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*Original Research Article*

# Anti-Diabetic potential of *Girardinia heterophylla* in alloxan induced rat model

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**ABSTRACT**

Diabetes mellitus is the most common and significant chronic endocrine disorder affecting approximately 200 million individuals worldwide. The current availability of allopathic medicines is not giving optimum response to cure these conditions. In this regard researchers are trying their best to resolve this worsen condition. The present study was designed as phytochemical and pharmacological investigation of bipolar solvent extracts of *Giardinia heterophylle* leaves in Alexon induced diabetic rats' model.

The fresh shade dried leaves extracts were subjected to qualitative chemical tests like steroids, triterpenoids, carbohydrates, glycosides etc. and the result was found to be presence of sterol, triterpinoids, carbohydrate, tannins and phenolic compounds. The presence of steroids was confirmed by TLC & HPTLC. The effect of different extracts such as ethanolic, petroleum-ether, aqueous and chloroform of *Girardinia heterophylla* leaves were evaluated for their anti-diabetic activity by using alloxan induced diabetes model (albino rats) and Glibenclamide (10 mg/kg. p.o.) used as standard drug. $LD_{50}$ cut-off dose for *Girardinia heterophylla* leaves extracts of petroleum ether, ethanolic, chloroform and aqueous extracts were found to be 2000 mg/kg. $1/10^{th}$ of this lethal dose of leaves extracts i.e. 200 mg/kg body eight was taken for screening of antidiabetic potential. The experimental results confirms that ethanolic, aqueous and chloroform extracts showed significant improvement in diabetic animals.

## 1. Introduction

*Girardinia heterophylla*, commonly known as '*DansKandali'*, is found in temperate and sub-tropical region of the Himalayas. The species of *Girardinia heterophylla* growing extensively as an underutilized biomass in the forest areas situated in Mussoorie hills of Dehradun district of Uttarakhand state. The leaves of this plant are boiled and cooked for vegetables. The whole plant is also used as cattle fodder to improve milk production. The stem portion of the plant yields the valuable fiber, which has been traditionally used by the tribal for making rope based products used for packing grains and transportation purpose. After extracting the fiber from the stem, residue of the bark portion is used as fuel wood. Traditionally, bark powder is also used as a bandage material for faster healing of wounds and setting of broken bones**[1].**

*Girardinia heterophylla* is among those plant species which have not been systematically studied for their biological active constituents in order to establish their potential use through its different parts viz. leaves, stem and roots. It is of immense importance to screen the medicinal plants growing in nature to find the additional source of bioactive compounds to be used for human welfare. The increasing global interest towards this interface between chemistry and biology has gained more importance and the public demand is continuously rising for the cost effective medications and biological agents from sustainable and natural resources**[2].**

This study was primarily designed to investigate the pharmacognostic activity as; sterols, triterpenoids, carbohadrate, tannins, and phenolic compounds extracted from *Girardinia heterophylla* leaves and pharmacological investigation as blood glucose, differential cholesterol levels, lipid profiles, biochemical parameters and antioxidant effect on alloxan induced diabetic rats' model.

Diabetes mellitus is one of the recurrent metabolic disorders initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or its action**[3].**

The main cause of diabetes is due to the shortage of insulin or insulin resistance. Glucose is the ultimate source of energy for all metabolic processes.

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Insulin, a hormone secreted by the β-cells of pancreas plays a vital role in regulating the movement of glucose and its level in blood**[4].**

An adequate amount of insulin is required for absorbing sufficient glucose from the blood into the cells. If glucose level in the blood remains high (hyperglycemia) over a long period of time, this can result in continuing damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood arteries. People with diabetes are in a risk of other complications associated with damaged tissue of vital organs may lead to death**[5].**

In the mid of 21$^{st}$ century, diabetes mellitus has considered as one of the main threats to human health. In developing countries, the prevalence of diabetes is increasing spontaneously and estimated by the World Health Organization (WHO), around 70 million people suffering from diabetes mellitus**[6].** Over the last century, changes in human behaviour and lifestyle have resulted in a dramatic increase in the occurrence of diabetes worldwide and any person can be affected by this disease at any age**[7].** Diabetes is also defined as chronic disorders of carbohydrate metabolism due to the lack of insulin result in the hyperglycemia and glycosuria.[8] The type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM) is a multi-factorial autoimmune disease,[9] which susceptibility is determined by a combination of genetic and environmental factors. Diabetes mellitus is one of the most common chronic disorders of childhood**[10].**

## 2. Materials and methods

### 2.1 Plant material

The leaves of *Girardinia heterophylla* was collected from the Mussoorie and Dhanaulti, district Dehradun situated in middle Himalayas region at the altitudinal range of 22,00 to 25,00 meter in the month of October-November. The plant sample was authenticated from systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand, India.

### 2.2 Preparation of extracts

The collected leaves of *Girardinia heterophylla* were dried in shade and powdered. The powered leaves were subjected to successive hot-solvent extraction process with the solvents in order of increasing polarity, viz. petroleum ether (40-60$^0$C), chloroform, ethanolic and aqueous. Aqueous extract was performed by cold maceration process. The extracts were subjected to qualitative chemical tests. Steroids were isolated from ethanolic extract of *Girardinia heterophylla* leaves.

### 2.3 Phytochemical investigations

The fresh shad dried leaves powder was subjected to extraction with petroleum ether, chloroform, ethanol and water. The percentage yield obtained from the non-polar solvents i.e. petroleum ether and chloroform (2.65% and 1.66% respectively), which was lesser the other yield obtained from the polar solvents i.e. ethanol and water (11.50% and 13.43% respectively). The percentage yield obtained from the successive extraction using the solvents petroleum ether, chloroform, ethanol and aqueous extract obtained by hot and cold maceration process**[11].** Preliminary Phytochemical screening of extracts of *Girardinia heterophylla* leaves was done for their chemical constituents. The presence of different chemical constituents was confirmed by treatment of the extract with different chemical reagents. For instances, Alkaloids with Dragendroff's reagent, flavonoids with metallic magnesium plus HCl, saponins with the ability to produce foam, reducing sugars with Fehling's reagent, glycosides with Lieberman's test, tannins with ferric chloride and polysaccharides with iodine solution**[17].**

### 2.4 Physicochemical investigations

In present study the powder of *Girardinia heterophylla* leaves was investigated for the physical constants such as total ash, loss on drying and extractive values. The total ash found in leaves was 16.5% w/w, acid insoluble ash 4.36% w/w and water soluble ash 7.1% w/w with absence of any foreign matter and loss on drying found to be 15.0% w/w. The extractive value was obtained maximum in aqueous extract of leaves 29.34% w/w as compare to alcoholic soluble extract i.e. 8.0% w/w**[12].**

### 2.5 Quantitative investigation

The Quantitative microscopy of leaves ware determines palisade ratio (1:2), stomata number (3.5), stomata index (1.6-3.4-5.3), vein-islet number (21-23) and veinlet termination number (09-15) carried out on epidermal strips. Other parameters determined for the powdered leaves were moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol (90 % ethanol) and water soluble extractive values**[13].**

### 2.6 Drug and chemicals

All the chemicals and drugs used in the study was analytical grade and procured from local supplier Himgiri traders Dehradun. Glibenclamide and alloxan monohydrate drug were obtained from CHD drugs limited, Noida as a gift sample. Chemical like ethanol, chloroform, petroleum ether and other phytochemical reagents were provided by Institute. Glucose kit (GOD/POD) was also purchased from local market.

### 2.7 Experimental Animals

Wistar albino rats weighing 200-250g of either sex was maintained in the department of animal house for experimental purpose. Then all the animals were acclimatized for two weeks under standard husbandry conditions i.e. room temperature of 25±1°C; relative humidity 45-55% and a 12:12h light/dark cycle. The animals had free access to standard diet with water supplied *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of nonspecific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of SLT Institute of Pharmaceutical Sciences, Bilaspur (Chhattisgarh) was taken

prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to Institutional Animal ethical Committee guidelines (Reference No. IAEC/Pharmacy/2011/51) provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (Approval No. 1489/PO/a//11/CPCSEA).

The extracts of *Girardinia heterophylla* leave was evaluated for the antidiabetic activity using following experimental model. All the animals were divided into 7 groups each containing 6 rats as follows; Group I- Control group: 2ml Saline p.o.; Group II- Diabetic Control: 2ml Saline p.o.; Group III- Petroleum Ether Extract: 160 mg/kg p.o.; Group IV- Chloroform Extract: 200 mg/kg p.o.; Group V- Ethanol Extract: 240 mg/kg p.o.; Group VI- Aqueous Extract: 180 mg/kg p.o.; Group VII- Glibenclamide: 10 mg/kg p.o. All the animals were fasted over night and then blood glucose level was determined immediately before treatment and then 7 hrs after treatment**[14].**

### 2.8 Oral glucose tolerance test (OGTT)

Animals were fasted for 12 h before the OGTT. Glucose (1g/kg) was administered by gavages 30min after oral administration of 250mg/kg of *Girardinia heterophylla* leave extracts. Glibenclamide at dose of 10mg/kg was used as standard drug.[15] Blood glucose level was measured each hour after glucose loading in rats under light ether anesthesia. Blood was obtained from retro orbital puncture by using heparinised capillary tube and immediately centrifuged for 5 min. Plasma was analyzed for glucose content using a glucose oxygenase method (Sigma diagnostics centre, Dehradun)**[16].**

### 2.9 Statistical Analysis

Results of Anti-diabetic activity were reported as Mean ± SEM. Significant intergroup difference of each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out. The calculated mean tabulated along with the Dunnet's't' test was used for individual comparison.

## 3. Results and discussion

The result of qualitative chemical investigation of *Girardinia heterophylla* leave extract has indicated the presence of sterols, triterpenoids, carbohydrate, tannins and phenolic compounds. Petroleum ether (40-60$^0$C) extract contains (Steroids, triterpenoids, fats and oils), Chloroform extract (Steroids, triterpenoids, carbohydrates), Ethanolic extract (Steroids, triterpenoids, carbohydrate, tannins and phenolic compound), Aqueous extract consist of (Carbohydrate, steroids, triterpenoids, tannins and phenolic compounds).

Further Petroleum ether (40-60$^0$C), chloroform, Ethanol and aqueous extracts of *Girardinia heterophylla* leaves were subjected to assessment of acute oral toxicity study and antidiabetic activity in alloxan induced albino rats in both acute and prolonged treatment.

### 3.1 Acute Oral Toxicity Study

Acute toxicity study was carried out according to OECD guidelines. The animals were fasted 3 hrs prior to the experiment, up and down procedure (OECD guideline no. 425). 5 Animals were administered with single dose of extracts dissolved in 2% w/v acacia and observed for its mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the dose of the next animals was determined as per as OECD guideline 425. All the animals were also observed for long term toxicity (14 Days). The $LD_{50}$ of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA**[18].**

The following $LD_{50}$ values of *Girardinia heterophylla* were obtained for various extracts i.e. Petroleum ether extract having $LD_{50}$ Cut-off value 1600 mg/kg b.w.; Chloroform extract (2000 mg/kg b.w.); Ethanolic extract (2400 mg/kg b.w.) and Aqueous extract (1800 mg/kg b.w.) after observing for long term toxicity study. 1/10$^{th}$ of this lethal dose was taken as effective dose (therapeutic dose) for subsequent antidiabetic activity i.e. 200 mg/kg b.w.

### 3.2 Antidiabetic activity

The petroleum ether (40-60$^0$C), chloroform, ethanol and aqueous extracts were given orally at a dose of 200 mg/kg b.w. with the help of gastric livages tube in alloxan induced diabetic rats. Further the blood glucose level was analyzed initially (0 hr), 1$^{st}$ hr, 3$^{rd}$ hr, 5$^{th}$ hr and 7$^{th}$ hr after single dose and 7$^{th}$ day after prolonged treatment of leave extracts.

Normal control and diabetic control animals received equal volume of normal saline and Glibenclamide (10 mg/kg b.w.) served as standard control. Blood glucose level was measured in all groups by using glucometer (Pulsatum, Pulsatum Health Care Pvt. Ltd., Bangalore) and glucose test kit.

Random blood glucose level after single dose administration of reference and test drug in Alloxan induced diabetes rats indicating that chloroform, ethanol and water extract showed significant ($p \leq 0.005$) reduction in blood glucose in comparison with diabetic control group at every alternative hour for 7 hour observation. The results were comparable with standard drug Glibenclamide and Ethanolic extract of *G. heterophylla*, which shows statistically significant ($p \leq 0.005$) improvement in anti-diabetic activity on single dose treatment at 7$^{th}$ hour compared to diabetic control group (table 1).

**Table No. 1:** Effect of random blood glucose level after single dose of drug administration

| Group (n) | Dose | Random blood glucose level after single dose administration | | | | |
|---|---|---|---|---|---|---|
| | | Initial | 1 hour | 3 hour | 5 hour | 7 hour |
| Normal control | 2 ml saline | 95.88±2.463 | 97.24±1.616 | 98.02±0.982 | 96.97±1.284 | 98.14±1.830 |
| Diabetic control | 2 ml Saline | 253.58±2.601$^{\#}$ | 268.36±2.823$^{\#}$ | 270.14±3.013$^{\#}$ | 277.122±2.830$^{\#}$ | 282.50±3.082$^{\#}$ |
| Pet. ether extract | 160mg/kg b.w. | 265.08±3.021 | 261.08±3.160 | 250.02±2.722$^{**}$ | 243.16±2.885$^{**}$ | 240.11±3.263$^{**}$ |
| Chloroform extract | 200mg/kg b.w. | 258.46±2.687 | 254.20±3.190$^{**}$ | 238.20±3.072$^{**}$ | 197.68±3.055$^{**}$ | 180.44±2.607$^{**}$ |
| Ethanol extract | 240mg/kg b.w. | 235.50±3.163$^{**}$ | 222.34±3.031$^{**}$ | 186.34±3.015$^{**}$ | 163.52±3.141$^{**}$ | 136.35±2.872$^{**}$ |
| Aqueous extract | 180mg/kg b.w. | 247.28±3.025 | 230.25±3.000$^{**}$ | 218.60±3.087$^{**}$ | 183.25±2.751$^{**}$ | 166.80±2.970$^{**}$ |
| Glibenclamide | 10mg/kg b.w. | 233.37±3.241$^{**}$ | 200.83±3.130$^{**}$ | 168.22±3.100 | 156.55±3.022$^{**}$ | 132.00±2.803$^{**}$ |

Values are given as Mean±SEM and expressed in mg/dl. $^{\#}p\leq0.05$ *r*epresents statistical significance against normal control and $^{**}p\leq0.005$ *r*epresents statistical significance against diabetic control group.

Observation of random blood glucose level after prolonged drug administration shows, the initial effect of Ethanolic extract (235.50±3.163) nearly equal to that of the reference drug Glibenclamide (233.37±3.241). On other hand at the end of study it was clearly established that, Ethanolic extract (135.53±3.166) almost equal to reference drug (128.84±3.873) and shows statistical significance ($p\leq0.005$) reduction in blood glucose level against diabetic control group. These findings clearly established that Ethanolic extract of *G. heterophylla* exhibited better anti-diabetic activity than other extracts (Table 2).

**Table No. 2:** Effect of random blood glucose level after prolonged drug administration

| Group | Dose | Blood glucose level after prolonged treatment | |
|---|---|---|---|
| | | Initial | End of study |
| Normal Control | 2 ml saline | 95.88±2.463 | 93.44±2.753 |
| Diabetic Control | 2 ml Saline | 253.58±2.601$^{\#}$ | 202.45±3.578$^{\#}$ |
| Petroleum ether extract | 160mg/kg b.w. | 265.08±3.021$^{**}$ | 188.77±4.204$^{**}$ |
| Chloroform Extract | 200mg/kg b.w. | 258.46±2.687 | 163.67±3.845$^{**}$ |
| Ethanol extract | 240mg/kg b.w. | 235.50±3.163$^{**}$ | 135.53±3.166$^{**}$ |
| Aqueous Extract | 180mg/kg b.w. | 247.28±3.025$^{**}$ | 170.40±4.072$^{**}$ |
| Glibenclamide | 10mg/kg b.w. | 233.37±3.241$^{**}$ | 128.84±3.873$^{**}$ |

Values are given as Mean±SEM and expressed in mg/dl. $^{\#}p \leq 0.05$ *r*epresents statistical significance against normal control. $^{**}p \leq 0.005$ *r*epresents statistical significance against diabetic control.

The serum lipid profile shows the values in table 3 as total cholesterol, triglycerides LDL and HDL; which increased significantly ($p \leq 0.001$) in diabetic animals in comparison to normal control. The Ethanolic extract shows greater improvement in elevated levels of total cholesterol, triglycerides LDL in comparison to other form of extract, which was statistically significant ($p<0.001$) when compare with diabetic animals. The result also showed that treatment with Ethanolic extract of *G. heterophylla* along with reference drug (Glibenclamide) have significantly ($p<0.001$) comparable of all elevated parameters except HDL at the end of study.

**Table No. 3:** Effect of Biochemical parameters (lipid profile) in serum

| Group | Total cholesterol | | Triglycerides | | Low density lipoprotein | | High density lipoprotein | |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 |
| Normal Control | 3.1±0.21 | 2.5±0.10 | 2.85±0.14 | 2.94±0.19 | 2.16±0.13 | 2.18±0.20 | 2.48±0.21 | 2.86±0.15 |
| Diabetic Control | 3.2±0.15$^{\#}$ | 2.8±0.22 | 3.16±0.11$^{\#}$ | 2.24±0.14$^{\#}$ | 2.65±0.17$^{\#}$ | 2.23±0.12$^{\#}$ | 3.01±0.19$^{\#}$ | 2.65±0.11 |
| Pet. ether extract | 2.4±0.18$^{*}$ | 2.7±0.16 | 2.88±0.15$^{*}$ | 2.19±0.10 | 2.45±0.21$^{*}$ | 2.68±0.15$^{*}$ | 3.67±0.15$^{*}$ | 2.93±0.13$^{*}$ |
| Chloroform Ext. | 3.3±0.25 | 3.0±0.24 | 3.11±0.20 | 2.91±0.14$^{*}$ | 3.05±0.09$^{*}$ | 2.68±0.14$^{*}$ | 2.86±0.16$^{*}$ | 2.99±0.15$^{*}$ |
| Ethanol extract | 2.6±0.25$^{*}$ | 2.0±0.20$^{*}$ | 2.79±0.26$^{*}$ | 2.00±0.12$^{*}$ | 3.11±0.12$^{*}$ | 2.86±0.11$^{*}$ | 3.21±0.14$^{*}$ | 2.58±0.15 |
| Aqueous Extract | 2.8±0.22$^{*}$ | 3.3±0.18$^{*}$ | 2.19±0.14$^{*}$ | 2.41±0.16$^{*}$ | 2.56±0.13$^{*}$ | 2.83±0.17$^{*}$ | 2.88±0.11$^{*}$ | 2.98±0.20$^{*}$ |
| Glibenclamide | 3.0±0.28 | 2.8±0.33 | 2.42±0.09$^{*}$ | 2.11±0.11$^{*}$ | 2.87±0.20$^{*}$ | 2.40±0.25$^{*}$ | 3.00±0.12 | 2.03±0.17$^{*}$ |

Values are given as Mean±SEM and expressed in mM/L. $^{\#}p \leq 0.001$ *r*epresents statistical significance against normal control. $^{*}p \leq 0.001$ *r*epresents statistical significance against diabetic control.

Table 4 shows the effect of *G. heterophylla* leave extracts on biochemical parameters in serum that, the elevated levels of Fasting blood glucose and creatinine in serum of diabetic animals decreased significantly ($p<0.05$) when treated with Ethanolic extract of *G. heterophylla* along with reference drug (Glibenclamide). While fasting plasma insulin level did not show any significant improvement of the entire treatment group in both initial and final day of observation.

**Table No. 4:** Effect of *G. heterophylla* leave extract on biochemical parameters in blood sample

| Group | Fasting blood glucose | | Serum creatinine | | Fasting plasma insulin | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 |
| Normal Control | 88.67±0.54 | 92.45±0.87 | 0.70±0.08 | 0.68±0.05 | 12.01±0.50 | 11.44±0.65 |
| Diabetic Control | 100.23±0.67$^{\#}$ | 158.32±0.83$^{\#}$ | 0.70±0.02 | 0.92±0.06$^{\#}$ | 11.23±0.61 | 13.01±0.42 |
| Pet. ether extract | 99.65±0.94 | 133.06±0.84$^{**}$ | 0.71±0.03 | 0.83±0.06 | 10.88±0.76 | 11.23±0.73 |
| Chloroform Ext. | 88.64±0.92$^{**}$ | 125.30±0.80$^{**}$ | 0.67±0.06 | 0.82±0.04 | 11.25±0.65 | 10.88±0.57$^{**}$ |
| Ethanol extract | 96.86±0.74 | 99.46±0.85$^{**}$ | 0.66±0.02 | 0.62±0.06$^{**}$ | 10.98±0.79$^{**}$ | 11.09±0.86$^{**}$ |
| Aqueous Extract | 84.87±0.34$^{**}$ | 124.86±0.64$^{**}$ | 0.69±0.06 | 0.78±0.07 | 12.01±0.68 | 13.00±0.22 |
| Glibenclamide | 94.54±0.94 | 100.80±0.56$^{**}$ | 0.72±0.02 | 0.62±0.05$^{**}$ | 10.76±0.71$^{**}$ | 10.28±0.63$^{**}$ |

Values are given as Mean±SEM and expressed in mg/dl and mM/L. $^{\#}p \leq 0.05$ *r*epresents statistical significance against normal control. $^{**}p \leq 0.05$ *r*epresents statistical significance against diabetic control.

Table 5 shows the effect of *G. heterophylla* leave extracts on biochemical parameters in urine that, the elevated levels of uric acid, urea and BUN of diabetic animals increased significantly ($p<0.001$) in comparison with normal control. The Ethanolic extract group of *G. heterophylla* along with reference drug (Glibenclamide) shows comparable and statistically significant ($p<0.001$) improvement in comparison to other treatment groups.

**Table No. 5:** Effect of *G. hetrophylla* leave extract on biochemical parameters in urine sample

| Group | Biochemical parameters at the end of study | | |
|---|---|---|---|
| | Uric acid | Urea | BUN |
| Normal Control | 1.023 ± 0.043 | 44.67 ± 0.83 | 20.00 ± 0.35 |
| Diabetic Control | 4.017 ± 0.028$^{\#\#}$ | 79.88 ± 0.67$^{\#\#}$ | 39.18 ± 0.39$^{\#\#}$ |
| Pet. ether extract | 3.002 ± 0.363$^{**}$ | 53.67 ± 0.85$^{**}$ | 27.64 ± 0.48$^{**}$ |
| Chloroform Ext. | 2.978 ± 0.456$^{**}$ | 57.82 ± 0.67$^{**}$ | 25.96 ± 0.53$^{**}$ |
| Ethanol extract | 1.065 ± 0.035$^{**}$ | 44.94 ± 0.57$^{**}$ | 21.34 ± 0.56$^{**}$ |
| Aqueous Extract | 2.139 ± 0.038$^{**}$ | 50.47 ± 0.54$^{**}$ | 25.74 ± 0.36$^{**}$ |
| Glibenclamide | 1.045 ± 0.157$^{**}$ | 46.05 ± 0.64$^{**}$ | 21.45 ± 0.45$^{**}$ |

Values are given as Mean ± SEM and expressed in mg/dl. $^{\#\#}p<0.001$ *r*epresents statistical significance against normal control and $^{**}p<0.001$ *r*epresents statistical significance against diabetic control.

Liver function test shows the value of SGOT, SGPT and total bilirubin in table 6 in serum sample. The activities of these enzymes were found to be significantly increased ($p < 0.001$) in the serum of diabetic rats in comparison with normal control. There were no significant changes in the total Bilirubin levels of the Glibenclamide animals in respect to normal control. Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) elevated levels after oral administration of Ethanolic extract group of *G. heterophylla* along with reference drug (Glibenclamide) at the end of treatment shows comparable and returned significantly ($p<0.001$) near to normal in comparison to other treatment groups.

**Table No. 6:** Effect of *G. hetrophylla* leave extract on liver parameters in serum

| Group | Liver function test at the end of study | | |
|---|---|---|---|
| | SGPT | SGOT | Total bilirubin |
| Normal Control | 68.63 ± 1.72 | 168.5 ± 1.52 | 0.18 ± 0.04 |
| Diabetic Control | 98.68 ± 3.01$^{\#\#}$ | 234.3 ± 0.85$^{\#\#}$ | 0.28 ± 0.03$^{\#\#}$ |
| Pet. ether extract | 74.06 ± 3.16$^{**}$ | 185.8 ± 2.02$^{**}$ | 0.27 ± 0.04 |
| Chloroform Ext. | 84.67 ± 3.41$^{**}$ | 177.5 ± 2.08$^{**}$ | 0.25 ± 0.02$^{**}$ |
| Ethanol extract | 78.56 ± 1.86$^{**}$ | 168.6 ± 1.86$^{**}$ | 0.24 ± 0.05$^{**}$ |
| Aqueous Extract | 75.93 ± 2.08$^{**}$ | 179.4 ± 2.00$^{**}$ | 0.26 ± 0.03 |
| Glibenclamide | 70.33 ± 1.98$^{**}$ | 170.5 ± 1.88$^{**}$ | 0.20 ± 0.05$^{**}$ |

Values are given as Mean ± SEM and expressed in mg/dl. $^{\#\#}p<0.001$ represents statistical significance against normal control and $^{**}p<0.001$ represents statistical significance against diabetic control.

### 3.3Antioxidant activity

Table 7 shows the concentration MDA in kidneys of both controls and experimental groups of rats. There was a significant (p<0.001) elevation in tissue malonaldehide level of diabetic rats in respect to control group. Oral administration of Ethanolic extract of *G. hetrophylla* and Glibenclamide significantly (p<0.05) reduced the elevated levels of MDA in comparison to diabetic rats and which was almost comparable with normal control animal.

The concentration of super oxide dismutase was found significantly (p<0.05) decreased in diabetic rats when compared with normal control. While Ethanolic extract of *G. hetrophylla* and Glibenclamide significantly (p<0.05) improve the elevated levels of SOD in comparison to diabetic rats and almost comparable with the value of normal control group.

Catalase and Glutathione shows statistically significant (p<0.05) lowering the enzyme levels in the diabetic animals compare to normal controls and it was observed that these elevated level significantly (p<0.001) improved in treatment with Ethanolic extract of *G. hetrophylla* and Glibeclamide groups.

**Table No. 7:** Effect of *G. hetrophylla* leave extract on enzyme activity in serum sample

| Group | Antioxidant enzymes at the end of study | | | |
|---|---|---|---|---|
| | MDA[a] | SOD[b] | CAT[c] | GSH[d] |
| Normal Control | 19.86 ± 2.10 | 1.83 ± 0.23 | 0.76 ± 0.04 | 148.78 ± 3.45 |
| Diabetic Control | 53.56 ± 2.32$^{\#}$ | 0.76 ± 0.31$^{\#}$ | 0.23 ± 0.02$^{\#}$ | 86.32 ± 3.00$^{\#}$ |
| Pet. ether extract | 31.22 ± 2.46$^{**}$ | 1.54 ± 0.51$^{**}$ | 0.45 ± 0.06$^{**}$ | 120.69 ± 3.23$^{**}$ |
| Chloroform Ext. | 28.08 ± 2.54$^{**}$ | 1.64 ± 0.46$^{**}$ | 0.54 ± 0.07$^{**}$ | 131.27 ± 2.86$^{**}$ |
| Ethanol extract | 21.00 ± 2.51$^{**}$ | 1.79 ± 0.55$^{**}$ | 0.75 ± 0.03$^{**}$ | 147.55 ± 3.03$^{**}$ |
| Aqueous Extract | 28.87 ± 3.03$^{**}$ | 1.48 ± 0.65$^{**}$ | 0.63 ± 0.05$^{**}$ | 132.76 ± 2.54$^{**}$ |
| Glibenclamide | 20.88 ± 2.20$^{**}$ | 1.80 ± 0.44$^{**}$ | 0.78 ± 0.07$^{**}$ | 149.00 ± 3.00$^{**}$ |

Values are given as Mean ± SEM with 6 animals in each group. $^{\#}p<0.05$ represents statistical significance against normal and $^{**}p<0.001$ represents statistical significance against diabetic group.

a= n mole of MDA/mg of protein. b= Units/mg of protein

c= µ mole of $H_2O_2$ consumed/min/mg of protein. d= µg/mg of protein.

### 3.4 Histopathology

The Histopathological report of the kidney (figure 1) showing the normal tubular structure, tubular epithelium attached with base line of the normal control animals (A). The diabetic animals showing induced nephropathy in which cell cytoplasm completely warned out & the nucleolus is pyknotic & lumen of tubule shown cell debris (B). The animals treated with Petrolium ether extract showing the partial recovery with few damaged tubules, damaged cells with pyknotic nucleolus (C). The animals treated with Chloroform Extract showing less recovery, damaged tubules still present with cell debris in lumen of tubule (D). The group treated with Ethanol extracts has observing good recovery from the damaged tubules and new cells were replacing the damaged ones of the tubules (E). The group administered with Aqueous Extract showing ongoing partial recovery from damage (F). The Glibenclamide treated group was showing the good recovery with apparently normal tubules (G).

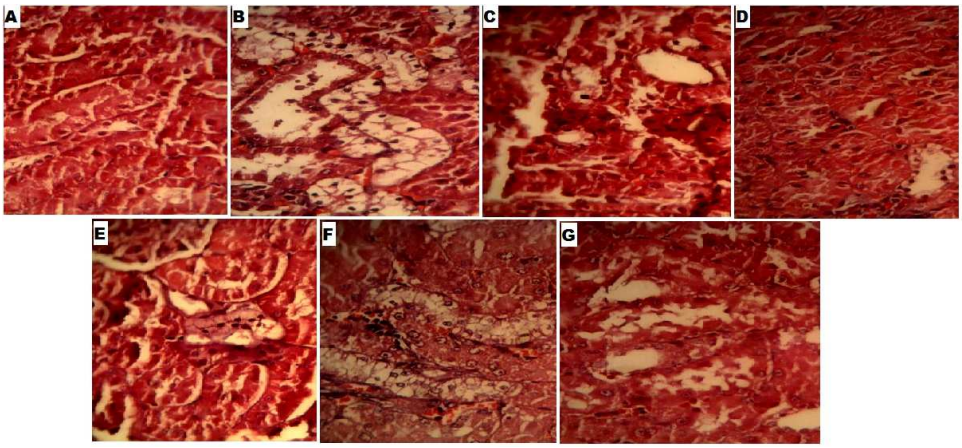


**Figure No. 1:** Histopathology of kidney tissues; (A) Normal Control, (B) Diabetic Control, (C) Petroleum ether extract, (D) Chloroform Extract, (E) Ethanol extract, (F) Aqueous Extract, (G) Glibenclamide by Hematoxylin and eosin stain with Bar 100 mm.

## 4. Conclusion

The overall results of the antidiabetic activity have led to the conclusion that ethanolic extract has exhibited more significant antidiabetic activity which compared with other extracts as it contains major chemical constituents viz. steroids and tannins. Aqueous and chloroform extracts also showed significant activity as compared to diabetic control which may be due to the combination of various active constituents. However, this claim demands for further study to pinpoint the mechanism of the extracts and formulation of *Girardinia heterophylla* leaves in combination or single for the development of herbal formulation.[19,20]

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