polyherbal solution both of the powder from the root of *Berberis lyceum* and leaves of *Hedra helix* mixed in 1L distilled water for 7days at room temperature. Then filtered and filtrate concentrated by rotary evaporator under vaccum to a semisolid mass. Methanolic polyherbal extract also made by using same procedure.

Experimental animlas:

For one week 42 local strain healthy rabbits (*Oryctolagus cuniculus*) weighing 1000-1500g kept under observation. Rabbits should fed normally with green grasses and vegetables before experimentation. The rabbits then divided into three major groups:

Group 1: control group having 6 rabbits.
Group 2: Non diabetic group having 18 rabbits. This group is further subdivided into three groups.
Group 2a: 6 rabbits tested for polyherbal aqueous extract.
Group 2b: 6 rabbits tested for polyherbal methanolic extract.
Group 2c: 6 rabbits tested for Metformin (oral hypoglycemic drug).
Group 3: Alloxan induced diabetic 18 rabbits. This group also further subdivided into three groups.
Group 3a: 6 rabbits for polyherbal aqueous extract.
Group 3b: 6 rabbits for polyherbal methanolic extract.
Group 3c: 6 rabbits for pioglitazone (oral hypoglycemic drug)

Alloxan monohydrate 150mg/kg is used to induce diabetes in rabbits. This alloxan is administered by injecting in rabbits marginal ear vein. One week before blood testing rabbits were made diabetic by *alloxan* monohydrate injections. Diabetic criteria for rabbits is having 250-400mg/dl blood glucose level .Group1 rabbits called as control group served with 20ml normal saline while both 2a,2b received both aqueous and methanolic polyherbal extracts respectively. Dose administered to 2a, 2b rabbits is 4g/kg body weight with 20ml of normal saline solution. Rabbits of group 2c treated with metformin 500mg/kg body weight with 20ml of normal saline solution. Group 3a, 3b rabbits received same doses but group 3c received pioglitazone with 20ml of normal saline solution. Blood glucose level of all rabbits recorded by Accu-Check easy glucometer at hour interval of 0, 2, 4, 8, 12 & 24. By Wet digestion and Dry ashing procedure hypoglycemic trace elements (Chromium, Mgnesium and Zinc) determined using flame photometry, SP 1991 PYE UNICAM Atomic Absorption Spectrophotmeter.

Results:

Both aqueous and methanolic polyherbal extract show anti diabetic effects in rabbits. And same with the oral medications administered. But the aqueous polyherbal extract show better efficacy as compared to methanolic polyherbal extract and metformin respectively.

Conclusion:

Metformin badly effects our body others systems so by focusing on herbal medications diabetes mellitus should be cured efficiently. Herbal medications like polyherbal extracts had no side effects. It may not harm our body's other systems so pharmacologist should focus on this side. Alkaloid namely berbrine present in the *Berberis lyceum* species found in Azad Kashmir have much more anti diabetic potential as compared to species present in other regions of Pakistan. So this should be one important factor used for controlling diabetes.

References:

Abdul Mannan Md, Beauty Akter Rupa, Md. Nur Kabidul Azam, Md. Nasir Ahmed, Md. Nazmul Hasan. A Quick Review on Anti-diabetic Plants and Action of Phytochemicals International Journal of Advanced Research (2014), Volume 2, Issue 5, 227-249.

N. H. Aboelsoud. (2012). Herbal medicine in ancient Egypt. Journal of Medicinal Plants Research Vol. 4(2), pp. 082-086.

Papaspyros NS. (1964). The history of diabetes. In: Verlag GT, ed. The History of Diabetes Mellitus. Stuttgart: Thieme; :4–5.

Sanders LJ, (2002). From Thebes to Toronto and the 21st century: an incredible journey. Diabetes Spect. 15; 56–60.

Medvei VC (1993). The Greco – Roman period. In:Medvei VC, ed. The History of Clinical Endocrinology: A Comprehensive Account

of Endocrinology from Earliest Times to the Present Day. New York: Parthenon Publishing; 34, 37.

Medvei VC. (1993). Mediaeval scene. In: Medvei VC, Ed. The History of Clinical Endocrinology: A Comprehensive Account of

Endocrinology from Earliest Times to the Present Day. New York: Parthenon Publishing; 46, 49.

Medvei VC. (1993). The 16th century and the Renaissance. In: Medvei VC, ed. The History of Clinical Endocrinology: A

Comprehensive Account of Endocrinology from Earliest Times to the Present Day. New York: Parthenon Publishing:55–56

Medvei VC. (1993). The 18th century and the beginning of the 19th century. In: Medvei VC, ed. The History of Clinical Endocrinology:

A Comprehensive Account of Endocrinology from Earliest Times to the Present Day. New York: Parthenon Publishing:97.

Schullian DM. John Rollo's patient. (1965). J Hist Med ;2:163–164.; 1

Bliss M. Triumph (1982). In: Bliss M, ed. The Discovery of Insulin. Chicago: University of Chicago Press: 104–128.

MacCracken J. (1997). From ants to analogues. Puzzles and promises in diabetes management. Postgrad Med; 4:138–150.

Nerup, J. et al., (1976). HLA, autoimmunity, and insulin-dependent diabetes mellitus. In The Genetics of Diabetes Mellitus. Berlin, Springer-Verlag,

Cudworth, A. G., and Woodrow, J. C. (1976). Genetic susceptibility in diabetes mellitus: analysis of the HLA association. Br. Med. J. 2; 846.

Mohammad Rahimi Madiseh, Esfandiar Heidarian, Mahmoud Rafieian-kopaei1, (2014). Biochemical components of Berberis lycium fruit and its effects on lipid profile in diabetic rat J HerbMed Pharmacol.; 3(1): 15-19.

O'Sullivan, J. M. et al., (1971). Treatment of verified prediabetes in pregnancy.J. Reprod. Med. 7(21).

Alam K, Mahpara S. (2003). Role of diet, nutrients, spices and natural products in diabetes Mellitus. Pak J Nutr; 2(1):1-12.

Veeramani C, Pushpavalli G, Pugalendi VK. (2008). Antihyperglycaemic effect of cardiospermum halicacabum Linn. Leaf extract on STZ-induced diabetic rats. J Appl Biomed 6:19-26.

Carmena R. (2005). Type 2 diabetes, dyslipidemia, and vascular risk: Rationale and evidence for correcting the lipid imbalance. Ame Heart J;150(5):859-70.

Defronzo RA, Ferrannini E. (2005). Insulin resistance: A multifaceted syndrome responsible for niddm, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care ;14(3):173-94.

Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG.(1973). Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest; 52(7):1544-68.

Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, et al,. (2001). Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. Circ Res; 88(12):1291-8.

Uemura S, Matos C, Moutinho C, Queiroz Gr, Gomes Lg R.(2009). Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern of Portugal). J Ethnopharmacol; 124(2):270-83.

Tyler VE. Herbs of choice. (1994). The therapeutic use of phytomedicinals. Binghamton: Pharmaceutical Products Press (imprint of Haworth Press, Inc.).

Farnsworth NR. (1998). Screening plants for new medicines. Chicago: Biodiversity.

Derosa G, Bonaventura A, Bianchi L, Romano D, D' Angelo A, Fogari E, et al., (2013). Effects of Berberis aristata/Silybum marianum association on metabolic parameters and adipocytokines in overweight dyslipidemic patients. J Biol Regul Homeost Agents; 27(3):717-28.

Baby Joseph and D. Jini. (2011). Insight into the Hypoglycemic Effects of Traditional Indian Herbs used in the Treatment of Diabetes.

Cowan MM. (1999). "Plant products as antimicrobial agents". Clinical microbiology reviews; 12.4: 564-582.

Imtiaz UH and Manzoor H. (2003). "Medicinal plants of Mansehra". Hamdard medicus 36: 69.

Sabir S., et al., (2013). "Phytochemical and antioxidant studies of Berberis lycium."Pakistan journal of pharmaceutical sciences 26: 1165-1172.

Ali MN and Khan AA. (1978). "Pharmacognostic studies of Berberis lycium Royle and its importance as a source of raw material for the manufacture of berberine in Pakistan". Pakistan Journal of Forest.

Ahmad M., et al.,(2009). "Effect of Berberis lyciumRoyle on lipid profile in alloxan induced diabetic rabbits". Ethnobotanical leaflets 13: 702-708.

Jafri SMH. "Berberidaceae". Flora of Pakistan.

Irshad AH., et al., (2013). "Antibacterial activity of Berberis lycium root extract". Trakia Journal of Sciences 11: 88-90.

Ahmad M and Alamgeer Sharif T. (2009). "A potential adjunct to Insulin: Berberis lycium Royle." Diabetologia Croatica 38 (2009): 13-18.

Malik TA., et al., (2013). "In vivoanticoccidial activity of berberine [18, 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3 benzodioxolo (5,6-a) quinolizinium]-An isoquinoline alkaloid present in the root bark of Berberis lycium".Phytomedicine 21: 663-669.

Gulfraz M., et al., (2008). "Comparison of the antidiabetic activity of Berberis lyceumroot extract and berberine in alloxan-induced diabetic rats". Phytotherapy Research 22 : 1208–1212.

Ahmad M., et al., (2009). "Effect of Berberis lycium Royle on lipid profile in alloxan induced diabetic rabbits". Ethnobotanical leaflets 13: 702-708.

Gulfraz M., et al., (2007). "Antihyperglycemic effects of Berberis lyceum Royle in alloxan induced diabetic rats". Diabetologia croatica 36: 49-54.

Winder WW, Hardie DG. (1999). AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol; 277(1 Pt 1):E1-10.

Viollet B, Mounier R, Leclerc J, Yazigi A, Foretz M, Andreelli F. (2007). Targeting AMP-activated protein kinase as a novel therapeutic approach for the treatment of metabolic disorders. Diabetes Metab ; 33(6):395-402.

Zhang H, Wei J, Xue R, et al., (2009). Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. Metabolism; 59(2):285-92.

Kong WJ, Zhang H, Song DQ, et al., (2009). Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression.Metabolism ; 58 (1):109-19.

Yin J, Gao Z, Liu D, Liu Z, Ye J. (2008). Berberine improves glucose metabolism through induction of glycolysis. Am J Physiol Endocrinol Metab; 294(1):E148-

Yin J, Gao Z, Liu D, Liu Z, Ye J.(2008). Berberine improves glucose metabolism through induction of glycolysis. Am J Physiol Endocrinol Metab; 294(1):E148-56.

Lu SS, Yu YL, Zhu HJ, et al.,(2009). Berberine promotes glucagon-like peptide-1(7-36) amide secretion in streptozotocin-induced diabetic rats. J Endocrinol; 200(2):159-65.

Al-masri IM, Mohammad MK, Tahaa MO. (2009). Inhibition of dipeptidyl peptidase IV (DPP IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. J Enzyme Inhib Med Chem ;24(5):1061-6

Xia X, Yan J, Shen Y, Tang K, Yin J, Zhang Y, Yang D, Liang H, Ye J, Weng J. (2011). Berberine improves glucose metabolism in diabetic rats by inhibition of hepatic gluconeogenesis. PLoS One 3;(2):e16556.