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BIOACTIVITY EVALUATION OF BERGENIA CILIATA

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ABSTRACT

Bergenia ciliata was subjected to bioactivity analysis. The records of these investigations are described in this communication. A study made on the bioactivity analysis of medicinal herb *Bergenia ciliata*, which in folkloric medicine is used to cure hypoglycemic activity. A battery of assays was performed on different extracts of *Bergenia ciliata* which include hypoglycemic activities, toxic evaluations such as acute systemic and intracutaneous toxicity as well hemolysis test. *Bergenia ciliata* has been employed in folklore medicine to treat symptoms of diabetise mellitus. All the extracts except chloroform extract of root and leaves of *Bergenia ciliata* were found to possess hypoglycemic activity in Streptozotocin (STZ) treated rats. Therefore the plant can be classified as hypoglycemic, hypoglycemic activity in experimental diabetes ranging from 40-70% of its onset to reduce blood glucose level.

The toxicological investigations of *Bergenia ciliata* with particular reference to acute systematic toxicity and intracutaneous toxicity in experimental animals displayed that it ellicit severe toxicity. The symptoms of toxicity in intracutaneous test showed erythema and edema whereas assessment of acute systemic toxicity frequently observed breathing problem and initiations of diarrhea with blood in stool of experimental model and caused gastero-intestinal syndrome. *Bergenia ciliata* can produce toxicity suggesting a role in certain diseases. It is therefore, premature to speculate about mechanism of effect until toxin(s) is unequivocally identified. The hemolysis test on the extract of *Bergenia ciliata* was almost devoid of activity.

INTRODUCTION

Bergenia ciliata (Haw.) Sternb. belongs to family saxifragaceae. The genus Bergenia camprises of about 6 species, distributed in the temperate Himalayas and Central and East Asia, represented in Pakistan by two species namely Bergenia ciliata and *Bergenia stracheyi*. It is perennial herb upto 50 cm tall, succulent, distributed in temperate Himalayan region (from Kashmir to Nepal) from 2000-2700 m. very common rocks in and around the Murreee area (Ghazanfar S., 1977).

The root of Bergenia has been considered to enjoy all the useful attributes of Gentirna root and has been regarded as demulent and deobstruent, relieves pain in ribs and chest due toexcessive cold hmours, acts as effective diuretic and emmenagogue. Get-rid of kidney's and bladder stones and obstructions or toxic waste products, which remain in the alimentary canal, and urinary excretory system. The infusion is considered to be more active than root. In asthma, bronchitis, epilepsy and spasmodic affections and to relieve flatulent colic in children. Root is effective to cambat chronic venereal diseases (Usmanghani K. *et al.,* 1997).

Hypoglycemic Activity:

Hypoglycemic activity of different extracts of *Bergenia ciliata* was tested by determining blood sugar lowering effect on streptozotocin (STZ) induced rats (Basnet P. *et al.,* 1994). The

selected animals were housed in an air-conditioned animal house and fed on pellets (containing protein, lipid and carbohydrate) and water. The animals were made fasted by depriving them food for about 16 hours prior to the experiment, but were allowed free access to tap water. For preparation of diabetic model animals a single dose of 30, 40, 50, 60 and 70 mg/kg. of Streptozotocin in 10 m M citrate buffer (pH 4.5) was administered intravenously into the tail vein of the fasted rats. The animals were supplied with normal food and water after 30 minutes of the streptozotocin IV injection. The blood glucose level were measured at different time intervals of 0 hour, 6, 24,48, 72 hours, 7 days, 14 days and 21 days, after STZ administration.

***Materials**:*

Male adult albino rats weighing 120-140 gm, Streptozotocin, Plastic tubes, Heparin, Insulin syringes, Glucometer (Lifescan), Mice trap.

Procedure:

For the detection of hypoglycemic activity of the root and leaves extracts of *Bergenia ciliata*: **1.** The model rats were prepared by administering the STZ in 10 m M citrate buffer (pH4.5) through I.V. route at a dose of 50 mg/kg. **2.** The blood glucose level was examined after 4 days of STZ administration. **3.** The animals having high blood glucose level (more than 300 mg/dl and less than 550mg/dl) were considered as diabetic animals and divided into groups. **4.** On the 5^{th} day of STZ administrations the solution of the extracts were administered to the animals intraperitoneally. **5.** Five doses of drugs twice a day were administered at the dose of 200mg/kg body weight. **6.** Blood glucose level was examined 6 hours after giving the last dose. **7.** For the positive control a mixture of tolbutamide (200 mg/kg) was used. **8.** For the negative control equal volume of physiological saline was used. **9.** Blood samples were syringed out through the tail vein and immediately transferred into plastic tubes rinsed with heparin. **10.** The glucose level in the whole blood samples were analyzed within an hour by Glucometer using glucose strips.

Table 1

Effect of various single dose streptozotocin (STZ) on rat's blood glucose level.
Results are expressed as mean $\pm$ SE, n=6

Time interval	Control	30mg/kg	40mg/kg	50mg/kg	60mg/kg	70mg/kg
0 hour	140	140	140	139	141	142
6 hours	141	220	300	360	350	355
24 hours	141	350	410	500	520	530
48 hours	150	345	450	505	520	530
96 hours	152	410	460	520	530	535
1 week	150	440	460	475	540	555
2 weeks	152	415	450	480	540	550
3 weeks	160	410	455	485	530	545

Table 2
Effects of leaves extract of *Bergenia ciliata* (each five doses twice a day, intraperitoneally) on blood glucose level in STZ-induced diabetic rats

Groups	Dose (mg/kg)	No. of animals	Blood glucose level (mg/dl)		
			Before drug	After drug	Decrease (%)
Control	200	6	422.9 ± 28.6	454.8 ± 20.3	N/A
Positive Control	200*	6	429.2 ± 42.4	294.0 ± 22.4	36.37
Ethanol	200	6	428.8 ± 20.9	137.8 ± 6.0	70.13
Hexane	200	4**	425.8 ± 26.5	420.20 ± 20.8	N/A
Ethyl Acetate	200	6	430.0 ± 22.3	251.5 ± 55.1	45.67
Chloroform	200	6	455.2 ± 26.8	283.7 ± 21.9	42.13
Butanol	200	6	430.4 ± 26.5	428.22 ± 20.8	N/A
Aqueous	200	6	436.6±20.0	134.8±13.8	71.34

Results are expressed as mean ± SE. *Positive control group was administered 5 doses of a mixture of 200 mg/kg of tolbutamide. **2 of the experimental animals died. N/A (non or almost negligible activity).

Table 3
Effects of roots extract of *Bergenia ciliata* (each five doses twice a day, intraperitoneally) on blood glucose level in STZ-induced diabetic rats

Groups	Dose (mg/kg)	No. of animals	Blood glucose level (mg/dl)		
			Before drug	After drug	Decrease (%)
Control	200	6	422.9 ± 28.6	454.8 ± 20.3	N/A
Positive Control	200*	6	429.2 ± 42.4	294.0 ± 22.4	36.37
Ethanol	200	6	436.6 ± 20.0	134.8 ± 13.8	71.34
Hexane	200	6	428.8 ± 20.9	137.8 ± 6.0	70.13
Ethyl Acetate	200	6	460.4 ± 19.5	208.6 ± 44.5	57.88
Chloroform	200	6	392.8 ± 32.3	396.4 ± 20.2	6.13
Butanol	200	6	391.0 ± 41.1	217.5 ± 18.9	48.27
Aqueous	200	6	460.4 ± 19.5	208.6 ± 44.5	57.88

Results are expressed as mean ± SE. *Positive control group was administered 5 doses of a mixture of 200 mg/kg of tolbutamide. N/A (non or almost negligible activity).

Preparation of Test Sample:

The sample (extract) weighing 1.0 gram was dissolved in 200 ml water, to make the solution 0.5% (w/v). Before injection the solution was passed through 0.2 µ membrane filter. Each extract was agitated vigorously prior to withdrawal of injection doses to ensure even distribution of the extract. This procedure was performed for all the leaves and roots extracts, subjected for injection. Effect of various single dose STZ on rat's blood glucose in table 1, effect of leave extract of Bergenia ciliata on blood glucose level in table 2 and Effects of roots extract of *Bergenia ciliata* are given in table 3.

Acute Systemic Toxicity Test:

Toxicity reactions of all roots and leaves extracts of *Bergenia ciliata* have been studied in healthy living albino mice. The animals were housed in an air-conditioned animal house and were fed on pallets and water. The animals were not previously used for test. The test samples were prepared from ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous extracts of the leaves and roots were prepared separately for the performance of the test. Each group comprised of 5 albino mice (British Pharmacopoeia 1993 and The United State Pharmacopoeia 1990).

Materials:

1. Male albino mice, weighing between 17 to 23 grams, **2.** Mice trap, **3.** Disposable, **4.** Insulin Syringes 1 ml, **5.** 0.2 µ membrane filter, **6.** fiteration assembly, **7.** Ethanol, **8.** Injectable Normal saline Solution.

Preparation of Test Sample:

The sample (extract) weighing 1.0 gram was dissolved in 200 ml water, to make the solution 0.5% (w/v). Before injection the solution was passed through 0.2 µ membrane filter. Each extract was agitated vigorously prior to withdrawal of Injection doses to ensure even distribution of the extract. This procedure was performed for all the leaves and roots extracts, subjected for toxicity test.

Control Solution:

Injectable Normal Saline Solution was used as control solution.

Procedure For Injection:

1. Healthy male mice were weighed on electric balance individually. **2.** Mice were fixed into the trap in a position that the tail was set free. **3.** The tails were disinfected with 70% ethanol. By using Insulin syringes 1.0 ml / 20 gm body weight, test solution was Injected Intravenously into the vein of the tail of the each mice. Time of the injection occupied about 15-30 seconds. **4.** Control Solution was also injected intravenously into the tail vein in the same manner as carried out for the test solution. **5.** Mice groups were transferred in different cages and marked with their identification.

Interpretation of Results:

Animals were observed immediately after injection. Again 4 hours after injection and then at 24 hours, 48 hours and 72 hours.

Biological reactivity includes erection of hairs, skin diseases, difficulty in breathing, gross behavioral effects and any other abnormal activities were observed.

The test results are given in table 4 and table 5.

Table 4
Acute toxicity test of roots extracts of *Bergenia ciliata*

Sample Name	Observation after				
	Immediately	**4 hours**	**24 hours**	**48 hours**	**72 hours**
Ethanol N=5	Breathing problem with Idleness	Recovered and started normal activity	Diarrhea with blood	Idleness	Normal activity
Hexane N=5	Breathing problem with idleness	Breathing problem with idleness	Normal activity	Normal activity	Mild erections of hair
Ethyl acetate N=5	Breathing problem with idleness	Recovered and started normal activity	Normal activity	Normal activity	Normal activity
Chloroform n=5	Breathing problem with idleness	Recovered and started normal activity	Normal activity	Normal activity	Normal activity
Butanol n=5	Normal activity	Normal activity	Loose motion	Mild erections of hairs	Normal activity
Aqueous n=5	Normal activity	Normal activity	Idleness	Idleness	Started normal activity
Control n=5	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity

Table 5
Acute toxicity test of leaves extracts of *Bergenia ciliata*

Sample Name	Observation after				
	Immediately	**4 hours**	**24hours**	**48 hours**	**72 hours**
Ethanol n=5	Breathing problem with Idleness	Recovered and started normal activity	Normal activity	Normal activity	Normal activity
Hexane n=5	Breathing problem with Idleness	Breathing problem with Idleness	Recovered and started Normal activity	Normal activity	Mild erection of hair
Ethyl acetate n=5	Breathing problem with Idleness	Recovered and started normal activity	Normal activity	Normal activity	Normal activity
Chloroform N=5	Breathing problem with Idleness	Recovered and started normal activity	Normal activity	Normal activity	Normal activity
Butanol n=5	Breathing problem with Idleness	Recovered and started normal activity	Normal activity	Normal activity	Normal activity
Aqueous n=5	Normal activity	Idleness	Idleness	Normal activity	Normal activity
Control n=5	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity

Intracutaneous Test:

The biological reactivity of the root and leaves extracts of *Bergenia ciliata* was determined by intracutaneous test according to the pharmacopoeia (The United State Pharmacopoeia 1990). Local responses like erythema, edema or necrosis have been determined for the leaves and root extracts of *Bergenia ciliata* following intra-cutaneous injection into the albino rabbits. The rabbits were housed in an air-conditioned animal house and were fed on pellets and water. The rabbits selected for tests were with thin, clean unblemished skin, free from mechanical irritation and trauma. For each sample two rabbits were used. The test samples were prepared from ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous extracts of the leaves and roots of the plant. The injectable solution 0.5% w/v was prepared separately for all the extracts.

Materials:

Albino rabbits weight not less than 2.5 Kg, Electric shaver, Disposable insulin syringes 1 ml., Normal saline, Ethanol, Isotonic NaCl solution.

Preparation of Test Samples:

The sample (extract) weighing 1.0 g. was dissolved in 200 ml water, to make the solution 0.5%. Before injection the solution was passed through 0.2 μ membrane filter. Each extract solution was agitated vigorously prior to withdrawal of injection doses for the assurance of even distribution in the solution. This procedure was performed for all the leaves and roots extract.

Blank Solution:

Isotonic normal saline solution (0.9%) was used as blank solution.

Procedure:

On the day of the test, the fur was closely clipped on animals back with the electric shaver on both sides of the spinal column over a sufficiently large test area. Mechanical irritations and trauma was avoided. Loose hairs were removed by means of vacuum. The skin was lightly swabbed with ethanol. Five spots 2.5 cm away from the spinal column and 2 cm away from each other were marked at one side of spinal column and similarly five spots at the other side of the spinal column were also marked.

- 0.2 ml sample solution for every spot was injected Intracutaneously on one side of the spinal column by using insulin syringes.

- 0.2 ml blank solution was also injected intracutaneously at the spots of other side of spinal column

The same procedure was repeated on the other rabbits with test and blank solutions.

Interpretation of Results:

In order to facilitate the readings, injection sites were lightly swabbed with diluted alcohol. The rabbits were examined immediately after injection, at 24, 48 and 72 hours for any tissue reaction, like, erythema, edema, or necrosis. The average reaction of the injected sites was compared with the site of the blank solution. Observations were noted on following numerical scale for the extract of the samples and for blank.

Table 6
Evaluation of skin reactions

Erythema and Eschar Formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4

Edema Formation	**Value**
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

Table 7
Intracutaneous test of the leave extracts of *Bergenia ciliata*

Sample Name	**Observation after**			
	Injection (immediately)	24 hours	48 hours	72 hours
Ethanol	Mild Irritation	Edema,erythema 2, 2	Edema, erythema 2, 3	Edema,erythema 2, 4 with puss formation
Hexane	Mild irritation	Edema 2	Edema 2	Edema 3
Ethyl acetate	Normal	Edema , erythema 1,2	Erythema 2	Erythema 1
Chloroform	Mild irritation	Erythema 2	Edema, erythema 1,1	Erythema 1
Butanol	Normal	Erthyema 1	Normal 0	Normal 0
Aqueous	Mild irritation	Erythema 3	Erythema, 2 puss formation	Erythema 1
Control	Normal 0	Normal 0	Normal 0	Normal 0

The results are mean of two rabbits with total 10 spots in each case.

Table 8
Intracutaneous test of the roots extract of *Bergenia ciliata*

Sample Name	Observation after			
	Injection (immediately)	24 hours	48 hours	72 hours
Ethanol	Mild Irritation 0	Edema, erythema 2, 3	Edema, erythema 4, 3	Edema, erythema 4, 2 with puss formation
Hexane	Mild irritation 0	Erythema 2	Erythema 2	Erythema 3
Ethyl acetate	Mild irritation 0	Edema , erythema 1, 1	Edema 2	Edema 1 With puss formation
Chloroform	Normal 0	Erythema 1	Normal 0	Normal 0
Butanol	Mild irritation 0	Erythema 2	Erythema 1	Normal 0
Aqueous	Normal 0	Erythema 2	Erythema 1	Erythema 1
Control	Normal 0	Normal 0	Normal 0	Normal 0

The results are mean of two rabbits with total 10 spots in each case.

Hemolysis Test:

Hemolytic effects of different extracts of *Bergenia ciliata* have been determined on rabbit's blood. The test samples were prepared from ethanol, hexane, ethyl acetate, chloroform, butanol, and aqueous extracts of leaves and roots of *Bergenia ciliata* (The Pharmacopoeia of Japan, 1991).

Materials:

Rabbit's blood (5 to 10 ml), Glass beads, Nessler cylinder, Pippet 1 ml, Three Test Tubes, Muslin clothes, Syringe 5 ml.

Preparation of Test Samples:

The sample (extract) weighing 1.0 gram was dissolved in 200 ml water, to make the solution 0.5% w/v. Before injection the solution was passed through 0. 2 µ membrane filter. This procedure was performed for all the leaves and roots extracts, subjected for hemolysis test.

Control Solutions:

Injectable 0.9% normal saline solution was used as negative control whereas distilled water was taken as positive control.

Preparation of Defibrinated Blood:

- 5 ml to 10 ml rabbit's blood was drawn from the ear vein of the rabbit and it was transferred in a Nessler cylinder containing 10 to 20 glass beads in it.
- Nessler cylinder was covered through a lid and shaken until the collision noise of glass beads stopped while shaking (this indicated complete coagulation of blood protein around the glass beads).
- The blood was filtered in a clean test tube through a Muslin cloth; test tube was covered with a stopper.

Test Procedure:

- Three tubes were marked as "sample", " negative control" and "positive control".
- 10ml test sample, Normal saline and distilled water respectively were taken in the tubes individually. 0.1 ml of defibrinated blood was added in all three tubes by a clean pipette. The tubes were covered properly and incubated at 37^{o}C for 24 hours.

Interpretation of Results:

The tubes were observed for hemolysis in the positive control and for button formation in negative control and samples were checked against the negative and positive controls.

Table 9
Hemolytic test of the leaves extract of *Bergenia ciliata*

Sample Name	Observation after	
	I hour	**Hours**
Ethanol	Weak lysis occurred. Slight yellow-ish red colour produced	Weak lysis occurred. Slight yellow-ish red colour produced
Hexane	Button formation at bottom. No hemolysis occurrence	Button formation at bottom. No hemolysis occurrence
Ethyl acetate	Weak lysis occurred. Slight yellow-ish red colour produced	Weak lysis occurred. Slight yellow-ish red colour produced
Cloroform	Button formation at the bottom appeared. No lysis occurred	Button formation at the bottom appeared. No lysis occurred
Butanol	Button formation at the bottom appeared. No lysis occurred	Button formation at the bottom appeared. No lysis occurred
Aqueous	Button formation at the bottom appeared. No lysis occurred.	Button formation at the bottom appeared. No lysis occurred.
Positive Control	Lysis occurred Red coloration formed	Lysis occurred Red coloration formed
Negative Control 0.9% saline sol.	Button formation at the bottom no lysis occurred	Button formation at the bottom no lysis occurred

Table 10
Hemolytic test of the roots extract of *Bergenia ciliata*

Sample Name	Observation after	
	I hour	**24 hours**
Ethanol	Weak lysis occurred. Slight yellowish red color produced	Weak lysis occurred. Slight yellowish red color produced
Hexane	Button formation at the bottom no lysis occurred	Button formation at the bottom no lysis occurred
Ethyl acetate	Button formation at the bottom no lysis occurred	Button formation at the bottom no lysis occurred
Chloroform	Button formation at the bottom no lysis occurred	Button formation at the bottom no lysis occurred
Butanol	Weak lysis occurred. Slight yellowish red color produced	Lysis occurred. Red coloration produced.
Aqueous	Button formation at the bottom no lysis occurred	Button formation at the bottom no lysis occurred
Positive Control	Lysis occurred Red coloration formed	Lysis occurred Red coloration formed
Negative Control 0.9% saline sol.	Button formation at the bottom no lysis occurred	Button formation at the bottom no lysis occurred

RESULTS AND DISCUSSION

Plants have been a source of medicinal compounds since pre-historic time. It is well established that all parts of plants were used in *Ayurvedic*, *Tibbi*, and *Unani* systems of medicine for centuries. However, the discovery and use of synthetic drugs led to a dramatic decline in the popularity of herbal products used in the therapy. Nevertheless the realization of harmful toxic effect of a large number of synthetic drug led to for alternative sources which would be safe and effective in various ailments.

A resurgence of interest in the study and use of medicinal plant taking place during the last three decades. A considerable growth has occurred in official and commercial interest in the use of natural products (Akerele O., 1992). In recent years there has been a growing trend to evaluate the bioactvity of the medicinal plants, so that a systematic approach could be adopted for their therapeutic utilization. The present study is an attempt to investigate and evaluate the bioactivity of *Bergenia ciliata*, which is of considerable medicinal importance.

As such the different chemical constituents elaborated by different spp. of *Bergenia* displayed are coumarin benzenoids, steroids and tannins and the antibacterial activity of *Bergenia ciliata* may emencipate due to either of these constituents.

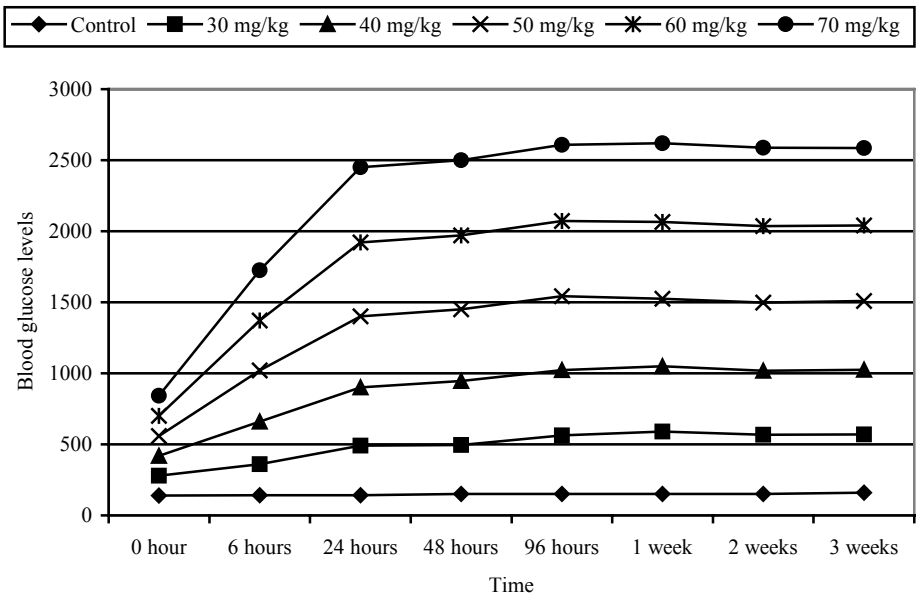

Fig. 1: Graphic representation of the effects of various doses of STZ on blood glucose level of rats.

Hypoglycemic Activity:

The hypoglycemic activity of the extracts of *Bergenia ciliata* was tested by determining the blood sugar lowering effects in rats. Streptozotocins (STZ) induced diabetic rats were prepared by inducing hypoglycemia with a single intravenous dose of 50mg/kg. After 4 days of STZ administration, blood glucose level was examined and on the fifth day of STZ administration, the solution of root and leaves extracts were administered to the animals intraperitoneally. A dose of 200 mg/kg body weight twice a day for 5 times were given to the animals. Each group comprised of 6 rats after five doses the blood glucose level of the animals were analyzed and compared with the control. The results are shown in fig. 1, exhibited that animals suffered with severe hypoglycemic upto 6 hours after the administration of STZ through intravenous route. The blood glucose level remained constantly high after 24 hours of STZ-administrations by all doses for 30-mg to 70mg. The experiment led to conclude that STZ by the dose of 60 and 70mg produces a severe diabetic model while 30 and 40 mg/kg produces comparatively mild diabetes in rats.

The effects of various *Bergenia ciliata* leaves extract on blood glucose levelare as shown in Fig. 2 and Table 2 revealed that the ethanol extract has significantly lowered 70.13% the blood glucose level (i.e. from 428.8±2.0 mg/dl to 137.8±6 mg/dl). The blood sugar level was reduced from 436.6±20.0 mg/dl to 134.8±13.8 mg/dl (71.34% reduction) in aqueous extract treated rats.

In chloroform extract treated rats blood sugar level reduced from 455.2±283.7±21.9 mg/dl (i.e. 42.13% decrease). Ethyl acetate extract treated rats have shown the decrease in blood glucose level from 443.0±22.3 mg/dl. Whereas hexane and butanol treated extract have not shown any hypoglycemic activity and no significant reduction in the glucose level has been observed in the animals treated with these extracts.

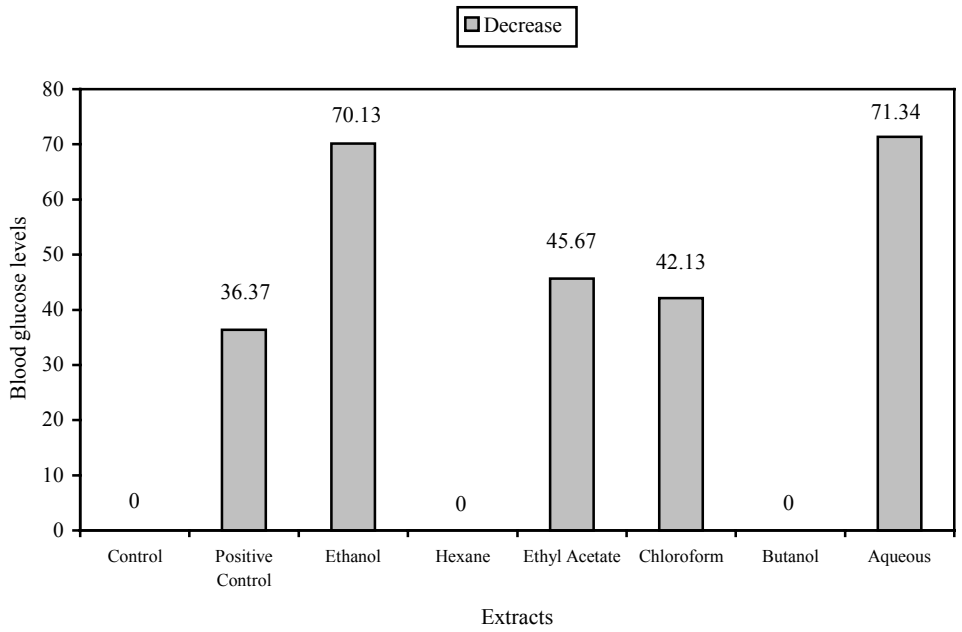

Fig. 2: Graphic representation of the effects of various leaves extracts on blood glucose level of rats.

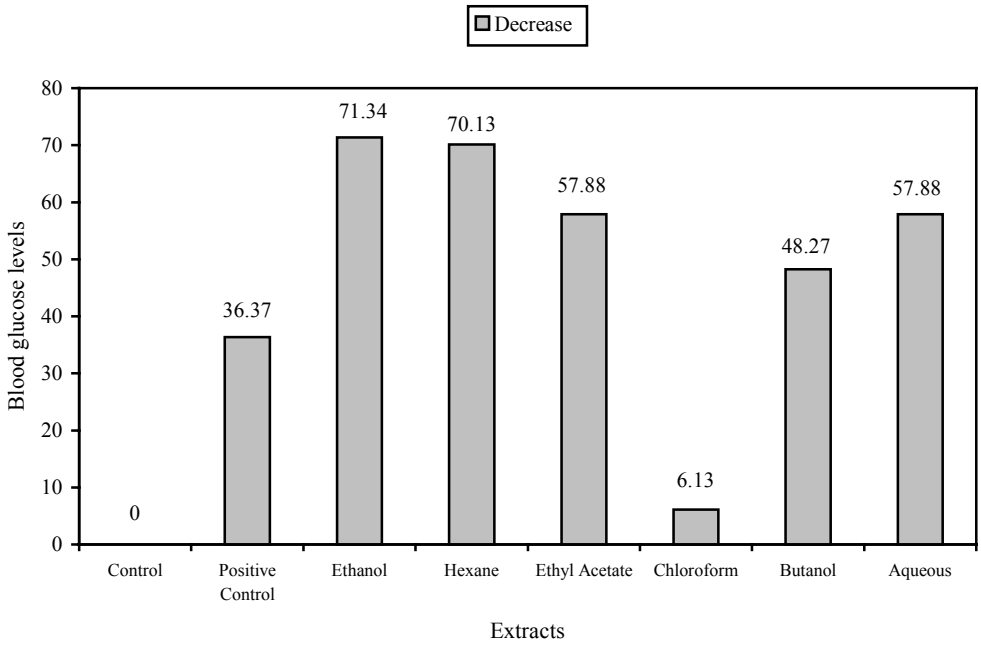

Fig.3: Graphic representation of the effects of various doses of roots extracts on blood glucose level of rats.

The hypoglycemic activity evaluation of the roots extract of *Bergenia ciliata* as shown through graphic representation in Fig. 3 and table 3 revealed that the ethanol extract treated rats has shown lowering of glucose level from 436.6±20.0 to 134.8±13.8 (71.34%). In hexane extract treated rats the glucose level reduced from 428.8±20.9 to 137.8±6.0 (70.13%) whereas ethyl acetate extract 460.4±90.5 to 208.6± 44.5 (57.88%) has exhibited moderate hypoglycemic activity. The butanol 391.0 ± 41.1 to 217.5 ± 18.9 extract treated rats has shown 48.27% decrease in blood glucose level in STZ induced animals. The aqueous 460.4± 19.5 to 208.6 ± 44.5 extract has exhibited 57.83% decrease in blood glucose level of the rats. The chloroform 6 %extract failed in showing any hypoglycemic activity in the rats.

Plant as a souce of antidiabetic agent have been widely screened, and out of 492 total number of plants tested 83 spp. were found to be active. Most widely used traditional antidiabetic plant cited are *Momordica catharanthus, catharanthus roseus, Anacardum occidentale, syzygium cumini, Lupium albus, Aloe vera, Allium cepa, Taraxacum officinale, Kyllinga monocephala, Phyllanthus emblica, Azadirachta indica, Morus alba, Poterium ancistroides* and *Daucus carota*.

The hypoglycemic natural products studied belongs to different chemical classes and the potential molecules cited are alkaloids, carbohydrate, coumarin cyanogenic glycoside, Flavonoids, glycopeptides, Peptides and amides, steroids and terpenoids (Marles R.J. & Farnsworth N.R., 1994) while different researchers have put up their studies with strong potential for the discovery of novel antidiabetic agents. But the increasing incidents of diabetic mellitus both Insulin dependent diabetic mellitus (IDDM) and non insulin dependent diabetic mallitus (NIDDM). In population of both developing and developed countries. The botanical source could be used for NIDDM controls and the natural sources could be of value and the great expense of modern therapy. Therefore there is clear need for the development of herbal strategies for diabetic therapy. Although *Bergenia* exhibited a positive response as an antidiabetic potential, but due to its toxicity a detail investigation is further needed to decifer its exact mechanism of actions.

Among the six extracts of roots of *Bergenia ciliata* five of them viz. ethanol, hexane, ethyl acetate, chloroform and aqueous extracts have shown significant hypoglycemic activity. Whereas among six leaves extracts of *Bergenia ciliata* four of them viz. Ethanol, ethyl acetate, chloroform and aqueous extracts have exhibited hypoglycemic activity. In the literature citation it is mentioned that *Bergenia stracheyi* ethanol-water (1:1) extract was subject to hypoglycemic activity analysis through oral administration (gastric intubation) of rats and it was determined that dose of 250 mg/kg is actively to lower the sugar level in the experimental animals (Aswal B.S. *et al.*, 1984). However the hypoglycemic carried out on *Bergenia ciliata* was extensive in a way that extracts were administered intraperitoneally (ip) and that comparatively leaves and roots extracts showed almost the activity at 200 mg/kg which is rather significant.

Acute Toxicity Test:

The acute toxicity was determined in albino mice by employing the Pharmacopeial test method for toxicity assessment (British Pharmacopoeia, 1993 & The United States Pharmacopoeia, 1990). Doses of the extracts and control solution were given in a group of 5 mice each. The intravenous route was selected for the administration of the extracts of *Bergenia ciliata*. After intravenous administration of the extracts and control solution, animal were observed for biological reactivity, which includes erection of hairs, skin diseases, breathing conditions, any other abnormal activities and gross behavioral effects. Observations were made immediately just after the administration of every extract, 4 hours, 24 hours, 48 hours and finally after 74 hours. The results recorded are shown in table 4 and table 5.

Toxicity results of root extracts of *Bergenia ciliata* revealed (table 4) that animals immediately after the administration of ethanol, hexane, ethyl acetate and chloroform extracts exhibited problem in breathing and movement, though in the later stages i.e. after 4 hours animals recovered and started moving normally. Animals which were administered ethanol extract started diarrhea with blood after 24 hours, followed by idleness after 48 hours. After 72 hours animals started normal activity. Ethyl acetate and chloroform extracts tested groups of animals exhibited normal activity after 24 hours. The animals given hexane extract showed normal activity after 24 hours but after 72 hours slight erections of hair was noted. Animals injected with butanol extract acted normal for initial 4 hours, but later after 24 hours displayed toxic reactions and responded with diseased condition like loose motion and erection of hairs. Animals subjected to aqueous extract were also remained normal for initial 4 hours but after 24 hours developed manifestation idleness. This condition persisted for 48 hours but after 72 hours the animals resumed normal activity.

Results of Leave extracts of *Bergenia ciliata* revealed (table 5) that ethanol, hexane, ethyl acetate, chloroform and butanol extracts just after administration produced its effects and as a result animals started facing difficulty in breathing and their agility have remarkably reduced. It was noted that animals injected with ethanol, ethyl acetate, chloroform and butanol extracts recovered after 4 hours and resumed their normal activity throughout the evaluation period. The animals given hexane extract exhibited difficulty in breathing and movement comparatively for longer period than the ethanol, ethyl acetate, chloroform and butanol extracts. The animals administered hexane extract, responded abnormal behaviour and after 24 hours became normal but after 72 hours symptoms of toxic reaction appeared and animal developed mild erection of hairs. Animals injected aqueous extract exhibited no abnormal activity just after the administration of drug but after 4 hours idleness in the animals developed which continued and later after 48 hours the animals became normal.

Literature survey reveals that extracts of genus *Bergenia* produces toxic reactions in the animals (Dhar M.L. *et al.,* 1968). The present study also have produced the evidences of toxic reactions in the albino mice. Consequently it can be inferred that both root and leave extracts are capable of producing toxic reactions. In comparision it can be concluded that root extracts are more toxic than the leave extracts.

Intracutaneous Test:

The intracutaneous biological reactivity test has been carried out in albino rabbits in conformance with the testing procedure as mentioned in pharmacopoeia such as B.P, U.S.P. (British Pharmacopoeia, 1993 & The United States Pharmacopoeia, 1990). Tests for every extract was conducted on two rabbits. Skin of the dorsal part along the vertebral column was selected for the intracutaneous administration of both *Bergenia ciliata* extracts and blank solution. The animals were examined for biological reactivity, which includes edema, erythema, necrosis and gross behavioral effects followed by intracutaneous injection. Observations were made immediately after the administration of every extract, at 24 hours, 48 hours and finally at 72 hours.

The results of biological reactivity of roots extract of *Bergenia ciliata* are displayed in Fig. 4 and table 8. The results revealed that animals exposed to ethanol, hexane, ethyl acetate and butanol extracts have responded restlessness immediately. Animals, which were injected with ethanol extract after 24 hours have showed slight edema and moderate erythema (numerical value 2,3), while after 48 hours edema raised more than 1 mm and extending beyond the area of exposure (numerical value 4), while continuation of the experimental at 72 hours, the condition of the

edema remained same, whereas condition of erythema reduced (numerical value 2). Results of hexane extract exhibited well-defined erythema (numerical value 2) after 24 hours, and this continued till the examination at 72 hours, while the observation after 72 hours the erythema increased from moderate erythema to severe erythema (numerical value 3). Ethyl acetate extract has shown very slight edema and erythema (numerical value 1,1) after 24 hours, while at 48 hours erythema vanished but edema was increased (numerical value 2). At 72 hours edema reduced (numerical value 1) but puss has developed at the injection site. Animals, which were injected butanol extract, have shown well-defined erythema (numerical value 2) after 24 hours, however at 48 hours erythema was reduced in size and redness, and was barely perceptible (numerical value 1). Consequently at 72 hours the animal became normal. Unlike the ethanol, hexane, ethyl acetate and butanol extracts, chloroform and aqueous injected with animals, behaved normal like that of control solution animals, and after 24 hours very slight erythema was developed, culminating at 48 hours completely vanished and this condition remained normal throughout the evaluation period i.e. 72 hours.

Results of leave extracts of *Bergenia ciliata* as shown in Fig. 4 and table 7 revealed that animal immediately after the administration of ethanol, hexane, chloroform and aqueous extracts developed mild irritation. The animals which were administered ethanol extract at 24 hours have developed edema and erythema (numerical value 2 and 2), at 48 hours erythema raised from moderate to severe (numerical value 3) at 72 hours slight eschar formations occurred and erythema became severe (numerical value 4) whereas edema remained constant i.e. well defined (numerical value 2). Animals which were given hexane extract developed slight edema (numerical value 2) which persisted till the observation at 72 hours. At 72 hours size of the edema raised approximately 1mm (numerical value 3). Results of the animals administered hexane extract at 24 hours exhibited very slight edema and well-defined erythema (numerical value 1 and 2) at 48 hours edema recovered and well-defined erythema persisted. At 72 hours the condition of erythema gradually improved and very slight erythema (numerical value 1) left at this stage. Animals, which were administered chloroform extract initially at 24 hours, have shown well-defined erythema (numerical value 2). At 48 hours though the erythema reduced (numerical value 1) but very slight edema has appeared. At 72 hours edema was removed and erythemic condition also improved and very slight erythema (numerical value 1) left at this stage. Animals who received butanol extract initially have shown very slight edema (numerical value 1) but at later examinations it found without any signs of abnormal conditions (numerical value 0). Animals which were given aqueous extract exhibited moderate to severe erythema (numerical value 3), this condition gradually improved and erythema comparatively of smaller size at 48 hours (numerical value 2) which ultimately at 72 hours become very slight (numerical value 1). Unlike animals of ethanol, hexane, chloroform and aqueous extract, Ethyl acetate and butanol extract administered animals did not show any irritations and their behavior was same like control solution animals.

The result of intracutaneous toxicity testing of the extracts of root and leaves of *Bergenia ciliata* very clearly predicted that the extracts are exhibiting toxcity causing erythema and edema either simultaneously or one after the other. If a comparative account is chalked out of both the extracts, then it appears that root extract is showing more intracutaneous toxicity than that of leaves extracts. Therefore as such at the concentration of 0.5% w/v, this plant is not suitable to be administered intracutaneously.

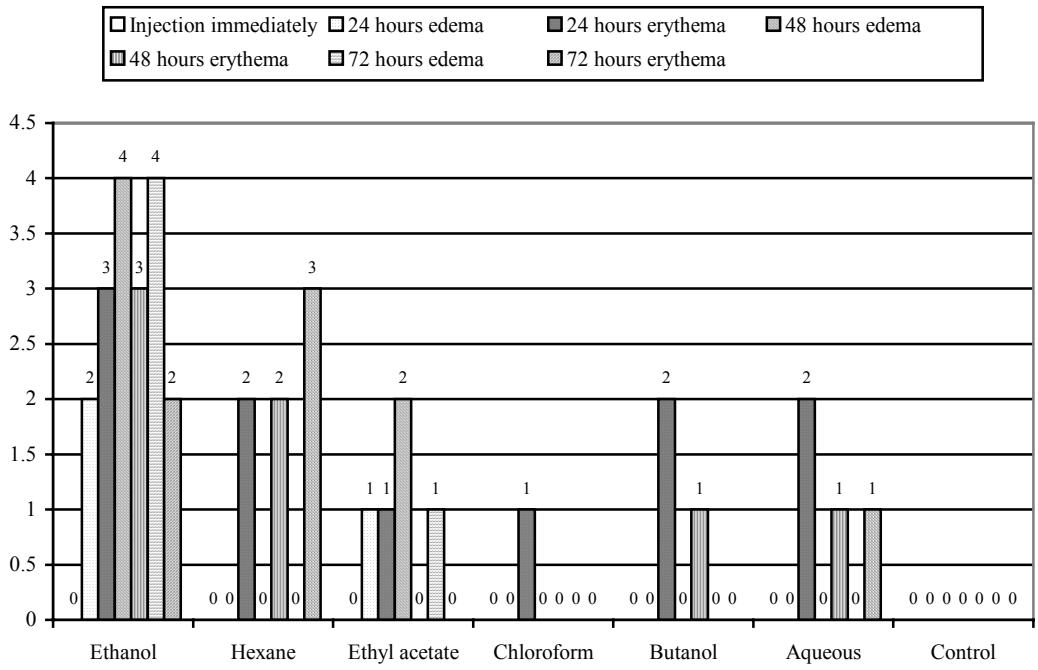

Fig. 4: Ggraphic representation of intracutaneous reactions of root extracts of *Bergenia ciliata*

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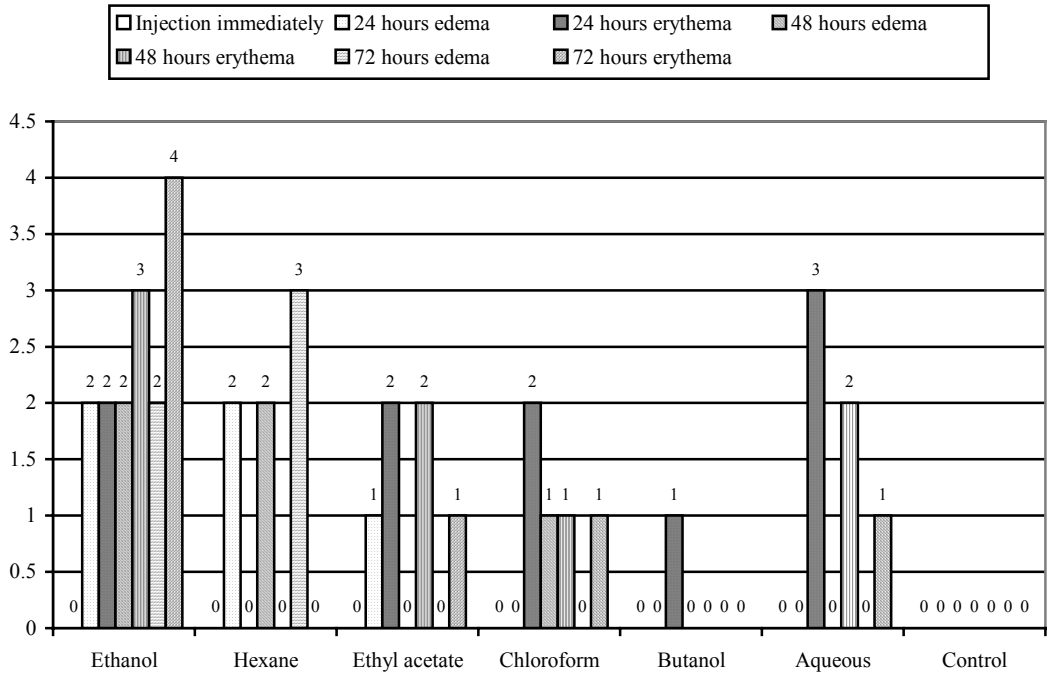

Fig. 4. Grhaphic representation of intracutaneous reactions of leaves extracts of *Bergenia ciliata*.

Hemolysis Test:

The hemolysis test has been conducted on rabbit's blood by employing the Japanese pharmacopoeial test method [4]. Hemolytic effect of 0.5% w/v solution of ethanol, hexane ethyl acetate, chloroform, butanol and aqueous extracts have been studied. The hemolytic test results of the root and leave extracts are given in table 9 and table 10. The results of leave extracts of *Bergenia ciliata revealed* (table *9*) that slight yellowish red color developed after 1 hour which persisted with the same intensity even after 24 hours. This has shown that weak lysis has occurred. In hexane, chloroform butanol and aqueous extract button formation at the bottom of the tube has produced like the negative control. Thus showing that these extracts can not produced lysis and hence are devoid of hemolytic effects.

The hemolysis evaluation of root extracts as mentioned in table 10 of *Bergenia ciliata* revealed that ethanol and butanol have produced slight yellowish red color, exhibiting weak lysis. Whereas in hexane, ethyl acetate, chloroform, and aqueous extract button formation have occurred, showing no hemolysis in these experiments.

In the study of both leave and root extracts it has been appeared that either lysis did not produce and if it is produced it is of very weak nature. Consequently it can be concluded that the extracts of *Bergenia ciliata* are not hemolytic agents.

Usually saponin hemolyse red blood cells, and litrature search on chemical constituents of *Bergenia ciliata* revealed that so for no saponin have been reported from this species (WHO, 1992). Therefore the negative evidence obtained on the activity of hemolyzing red blood cells quite clearly provide evidence that the extract of *Bergenia ciliata* is devoid of hemolytic activity.

Table 11
Bio-activity data of roots extracts of *Bergenia ciliata*

Extracts	Hypoglycemic activity	Acute systemic toxicity	Intracutaneous	Hemolysis
Ethanol	71.34%	***	ΔΔΔ	□
Hexane	70.13%	*	ΔΔ	N/A
Ethyl acetate	57.88%	*	Δ	N/A
Chloroform	6.13%	*	N/A	N/A
Butanol	48.27%	***	N/A	□
Aqueous	57.88%	**	Δ	N/A

N/A : None to very slight activity
• : 01-05 mm
•• : 06-10 mm
••• : 11-15 mm
•••• : 16-more
□ : weak activity
Δ : minor reaction
ΔΔ : moderate reaction
ΔΔΔ : moderate to severe reaction
* : symptoms at 4 hours
** : symptoms at 24 hours
*** : symptoms at 48 hours
**** : symptoms at 72 hours

Table 12
Bio-activity data of leaves extracts of *Bergenia ciliata*

Extracts	Hypoglycemic activity	Acute systemic toxicity	Intracutaneous	Hemolysis
Ethanol	70.13%	*	ΔΔΔ	□
Hexane	N/A	****	ΔΔ	N/A
Ethyl acetate	45.67%	N/A	Δ	□
Chloroform	42.13%	N/A	Δ	N/A
Butanol	N/A	N/A	N/A	N/A
Aqueous	71.34%	**	ΔΔ	N/A

N/A : None to very slight activity
• : 01-05 mm
•• : 06-10 mm
••• : 11-15 mm
•••• : 16-more
□ : weak activity
Δ : minor reaction
ΔΔ : moderate reaction
ΔΔΔ : moderate to severe reaction
* : symptoms at 4 hours
** : symptoms at 24 hours
*** : symptoms at 48 hours
**** : symptoms at 72 hours

In conclusion, the summary of the biological activity evaluation of the leaves and root extracts of *Bergenia ciliata* can be delineated as follows:

The hypoglycemic activity exhibited effective control of blood glucose in all the extract except chloroform, and the range varies from 40-70% blood glucose reduction level. This provides clear evidence that *Bergenia ciliata* can be treated as an antidiabetic plant.

The acute toxicity is another dimension, which proved that *Bergenia ciliata* exhibited severe toxicity. The acute systemic toxicity caused irritation, erection of hairs, idleness and gastro-intestinal syndrome. The intracutaneous toxicity also gave positive result confirming that *Bergenia ciliata* has moderate to severe intracutaneous toxicity with manifestation of erythema and edema. Therefore, it can suggested that *Bergenia ciliata* is not suitable for injectable bioreactivity. However *Bergenia ciliata* showed no response on blood hemolysis, therefore, devoid of such type of biological responses.

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