Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2015, Volume 3, Issue 4
ISSN: 2321–3647(online)

Hypoglycemic Activity of Gloriosa Superba on STZ Induced Diabetic Rats

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ABSTRACT

Gloriosa Superba is an Indian medicinal plant demonstrated to expect multiple pharmacology effects. In light of traditional claim of the plant in treatment of diabetes, the present study was carried out to evaluate the hypoglycemic actvity of the plant in STZ induced diabetics rats. The diabetic's rats were given ethanolic extract of Gloriosa superb (EEGS) (50mg/kg, 100mg/kg, and 200mg/kg) and metformin (180mg/kg) for 28days.The effect of both treatments on body weight and blood glucose were assessed. Three doses of extract and metformin show significant hypoglycemic actvity in acute, sub-acute study. Body wt. of extract and metformin treated rats were maintained during the study period where as body wt. of untreated rats had gone down. The result obtained from the study scientifically proved the folkloric use of Gloriosa Superba as Hypoglycemic agent. Thus the plant can be key contributor in treatment of diabetes.

Keywords: Diabetes Mellitus, Gloriosa Superba, Metformin, STZ.

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Received 01 March 2015, Accepted 08 March 2015

Please cite this article as: Basaiye GR *et al*., Hypoglycemic Activity of Gloriosa Superba on STZ Induced Diabetic Rats. American Journal of Pharmacy & Health Research 2015.

INTRODUCTION

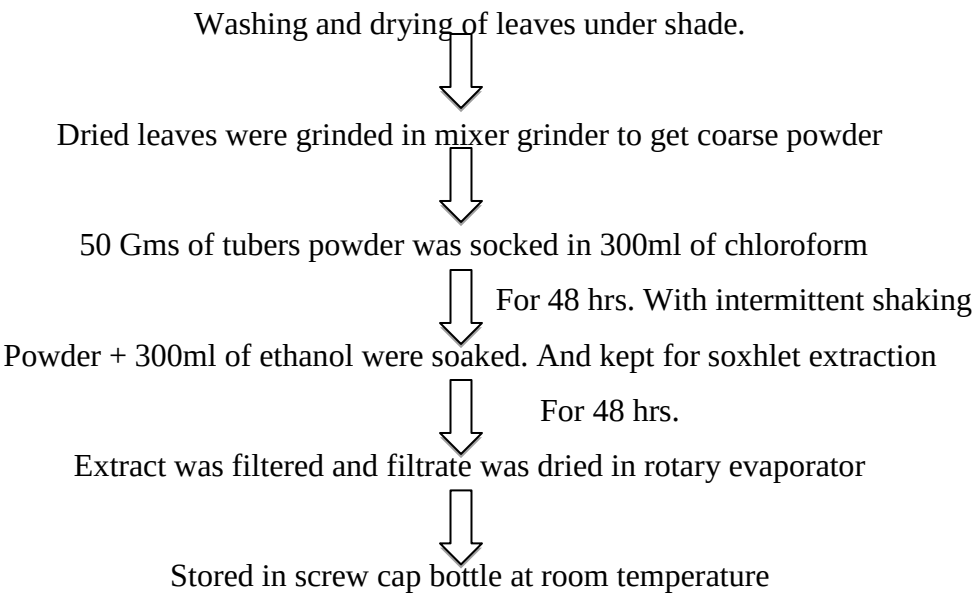
Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder. It is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases.The prevalence of diabetes mellitus is expected to reach up to 4.4% in 2030, and the occurrence was found to be high in India, China, and USA[1]. Diabetes claims a life every 10 seconds and in two decades time, 350 million people will be diabetic, if no action is taken the many complications that arise from the disease (IDF, 2008). So we say silent epidemic, diabetes continues to ignite and sustain motivation in finding a cure. Majority of the population in countries are therefore depending on their governments for health services, which is a heavy burden due to the high cost of medication. Not only is diabetes a heavy burden on the health budget, but diabetics depending on government support are often not treated optimally.[2]there are several medicinal plants with potent antidiabetic activity. One such medicinal plant *Gloriosa Superba Linn.*is selected for present study in our reserch protocol for Evaltion of treationally therapticus value. Upon extensive literature review with best of our knowledge the hypoglycemic activity of *Gloriosa Superba* tubers has not been documented. However, no scientific report on hypoglycemic actvity of tubers of title plant on type1 and type2 diabetes is recorded in literature so far. Therfore in present study an attment has been made to assess the antidiabetic effects study of *Gloriosa Superba*. One such plant cultivated for diffrent purposes such as medicine, Skin disease, respiratory disorders, and diabetes.[3]*Gloriosa Superba* tubers have showed the presence of glycoside, gloriosine, long chain fatty acids, flavonoids, tannins, alkaloids, 3-O-demethylcolchicine-3-O-α-D-glucopyranoside, 1,2-didemethyl colchicine, Glucoside, β and γ Lumicolichicines, β silosterol, Flucoside, 2,3-didemethyl colchicine, luterlin, N-formyl deacetyl colchicines, colchicocide, tannins, superbine, 2-hydroxy-6-methoxy benzoic and salicylic acid[4]. Tubers At low doses, has numerous medicinal applications. Tuber which looks like a hoe, it used for the treatment of bruises and sprains, colic, chronic ulcers, haemorrhoids, cancer, impotence, nocturnal seminal emission, and leprosy and also for including labour pains and abortions .[5] *Gloriosa superba* is the national flower of Zimbabwe.[6]

MATERIALS AND METHODS

- **Collection and identification of plant material**: The roots of *Gloriosa Superba L.* were collected from a local market in Pune City, Maharashtra, India and authenticated by Dr H. M

Pandit. (Botany) and Guru Nanak Khalsa College Matunga, Mumbai.400019. **(**Specimen#: grb 290314).[7]

- **Extraction of plant**:



Steps involved in Extraction of Gloriosa Superba L.

The crude EEGS was subjected to following studies after dissolve in water excluding phytochemical investigation.[8]

Pharmacological work

Animals used: Sprague–Dawley (SD) rats (180–200 g) of either sex were used throughout the experiment. The animals were procured fromVeterinary college lower parel &Bhart Serum vaccine pvt.Ltd. Bombay (Protocol no: AIEC/PCOL-22/2014.) The animals were acclimatized for fifteen days under standard laboratory condition. They were housed in polypropylene cages and maintained at, relative humidity 45c, 22±1^{0}C 12 hr light/dark cycle. The animals were fed with rodent pellet diet and water. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before initiation of the experiments.[9]

determination of acute toxicity (LD 50):

AccordingGuideline, OECD 425(Organization for Economic Cooperation and Development)

Acute Toxicity Studies

In acute toxicity study, there were no behavioural changes seen up to 4hrs and no mortality was observed up to the end of 48hrs even at the maximum tested dose level of 2000mg/kg per oral, it is considered as LD50. Thus, 1/10th of LD50 is taken as the effective dose. As a result, three doses of 50mg/kg, 100mg/kg and 200 mg/kg b.w was taken as an effective dose for the study.

Streptozotocin induced diabetes mellitus.

After fasting for overnight (36) rats were injected by intraperitoneally with a single dose of 50 mg/kg. Single intra peritoneal injection of a freshly prepared solution of STZ (50 mg/kg b.w.) in a 0.1 M citrate buffer (pH 4.5). After the injection they had free access to feed and water and were given 5% glucose solution to drink over night to counter the hypoglycemic shock .After7days, fasting blood glucose levels were measured and the animals showing blood glucose level 225 mg/dl were used for the present study, with signs of polyuria and polydipsia were considered to be diabetic and included in the study.[10]

Experimental protocol: The rats were divided into six groups (n = 6). Except group I which served as control all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) control. Groups III, IV and V, received (EEGS) at the dose of (50 mg/kg, 100mg/kg, and 2oo mg/kg.) Respectively; and group VI received the standard drug Metformin (180 mg/kg) daily for 28 days. Fasting blood glucose (FBG) level of each rat was measured on 7th, 14th, 21st&28thby using a one touch glucometer, while food and water intake, urine excretion in all rats were monitored before & after treatment.[11]. Body weight: The body weights of rats of each group were measured before and after 28 days of the treatments. Also, blood was withdrawn by retro-orbital puncture under mild anesthesia for analysis of blood. The animals were then sacrificed and then kidney, liver and pancreas were removed kept in 10% formalin solution for histopathological examination.

The parameters studied were as follows.

- Biochemical Tests: Fasting blood glucose, Serum total cholesterol, Serum total triglyceride.
- Morphological parameter: Body weight.
- Histopathological studies.

RESULTS AND DISCUSSIONS

Table 1: Blood Glucose Levels (mg/dl)

Effectof Ethanolic extract of *Gloriosa Superba L*on 1st, 7th, 14th & 21st, 28th day in rats.

GROUPS	Blood glucose (mg\dl)				
	1 DAY	7 DAY	14 DAY	21 DAY	28 DAY
Normal Control	92.83±0.874	94±1.506	92.00±1.342	92.00±1.342	93.33+1.687
Diabetic Control	459.3+8.476	538.7+5.818	560.5+6.761	571+4.380	586.8+3.05
Diseases control	465.8+7.120	453.2+7.565	283.8+2.227	183.8+2.227***	106.7+1.174***

Diabetic+ EEGS (50 mg\kg)	480.0+12.81	418±9.871	279.7+9.062*	179.7+9.062**	129.2+2.548
Diabetic+ EEGS (100 mg\kg)	457.7+8.505	418.8+9.871	268.0+8.363**	168.0+8.363	126.7+1.994**
Diabetic+ EEGS (200 mg\kg)`	445.3+6.206	416.5+16.75	244.7+10.34	164.7+10.34***	117.7+2.418***

Statistical significancefor Effect of Blood Sugar level (BSL).

*P<0.05, **P<0.01, ***P<0.001 was considered significant comparing to Diabetic control group. Values are given as mean ± SEM for groups of six animals in each group. ANOVA followed by Dunnet'Test.

Table 2: Serum Biochemical Parameters

Effect of Ethanolic extract of *Gloriosa Superba L* on Serum Biochemical Parameters after 4 weeks treatment.

GROUP	Urea	Uric acid	Creatinine
Control	32.10±2.359	1.203±0.05783	0.1471±O.6967
Diseases control	65.83±11.41**	3.2±0.4259	1.840±0.4140
Standard	25.47±1.554	0.9±0.008**	0.560±0.035
Test 1(50mg/kg)	37.34±1.228	1.313±0.2685**	0.6667±0.0484**
Test 2(100mg/kg)	30.31±4.521**	1.537±0.2167**	0.5800±0.041***
Test 3(200mg/kg)	29.79±1.648**	1.478±0.1477***	0.4033±0.0527***

Statistical Analysis of Urea, Uric acid, Creatinine.

*P<0.05, **P<0.01, ***P<0.001 was considered significant comparing to Diabetic control group. Values are given as mean ± SEM for groups of six animals in each group. ANOVA followed by Dunnet's *t*-test.

Table 3: Total Body Weight (Mg/Dl)

Effectof Ethanolic extract of *Gloriosa Superba L* on 1st, 7th, 14th & 21st, 28th day in rats.

GROUPS	Body weight (mg\dl)				
	1DAY	7 DAY	14 DAY	21 DAY	28 DAY
Normal Control	266.5±1.727	269.5±0.8851	270.7±0.6667	270.8±0.6009	271.7±0.6146
Diabetic Control	270.3±1.145	272.2±0.8724	268.1±1.065	264.0±0.8944	259.3±0.5578
Diseases control	265.2±1.662	269.3±1.430	269.0±1.706	270.7±0.8433	271.2±1.352
Diabetic+ EEGS (50 mg\kg)	266.8±1.515	270.7±0.667	271.7±0.4216	272.1±0.5774	273.2±0.4216**

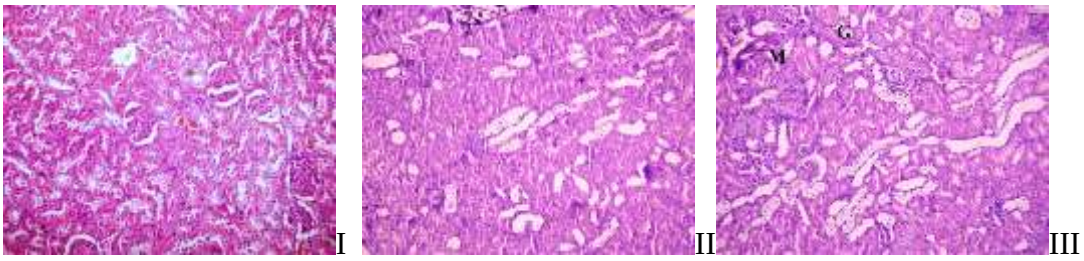
Diabetic+ EEGS (100 mg\kg)	266.0±1.366	268.2±1.108	271.8±0.8724	275.1±0.5164**	273.5±O.5627***
Diabetic+ EEGS (200 mg\kg)	274.5±1.008	274.5±1.008	275.8±0.833	278.2±0.6009*	279.2±0.4713***

*P<0.05, **P<0.01, ***P<0.001 was considered significant comparing to Diabetic control group. Values are given as mean ± SEM for groups of six animals in each group. ANOVA followed by Dunnet's *t*-test.

Table 4: Serum Biochemical Parameters (Mg/Dl)

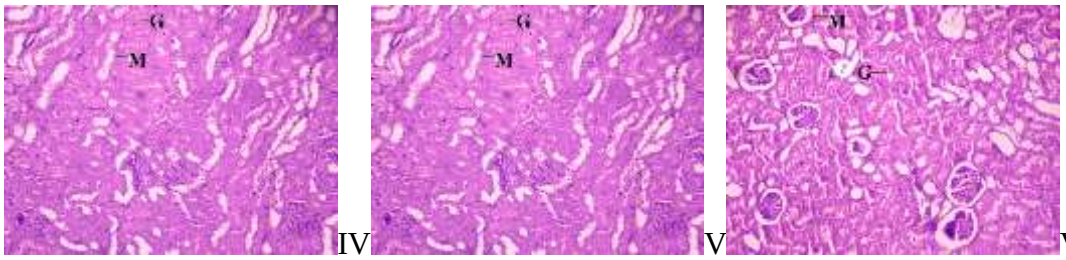
Effectof Ethanolic extract of *Gloriosa Superba L* on Serum Biochemical Parameters after 4 weeks treatment.

GROUP	TRIGLYCERIDE	CHOLESTEROL
Control	129.2± 3.005	100.8 ± 1.797
Diseases control	228.2± 3.978	222.5 ± 3.423
Standard	117.5 ± 3.731	120.3 ± 15.79
Test1(50mg/kg)	129.8 ± 1.078	136.3 ± 2.486
Test2(100mg/kg)	136.3± 1.430**	129.3 ± 2.305
Test3(200mg/kg)	142.8 ±2.400**	114.0±2.852***



I II III

Group I: Normalcontrol GroupII: Diseases control GroupIII: EEGS (50mg/kg)



IV V VI

Group Iv: EEGS (100mg/kg) Group V: EEGS (200mg/kg) Group VI: Std.Met (180mg/kg)

Figure: 1. Histopathology of Kidneys

Group I: Section of control rat Kideny tissue showingnormal structure of glomeruli and proximal and distalconvoluted tubules in kidneys.

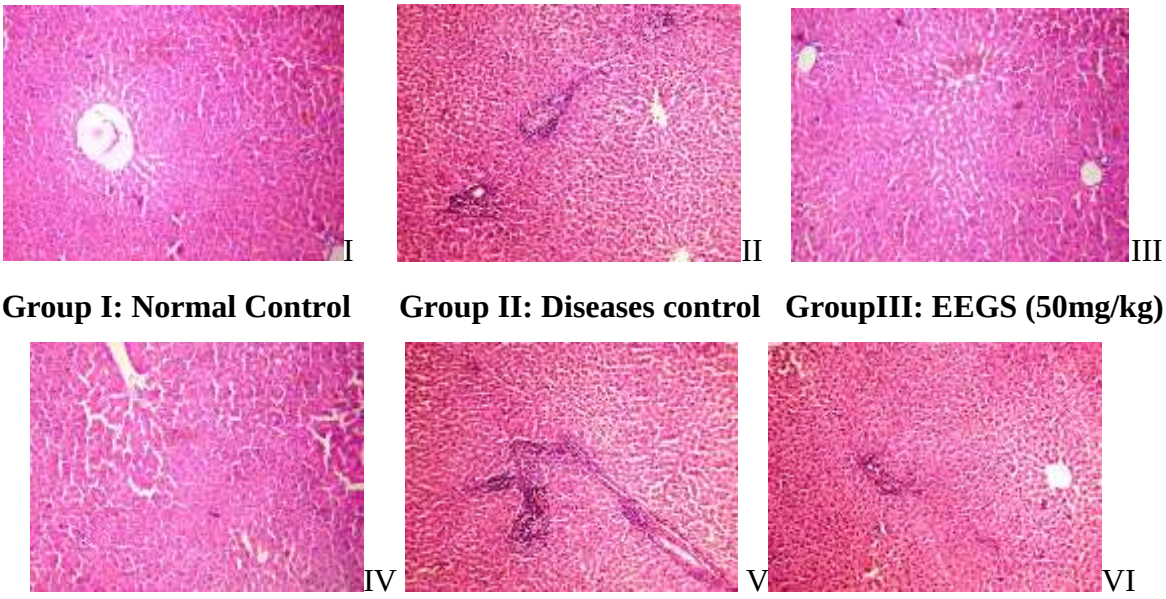
Group II: Section of Diabetic Control kidneys showed an increase in the mesangial cell and matrix of glomeruli and hyalinization of arterioles.

Group III: Section of Kidneys tissue from EEGS (50mg/kg) group showed kidney with less increase in the mesangial cell matrix of glomeruli and few hyalinized arterioles.

Group IV: Section of Kidneys tissue from EEGS (100mg/kg)group showed kidney with minimally multifocal mild degree vacuolar degeneration of tubular epithelium, mild degree granular degeneration of tubular epithelium.

Group V: Section of Kidneys tissue from EEGS (200mg/kg)showed kidney increase in the mesangial cell matrix of glomeruli and hyalinized arterioles.

Group VI: Metformin treated group showed normal kidney structure which appeared more.



Group I: Normal Control **Group II: Diseases control** **GroupIII: EEGS (50mg/kg)**

GroupIV: EEGS (100mg/kg) **GroupV: EEGS (200mg/kg)** **GroupVI: Std.Met180mg**

Figure 2: Histopathology of Liver:

Group I: Section of control rat liver tissue showing normal hepatocyte with vesicular nuclei.

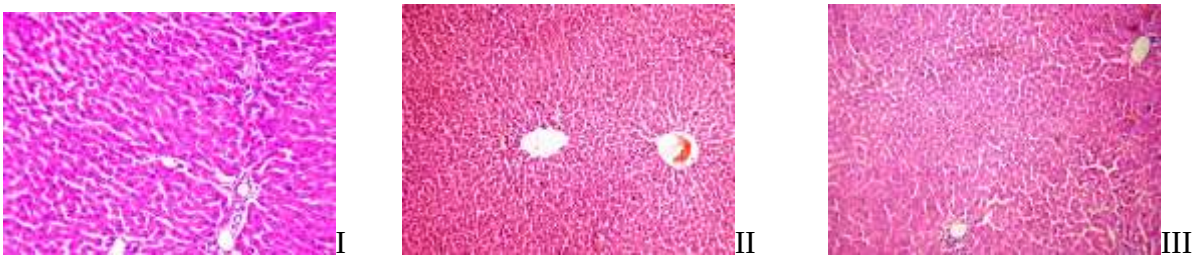
Group II: Disease Control section of diabetic rat liver tissue showing congested nuclei of the hepatocyte.

Group III: Section of liver tissue from EEGS (50mg/kg) treated diabetic rat showing Moderate degree granular degeneration, moderate degree fatty infiltration in the form of micro/ macro vesicular degeneration.

Group IV: Section of liver tissue from EEGS (100mg/kg/) treated diabetic rat showing Mild degree granular degeneration, mild degree fatty infiltration in the form of micro/ macro vesicular degeneration.

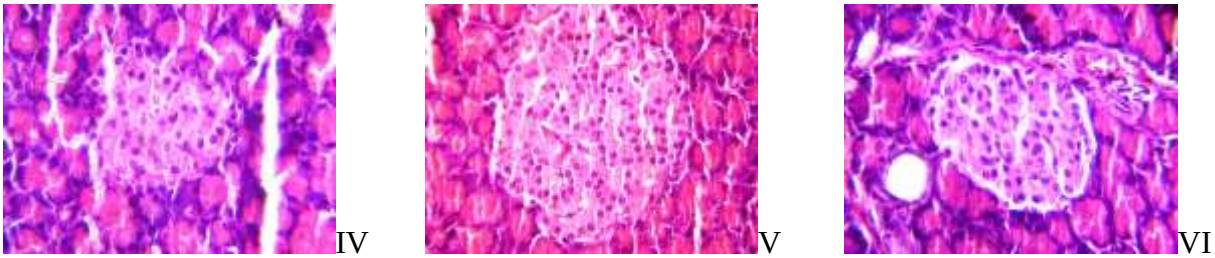
Group V: Section of liver tissue from EEGS (200mg/kg) treated diabetic rat showing Mild degree granular degeneration.

Group VI: Section of liver tissue from Metformin treated diabetic rat showing apparently normal architecture.



I II III

GroupI:Normal Control **GroupII:Diseases Control** **GroupIII:EEGS(50mg/kg)**



IV V VI

GroupIV: EEGS (100mg/kg) **GroupV: EEGS (200mg/kg)** **Group VI: Std (Met180mg/kg)**

Figure 3: Histopathology of Pancreas

Group I: Section of control rat pancreas tissue showing Normal acini and normal cellular population in islets of Langerhans of pancreas.

Group II: Disease Control of diabetic rat pancreas tissue showing Extensive damage to islets of Langerhans and reduced dimensions of islets.

Group III: Section of pancreas tissue from EEGS (50mg/kg) showing less restoration of cells of islet of Langerhans can be observed.

Group IV: Section of pancreas tissue from EEGS (100mg/kg) showing possible restoration of repair of cells of islet of Langerhans.

Group V: Section of pancreas tissue from EEGS (200mg/kg) Maximum restoration of the cells of islet of Langerhans can be observed.

Group VI: Section of pancreas tissue from Standard Metformin show, no Abnormality Detected. With well demarcated islet and normal acini structure (Exocrine part).

Diabetes mellitus ,a disorder of carbohydrate metabolism in which sugars in the body are not oxidized to produce energy due to lack of pancreatic hormone(insulin).Diabetes, that starts in childhood or adolescence(type1) is usually more severe than that beginning in middle or old age(type 2),but may also develop in young people. The present study was undertaken to investigate the hypoglycemiceffect of *Gloriosa superba* tubers in STZ induced diabetics rats, Metformin use as Standard drug. Ethanolic extract of *Gloriosa Suberba* tubers were subjected to

hypoglycemic actvity in dose of 50 mg/kg, 100mg/kg, 200mg/kg. Givenorally. Further blood glucose analyzed by 7^{th}, 14th, 21^{st}&28^{th} days. Control animal received equal volume of normal saline and metformin (180mg/kg) served as reference standard. Blood glucose level was estimated by using. Glucometer. The result indicated that the ethanolic extract showed highly significant. ($P< 0.001$) hypoglycemic actvity (117.7±2.418) compared to diabetics control (586.8±3.05). On prolonged treatment the EEGS showed significant actvity (117.7±2.418) nearly or equal to reference standard drug metformin (106.7±1.174). From this clearly established that the administration ethanolic extract exbitied better Hypoglycemic actvity. Diabetes mellitus causes failure to use of glucose for energy that leads to increased utilization and decreased storage of protein responsible for reduction of body wt. essentially by depletion of body proteins. In present study it was observed that *Gloriosa superba* tubers extract increased the body weight in diabetes rats when compared to diabetes control rats this may be due to protective effect of extract in controlling muscle wasting that is reversal of gluconeogenesis. STZ have been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the actions of stz induced free radical damage. Since the plant has been reported to possess to its anti-Oxidant actvity, and this could be linked to its anti-diabetes property. The result of present study in both experimental models indicates that extract can reduce the levels of serum urea, creatinine, and cholesterols. An increase confirms possibility that major function of extract are on protection of vital tissues (kidney, Liver) including pancreas, There by reducing causation of diabetes in experimental animals. Glorisa Superba as Liver protective agent. The improvement of liver function and subsequent increases in uptake of blood glucose and its utilization may be another mechanism of action of extract. STZ causes irreversible destruction of pancreatic beta cells due liberation of highly reactive free radicals. Thus hypoglycemic actvity might be due to extra pancreatic mechanism. The present study also showed that Gloriosa superba can partially inhibit STZ induced renal toxicity as observed from serum Urea and creatinine levels.Flavonids containing medicinal plant were reported to be having anti diabetes actvity. Preliminary photo-chemical screening revealed the presence of these phytochemical in test extract. Thus flavonoids present in extract may be suspected to possess hypoglycemic actvity.

CONCLUSION

The tuber of *Gloriosa superba* selected for the study was collected from a local market in Pune City, Maharashtra, India and authenticated by Dr H. M Pandit. (Botany) and Guru Nanak Khalsa College Matunga, Mumbai.400019. **(**Specimen#: grb 290314).

The powered drug was subjected to physicochemical characterization and standardization. Plant material was powdered using mixture grinder and subjected to soxhlet extraction with ethanol and water. A part of extract use for physiochemical investigations, remaining part was utilized for performing various biological activities. From detailed physiochemical investigation of all extract, it could be conclude that tubers contain carbohydrates, flavonoids, glycosides, protein &amino acids. All extract where study for acute toxicity study as per OECD guideline 425.The therapeutics doses for hypoglycemic actvity were selected according to LD_{50}. For diabetics study, blood glucose levels were noted on administration of 28^{th} days study. Glucose levels of respective drug extract treated group were compared with those of diseases control group. Ethanolic extract showed significant hypoglycemic actvity for four week of treatment also there is an increase in body weight, with Kideny and liver protection observed. After that blood was withdrawn by retro-orbital puncture under mild anesthesia for analysis of biochemical parameter. The animals were then sacrificed and then kidney, liver and pancreas were removed kept in 10% formalin solution and send for histopathological examination. In conclusion it must be stated that tubers of Gloriosa Superba may possess hypoglycemic properties based on the parameters examined. Gloriosa Superba significantly improved the fasting glucose status of diabetics animal over the period of 28^{th}days.There was also an observed increased in body mass. The biological effects of Gloriosa Superba tubers may be even higher after the isolation and purification of the compounds further studies in this direction are currently in progress.

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