ASSESSMENT OF ANTIDIABETIC ACTIVTY BY USING SILVERNANOPARTICLES OF ARCTIUM MINUS LEAVES IN WISTAR ALBINO RATS

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ABSTRACT

Diabetes Mellitus Type 2 is one of the most frequent metabolic illnesses, and it is characterized by an elevated blood sugar level owing to decreased insulin production or impaired insulin action or both. Diabetes Mellitus Type 1 is one of the rarest metabolic disorders. Metformin is most often used for the treatment of type 2 diabetes mellitus, but due to its slow mode of action and severe adverse effects, it exhibits a poor and delayed therapeutic response in patients. Now, researchers are working on creating nanomedicine to overcome these constraints. This study intends to explore the therapeutic potential of silver nanoparticles as an antidiabetic medication on streptozotocin-induced diabetic mice. The silver nanoparticles were prepared using the aqueous extract of *Arctium minus* and were characterized by UV, ATR-FTIR, TEM, EDAX and SEM analysis. Acute toxicity study was carried out in rats using OECD 423 and were found to be non-toxic. They were evaluated for their *in-vivo* antidiabetic activity using streptozotocin-induced diabetic rats. *Arctium minus* mediated silver nanoparticles possess strong antidiabetic potential and can further be explored as an effective and cheaper alternative option for treatment of Type 2 Diabetes Mellitus.

Keywords: Anti-diabetic activity; *Arctium minus;* Silver nanoparticles.

INTRODUCTION: The Diabetes Mellitus is persistent metabolic sickness. it's far a fast-developing international problem with huge social, fitness and monetary consequences. Hyperglycemia or high blood sugar is a commonplace effect of uncontrolled diabetes and over time leads to serious damage to a number of the body's structures, especially the nerves, kidney and blood vessels.

Herbal drug treatments do now not have any aspect outcomes, as they're loose from chemical compounds. they're additionally milder than allopathic drugs. The herbal detoxing method of the frame is efficaciously enhanced by way of natural medicines. They may be used to cleanse the colon, enhance digestion and food absorption. natural drugs are also superb in boosting the immune system[32].

Nanoparticles (NP) are particles discovered in natural, accidental, or synthetic substances, 50% or extra of which fall inside this length range of one- 100 nm. Inside the gift take a look at green

synthesis is seemed as an crucial tool to reduce the damaging outcomes associated with metabolic sickness

The fundamental techniques and mechanisms of “inexperienced” synthesis techniques are particularly for steel and metallic oxide [e.g., gold (Au), silver (Ag), copper oxide (CuO), and zinc oxide (ZnO)] nanoparticles the usage of natural extracts. Plant-based silver nanoparticles (AgNPs) are the various simplest to put together and to lessen and stabilize Ag ions the usage of a aggregate of biomolecules, along with polysaccharides, vitamins, amino acids, proteins, phenolics, saponins, alkaloids. where aqueous plant extract acts as a reducing in addition to the stabilizing agent of Silver Nanoparticles.

PLANT PROFILE

Figure No. 1: Whole plant of *Arctium minus*

Arctium minus

Herbalism or "Herbal medicines” have a long history to cure several kinds of human diseases from the various parts of the plants such as leaf, stem, bark, root, etc. *Arctium minus* is a biennial plant, normally found along roadsides. The leaves are basal and alternative, it is a tall, purple brush like flower heads which appears July to September. The common names are Burdock, cuckoo button, button burs, beggar’s button. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicines for some aspect of primary health care. It was realized from the various reports that *Arctium minus*, belongs to the family Rubiaceae, have displayed plethora’s of potential

biological activities such as anticancer, hepatoprotective, anti-inflammatory, anti-fertility, anti-amoebic, antinociptive etc. from its various parts .

II METHODOLOGY

PREPARATION OF EXTRACTION

The leaves were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder[41]. This powder was sequentially extracted to their increasing polarity with Petroleum ether, Ethyl acetate, Ethanol respectively. About 500gm of powdered leaves was uniformly packed into a thimble in a soxhlet apparatus and extracted with 1000ml Petroleum ether, Ethyl acetate and Ethanol, respectively[42]. Finally ethanol extract was collected and filtered.

SYNTHESIS OF SILVER NANOPARTICLES[29]:

Synthesis of silver nanoparticles (AgNPs) using leaves of Arctium minus was synthesized by a green method, through the reduction of silver nitrate using 90ml at a concentration of 1mM mixed with 10ml of *Arctium minus* extract. The reaction was carried out at 85^0C for 2hrs.

CHARACTERIZATION OF SYNTHESIZED AML-AGNPS[30]:

The synthesized AML-AgNPs were subjected to the following spectral study for characterization UV-vis spectroscopy, Energy Dispersive X-ray (EDX) and attenuated total reflectance Fourier transform infrared spectroscopy are among the analytical techniques used in the characterization (ATR-FTIR), Scanning electron microscopy (SEM) and High-resolution Transmission Electron Microscopy (HR-TEM).

UV-Spectrophotometry analysis of AML-AgNPs

UV-Vis Spectroscopy the nanoparticle solution was analysed using a UV-Visible spectrophotometer in the wavelength range of 300-800nm with a resolution of 1 nm to monitor the bioreduction of silver (Ag & AgO). Because of the colloidal suspension's high optical density (OD), a 1 ml aliquot of the solution was diluted with 3ml of distilled water. For 24 hous the absorbance spectra of silver nanoparticles was examined on a regular basis. As a control Milli Q water was employed.

Attenuated total reflectance fourier transform inflectance (ATR-FTIR) Analysis of AML-AgNPs

FTIR were employed to characterize the composition, structure, structure and morphology of the silver nanoparticle. The nanoparticle solution was centrifuged for 30mts at 6000rpm and the supernatant was discared. To eliminate any nonreacting molecules in the colloidal matrix the pellets in a hot air oven for 2hrs a powder sample was obtained. The Bruker Platinum-ATR spectrophotometer was used to conduct FTIR analyses on powder AM-AgNPs and crude extracts FTIR mesurements were performed at an average of 32 scans per sample in the wavenumber range 4000-400 cm l with a resolution of $4cm^{-1}$.

High resolution Transmission electron microscopy (HR_TEM) analysis of AML-AgNPs:

HR-TEM is a useful, extensively used and vital method for determining particle and grain size, size distribution and morphology. The distance between the objective lens and specimen as well as the distance between the objective lens its image plane, define the magnification of TEM particles were sonicated in 100% ehanol for 30 minutes to an hour. Allowing a drop of the suspension to dry on a carbon-coated formvar grid specimens were analysed using a 200 KV VEGA3 TESCAN SOC MK-University transmission electron microscope. Digital micrograph software was used to capture micrographs with a Gatan bottom mount camera.

Scanning Electron Microscopy (SEM) Analysis of AML-AgNPs:

Scanning electron microscopy (SEM) was carried out to determine the structure, size and forms of nanoparticles. Samples were placed on 12 mm aluminium specimen stubs using double-sided carbon tape, coated with gold palladium and analysed with a 10KV VEGA3 TESCAN SOC-MK University SEM.

X-ray Energy Dispersive (EDX) Analysis of AML-AgNPs:

Energy Dispersive X-ray (EDX) studies revealed the spherical nature of particles synthesized from silver metal. It reveals strong signal in the silver region and confirms the formation of AgNPs. Metallic silver nanocrystals generally show typical peak approximately at 3KeV due to surface plasmon resonance.

PHARMACOLOGICAL ACTIVITY:

Acute toxicity study

In the research study, when a drug is administered to a biological system there will be some interactions may happen .In most case these are desired and useful, but many effects are not advantageous. Acute, sub acute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug. Acute toxicity is involved in estimation of LD_{50} (It is the lethal dose (causing death) to 50% of tested group animals).

LD_{50} (median lethal oral dose)

LD_{50} (median lethal oral dose) is a statistically derived oral dose of a substance that can be expected to cause death in percent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of animal (mg/kg).

In this study acute toxicity study was carried out in Wistar Albino rats. The procedure was followed by using OECD 423(Acute toxic class method).The rats are fasted overnight, prior

to dosing. The three dose levels are administered by the help of oral feeding needle over the prior of 24 hours[45]. After the extract has been administered, food may be with held for a further 3-4 hours in rats. The purpose of sighting study is to allow selection of the appropriate starting dose for main study. The test substance is administered to a single animal in a sequential manner following from the fixed dose levels of 5, 50, 300 and 2000mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, Duration and severity of toxic signs[46]. The animal weight is recorded on weekly once. On the day fourteen all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg).Based on the mortality result on 14th day of observation, the doses for *invivo* study are selected.

Invivo anti-diabetic activity of nanoparticles _Arctium Minus_ leaf in streptozotocin induced diabetic Wistar Albino rats.

Wistar albino rats (150- 200 grams) of both sexes were procured from TANWAS laboratories, Chennai-TN, India. Prior to the experiment the rats were housed in a clean polypropylene cages (6 rats/ cages) for a period of 7 days under standard temperature (25 - 30^{O}C) , relative humidity (45 – 55%), dark / light cycle (12 /12 hrs). The studies were performed with the approval of CPCSEA (887/Po/Re/S/2005) IAEC/M.PHARM/07/2018. The animals were put in overnight fasting were deprived of food for 16 hrs but allowed free access of water.

Hypoglycemic Test

Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (2mg /kg), Group III

served as aqueous nanoparticles of *Arctium minus* – (200mg/kg), Group IV served as aqueous nanoparticles of *Arctium minus* – (400mg/kg). Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test

Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (2 mg /kg), Group III served as aqueous nanoparticles of *Arctium Minus* – (200mg/kg), Group IV served as aqueous nanoparticles of *Arctium Minus* – (400mg/kg). All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been administered to different groups and 30mins later all the groups of rats were treated with glucose orally at dose 10gm/kg body weight by using oral feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Induction of diabetes to animals

A single dose (100 mg/kg, i.p.) of streptozotocin monohydrate dissolved in sodium citrate buffer was used for the induction of diabetes in rats after overnight fasting. After 1 hr of streptozotocin monohydrate administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypo-glycaemic phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

Experimental design

Five groups of rats six in each groups received the following treatment schedule for 14 days.

Group Treatment

Group	Treatment
Group – I	Normal control (normal saline 10 ml /kg, P.O)
Group – II	Streptozotocin treated control (100 mg/kg, I.P)
Group – III	Streptozotocin (100 mg/kg, I.P) + Standard drug Glibenclamide (2 mg/kg, P.O).
Group – IV	Streptozotocin (100 mg/kg, i.p.) + AML-AgNPs (200 mg/kg, P.O)
Group – V	Streptozotocin (100 mg/kg, i.p.) + AML-AgNPs (400 mg/kg, P.O)

AML-AgNPs, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II to Group V were diabetic control rats. Group IV and Group V (which previously received streptozotocin 100mg/kg) were given fixed doses of AML-AgNPs (200 mg/kg, P.O, 400 mg/kg, P.O) and group III received standard drug Glibenclamide (2 mg/kg,P.O) for 14 consecutive days.(AML-AgNPs).

Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of rats at weekly intervals till the end of the study 1, 7, and 14 days.

Estimation of biochemical parameters Serum blood glucose

On 1, 7, and 14 days fasting blood samples were collected and analyzed the blood glucose.

Blood glucose level

The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

Statistical analysis

Statistical analysis was done by using Graph pad Prism 5.0.All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one- way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.

RESULT AND DISCUSSION

Hypoglycemic Test

Treatment	Dose mg/kg	Blood Glucose Level (mg/dl)		
		0 min	0.5hr	1 hr
Control Carboxymethyl Cellulose(Cmc)	0.5 %	68.8±2.50	68.1±2.6	71.8±2.3
Positive Control Glibenclamide	2	69.10±0.64	52.3±4.03**	32.8±1.6***
Aqueous AML-AgNPs	200	68.70±2.25	60.1±3.50*	59.3±3.6*
Aqueous AML-AgNPs	400	68.01±2.5	53.1±2.31**	34.1±1.1***

The glucose levels were analyzed by using glucometer and each value is the mean ± standard error (n= each group consist of 6 animals)($p<0.05$)*, ($p<0.001$)**& ($p<0.0001$)*** as compared to control & positive control group evaluated by one way, ANOVA followed by Dunnet 't' test.

The hypoglycemic test results have shown Table No:I, which indicated aqueous extract of *Arctium minus* treated animals 200 & 400, significantly decreased in blood glucose level (0.84± 1093↓ & 18.83±3.879↓) ($P<0.05$)*, ($P<0.001$)**& ($P<0.0001$)*** when compared to control and positive control.

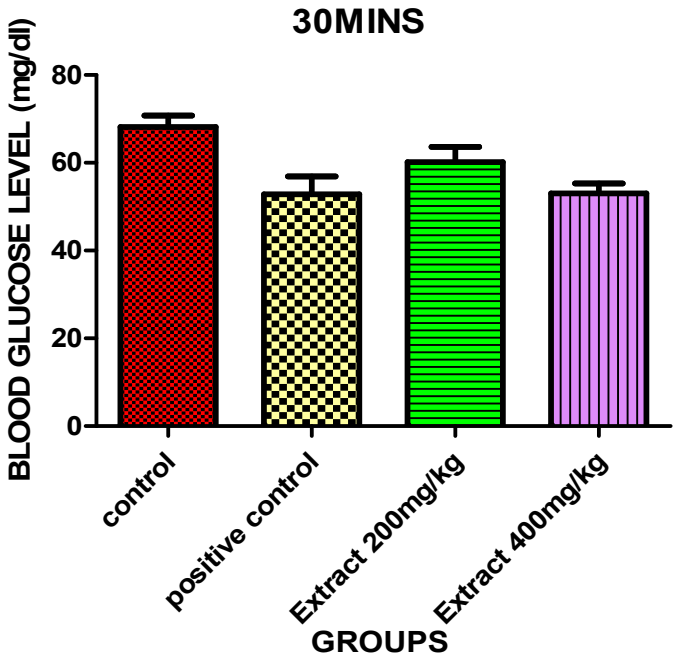

Figure No. 2: Hypoglycemic Test at 30 minutes

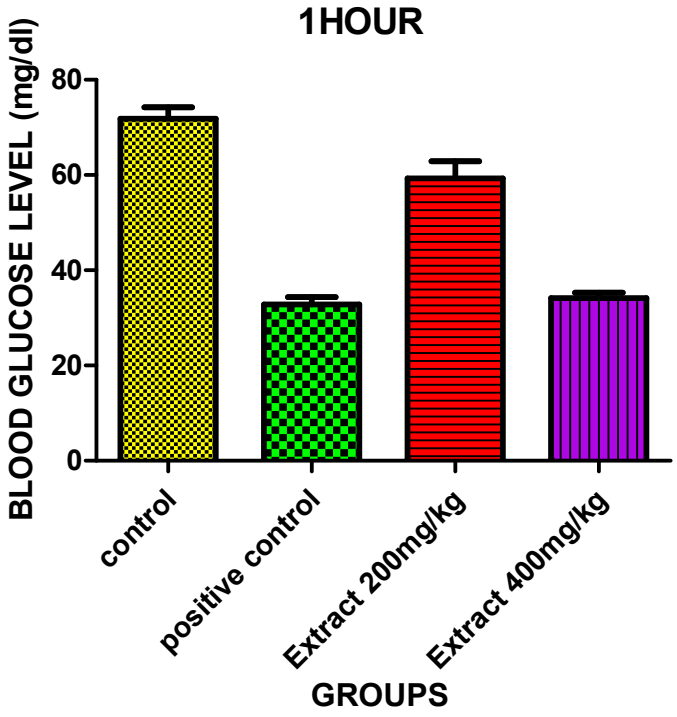

Figure No. 3: Hypoglycemic Test at 1 hour

***Invivo* antidiabetic study**

Table No. 8: Results of the effects of AML-AgNPs **on blood Glucose levels**

Sl. No.	Treatment	Blood glucose level (mg/dl) day		
		Day 1	Day 7	Day 14
1	Normal control 10 ml/kg P.O	79.9±2.8	75.8±4.2	76.8± 5.1
2	Negative control	265.2±3.8	270.2±2.9	275.2±3.5
3	Possitive control (Glibenclamide 2mg/kg) P.O	255.9±2.4	135.7±3.8***	112±3.8***
4	AML-AgNPs 200 mg/kg P.O	260.01±3.5	250.4±3.3**	245.2±4.2**
5	AML-AgNPs 400 mg/kg	263±4.5	173.2±2.9***	162.2±1.9***

	P.O			

(The values were expressed as Mean ± S.E.M. (n=6 animals in each group)

The experimental results have indicated on Table No:8. The negative control group glucose levels were significantly increased when compared to each other groups. All the groups of animals were affected in diabetes, which indicated blood glucose levels were slight changes in the blood glucose level (4.13±1.207↓& 1.±0.93↑) for normal control group at 7^{th} and 14^{th} days . On day 7^{th} glucose levels were significantly decreased glibenclamide 2mg/kg treated group (120.2±1.414↓& 23±1↓) (P<0.05)*, (P<0.001)**& (P<0.0001)*** when compared with control group at 7^{th} and 14^{th} days. The AML-AgNPs treated groups 200 & 400 mg/kg were dose dependent manner decreased (P<0.001)**& (P<0.0001)*** (10±0.362↓& 90±1.67↓) when compared with control group but positive control have more anti diabetic activity at 7^{th} day. The aqueous AML-AgNPs at the dose level 400mg/kg have potent activity (90±1.67↓&120.2±1.414↓when compared with positive control at 7^{th} day. The nanoparticles of *Arctium minus leaf* 200 & 400 mg/kg have been expressed dose dependent antidiabetic action (P<0.001) **& (P<0.0001) *** when compared to control and positive control. On day 14^{th}, nanoparticles of *Arctium minus leaf* treated animals 200 & 400 mg/kg significantly decreased and maintain the blood glucose level (5.1±0.07↓ & 11±1.08↓), (P<0.001) **& (P<0.0001) *** when compared to control and positive control

TREATMENT	DOSE mg/kg	Blood Glucose Level (mg/dl)						
		0 min	0.5 hr	1 hr	1.5 hr	2 hr	2.5 hr	3 hr
Control Carboxymethyl Cellulose	0.5 %	68.10 ± 2.5	142.5 ± 6.3	187.5 ± 9.5	172.5 ± 12.3	157.5 ± 12.4	153.5 ± 12.83	130.2 ± 13.4
Positive Control Glibenclamide	2	68.90 ± 0.6	104.2 ± 7.4**	110.5 ± 6.1***	93.67 ± 1.4***	83.67 ± 1.3***	77.17 ± 4.70***	74.33 ± 2.9***
Aqueous AML-AgNPs	200	68.80 ± 2.2	128.3 ± 6.1	147.3 ± 2.5*	138.5 ± 5.7*	128.5 ± 5.6*	113.8 ± 6.76**	108.8 ± 6.1**
Aqueous AML-AgNPs	400	68.00 ± 2.4	115.0 ± 6.2**	121.2 ± 6.2**	103.3 ± 4.8***a	93.33 ± 4.0*** a	86.6 ± 3.61***a	83.67 ± 2.9***a

Table No. 9: Oral Glucose Tolerance Test

The glucose levels were analyzed by using glucometer and all values are expressed as Mean± SEM (n=6), Group 2 was compared with group 1, Groups —3,4 were compared with group 2; *p<0.05, **p<0.01,p<0.001*** evaluated by one way, ANOVA followed by Dunnet ‘t’ test.

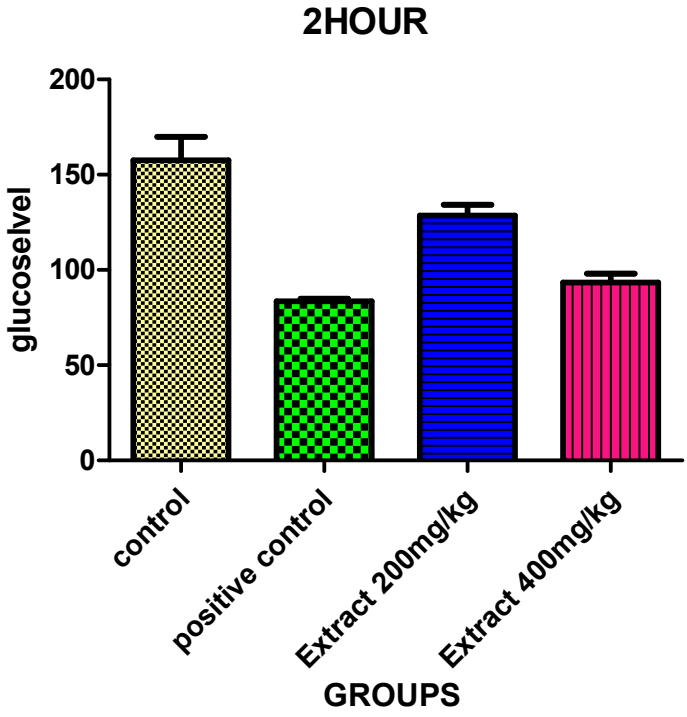

Figure No. 5: Oral Glucose Tolerance Test at 2 hours

Oral Glucose Tolerance Test (OGTT) results have been expressed on Table No:. Half hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased (74.5±3.863↑, 35.2±6.6905↑, 59.5±3.764↑ & 65.58±3.762↑). The blood glucose levels were significantly decreased for , aqueous nanoparticl of *Arctium minus* 200 & 400 mg/kg (59.5±3.764↓& 65.58±3.762↓) ↓), (P<0.001) **& (P<0.0001) ** when compared to control and positive control at 1hour and each and every ½ hour blood glucose levels(200 mg/kg : 8.8±3.26,10±0, 6.66±1.164 & 3±0.696, 400 mg/kg:17.9±1.422,9.97±0,14.7±1.093&5±0.696) (P<0.05)*, (P<0.001)**& (P<0.0001)*** were changes in the dose dependent manner extract treated group of animals compared to control and positive control but 400mg/kg produce the potent activity.

DISCUSSION

Sequential extraction was done according to increasing polarity order (petroleum ether, ethyl acetate and ethanol). In this present study Acute toxicity study was carried out in rats. The procedure was followed by using OECD 423 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with three animals of a single sex per step. The method used defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of EEKAGA was 2000 mg/kg body weight p.o as most of the crude extracts possessed LD50 value more than 2000 mg/kg p.o and also found to be the maximum safe dose. They were observed for signs of toxicity for 14 days. The onset of toxicity and signs of toxicity also noted[52].

Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study. Streptozotocin, a beta cytotoxic, induces diabetes in a wide variety of animal species by damaging the insulin secreting cells of the pancreas. Literature sources indicate that the Streptozotocin induced rats are hyperglycaemic. The treatment of lower doses of streptozotocin (100mg/kg b.w.) produced partial destruction of pancreatic β-cells even though the animals become permanently diabetic. Thus, these animals have surviving β-cells and regeneration is possible. It is well known that the sulfonylurea's act by directly stimulating the β-cells of the Islets of Langerhans, to release more insulin and these compounds are active in mild streptozotocin-induced diabetes. *Invivo* anti-diabetic screening was performed for the confirmation of above mechanism of action, of nanoparticles of *Arctium minus* in biological system. At the end nanoparticles of *Arctium minus (*200 mg/kg p.o, 400 mg/kg p.o.) showed statistically significant decrease in blood glucose levels. Hence, the silver nanoparticles of *Arctium minus* showed antidiabetic activity. This work may serve as a preliminary work, and may be used by researchers to evaluate experimental anti-diabetic models and its related diseases, to develop new entity, for the treatment of diabetes mellitus[56].

SUMMARY AND CONCLUSION:

Extraction of leaf was done by sequential extraction method. The leaves of *Arctium minus* was extracted using the solvent with increasing polarity order (petroleum ether, ethyl acetate and ethanol). Then Successfully synthesized AgNPs. The AgNPs exhibited potent carbohydrate degrading enzymes inhibitor for further animal studies and clinical methodology. Based on this result, it is suggested the AgNPs should be used as a nano antidiabetic drugs. Finally, the invivo anti-diabetic activity of silver nanoparticles of *Arctium minus* leaf was tested using streptozotocin-induced diabetic rats. Acute toxicity study was carried out in rats using OECD 423. 1/10th (200mg/kg) and 1/5th (400mg/kg) of the maximum safe dose (2000mg/kg) was selected for further study. Fasting blood sample were drawn from retino-orbital puncture of rats at weekly intervals till the end of the study (1, 7 and 14 days). On these days, fasting blood glucose were collected and analyzed for glucose. At the end of the study, (14th day) the silver nanoparticles of *Arctium minus* leaf (200mg/kg p.o and 400 mg/kg p.o) treated diabetic groups showed statistically significant decrease in blood glucose levels similar to the standard drug glibenclamide (2mg/kg). The synthesized silver nanoparticles showed higher level of antidiabetic activity by inhibiting the carbohydrate metabolizing enzymes. In addition, it also exhibited good antioxidant activity which will scavenge the free radicals produced due to hyperglycemia.

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