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Anthelmintic Activity of *Gloriosa superba* Linn (Liliaceae)

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Abstract: The ethanol and water extract of whole plant of *Gloriosa superba* Linn. (Liliaceae) were investigated for activity against Indian earthworms *Pheretima posthuma*. Various concentrations (20-60 mg mL^{-1}) of each extract were tested, which involved determination of time of paralysis and time of death of the worms. Both extract (aqueous and ethanol) exhibited considerable anthelmintic activity. Both extract (aqueous and ethanol) at the tested dose (20-60 mg mL^{-1}) level produced significant activity ($p<0.01$) when compared with piperazine citrate (15 mg mL^{-1}) which is included as standard reference and normal saline as control. The present study indicates the potential usefulness of *Gloriosa superba* whole plant as an anthelmintic.
Keywords: *Gloriosa superba*, anthelmintic activity, *Pheretima posthuma* **.**

INTRODUCTION

Gloriosa superba (Family: Liliaceae) is branched herbaceous climber common in low jungles almost throughout India[1]. Commonly known as kal-lavi, indai, khadyanag in Marathi. Gloriosa has gained the importance in medicine in recent years only and is indicated promising drug for the production of colchicine on commercial scale[2].

Traditional medicines are still commonly used in India for treatment of gout, inflammation. In our way to investigate the local medicinal plants for their potential therapeutic uses, the present study was undertaken to investigate the anthelmintic activity of aqueous and ethanolic extract of the plant *Gloriosa superba* (Family: Liliaceae).

Botanical classification:

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	liliales
Family	liliaceae
Genus	Gloriosa
Species	superba

MATERIAL AND METHODS

Plant material: The fresh whole plant of the plant *Gloriosa superba* (Liliaceae) were collected from different places at Satana, Dist-Nasik, Maharashtra, India in the month of June 2009 and it was identified and authenticated by Prof. Acharya Sir, Dept of Botany, C.T.Bora Science College, Shirur (Ghodnadi), Pune. A voucher specimen has been kept in our laboratory for future reference. The whole plant of *Gloriosa superba* were washed and cut into small pieces. Allowed it to shed dried for 15 days, powdered by a mechanical grinder to get coarse powder and stored in an airtight container for further successive extraction.

Preparation of Extract:

Alcoholic extract: by cold maceration method- About 250 g of the powdered plant material was extracted with ethanol 95% at room temperature by cold maceration method. The filtrate was collected and concentrated till a syrupy mass was obtained[3]. Then the extract was again dried by using rotary evaporator

under controlled condition of temperature and pressure.

The ethanolic and aqueous extracts were referred as AEGS and EEGS respectively. The dried extract were dissolved in normal saline (vehicle) and used for anthelmintic activity study.

Animals: Indian adult earthworm *Pheretima posthuma* were used to evaluate anthelmintic activity. The earthworms were collected from moist soil at Government compost and nursery, Shirur, Pune and washed with normal saline to remove soil and fecal matter. Earthworm were identified at Zoology Dept. C.T. Bora Science college; Shirur Dist–Pune (Maharashtra). The earthworms of 4 -6 cm in length and 0.2-0.3 cm in width were used for all experimental protocol.

Drug and chemicals used: Piperazine citrate was used as reference standard. Chemical Ethanol (95% v/v) AR (SD Fine Chemicals, Mumbai).

Preparation of Test Sample

Sample for experiment were prepared by dissolving extract to obtain a stock solution of 100mg/mL. From this stock solution, different working dilutions were prepared to get concentration range of 30, 40, 50 and 60 mg/mL and 20, 30, 40, and 50 mg/mL of aqueous extract solution and alcoholic extract solutions respectively.

Anthelmintic activity: The anthelmintic activity was evaluated in adult Indian Earthworm (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being[5][6][7][8]. The anthelmintic screening was carried out.[4]

The groups of equal sized Indian earthworms consisting of six earthworms in each group were released in 50mL of sample with desired concentrations, AEGS (30, 40, 50, 60mg mL^{-1}), EEGS (20, 30, 40, 50mg mL^{-1}). Group of earthworms in saline solution was used as Control group and Group of earthworms in piperazine citrate (15mg mL^{-1}) used as Reference. Observations were made for the time taken to paralysis and death of individual worms. Paralysis as said to occur when no movement of any sort could be observed except the worms was shaken vigorously. Death was concluded when the worms neither moved when shaken vigorously nor when dipped in warm water at 50 ^{o}C.

RESULT AND DISCUSSION

Preliminary Phytochemical analysis showed the presence of carbohydrates, alkaloids, phytosterols, fixed oil, fats, saponins, gum, mucilage, phenolic compounds and tannins like phytoconstituents (Table1) may be responsible to show a potent anthelmintic activity. From the observation made all the extracts of whole plant of *Gloriosa superba* was found to show a potent anthelmintic activity when compared to the standard drug,

After a brief stimulant effect, earthworm lost their motility on exposure to crude extract of plant *Gloriosa superba* Linn. Each aqueous extract containing 30, 40, 50 and 60 mg/mL and alcoholic extract containing 20, 30, 40 and 50 mg/mL produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. As shown in graph no. 1 and 2, alcoholic extract of plant *Gloriosa superba* Linn. and its different fractions exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis and death respectively with 50mg/mL. Also as shown in graph no. 3 and 4, aqueous extract of plant *Gloriosa superba* Linn. and its different fractions exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis and death respectively with 60mg/mL. Therefore potency of drug was found to be inversely proportional to the time taken for paralysis/ death of worms (Table2). The higher concentration of each crude extract produced paralytic effect much earlier and necrotic spots were observed externally on the worms, with higher concentrations. The effect of each crude extract was compared with piperazine citrate.

From the above results, it is concluded that all the extracts of whole plant of *Gloriosa superba* have potent anthelmintic activity when compared with conventionally used drug and is equipotent to standard anthelmintic drug. Further studies using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Gloriosa superba* as an anthelmintic drug. The drug may be further explored for phytochemical profile to identify the active constituent responsible for anthelmintic activity.

Table1: Preliminary phytochemical screening of the entire plant powder of *Gloriosa superba*

Test	Petroleum Ether	Chloroform	Ethanol	Water
Alkaloids	-	-	+	+
Carbohydrates	-	-	+	+
Phytosterols	+	-	-	-
Terpenes	-	-	-	-
Fixed oil and fats	+	-	-	-
Saponins	-	-	+	+
Phenolic compounds and tannins	-	-	+	+
Flavonoids	-	-	-	-
Gums and mucilage	-	-	-	+
Proteins	-	-	-	-

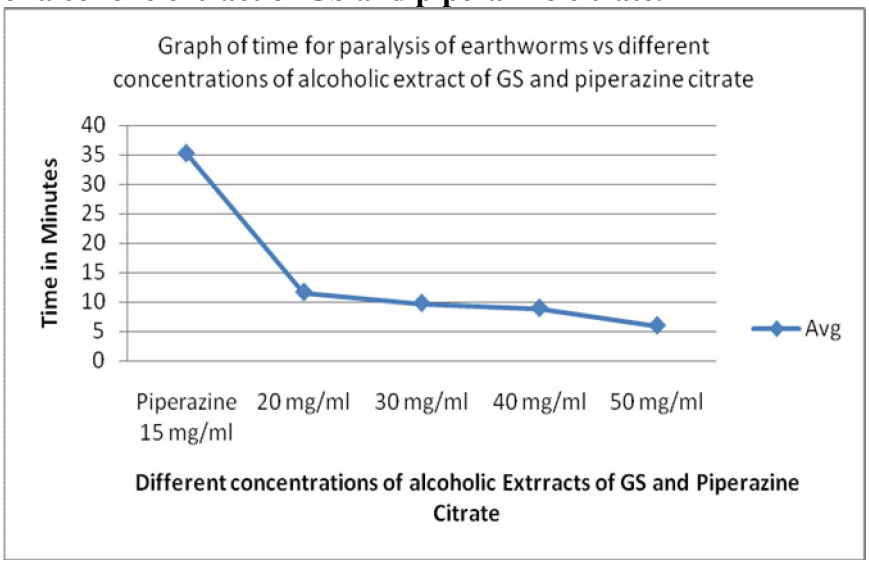
+ present, - absent

Table2: Anthelmintic activity of ethanol and aqueous extract of *Gloriosa superba*

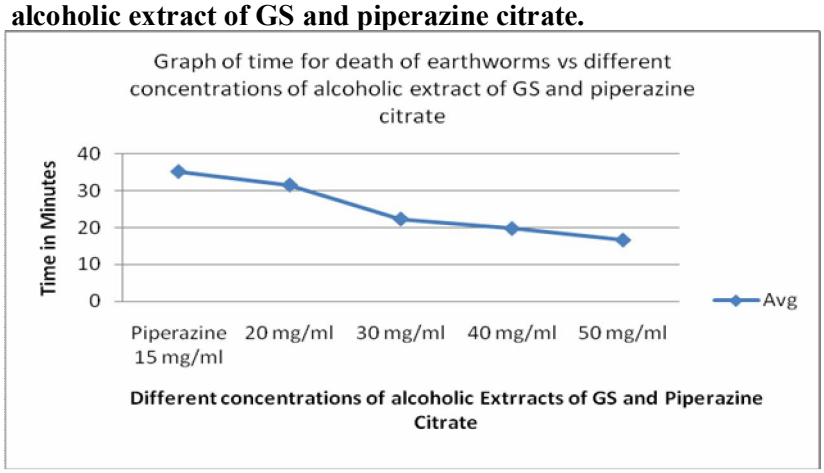
Groups	Treatments	Concentration used ($mgmL^{-1}$)	Time taken for Paralysis (min)	Time taken for Death (min)
1	Normal saline (control)	-	-	-
2	Piperazine citrate	15	21.20±0.87	35.21±2.42
3	Ethanol extract	20	11.66±1.04	31.70±0.94
4	Ethanol extract	30	9.80±0.52	22.39±0.55
5	Ethanol extract	40	8.95±1.60	19.83±1.90
6	Ethanol extract	50	5.99±0.37	16.73±2.17
7	Aqueous extract	20	39.93±1.06	56.54±1.11
8	Aqueous extract	40	25.08±1.16	35.54±1.71
9	Aqueous extract	50	20.36±1.30	31.67±1.82
10	Aqueous extract	60	13.73±0.13	22.83±0.98

Results are expressed as Mean±SEM from six observations -: worms alive upto 24 hr of observation, *p<0.01when compared to piperazine citrate as standard reference.

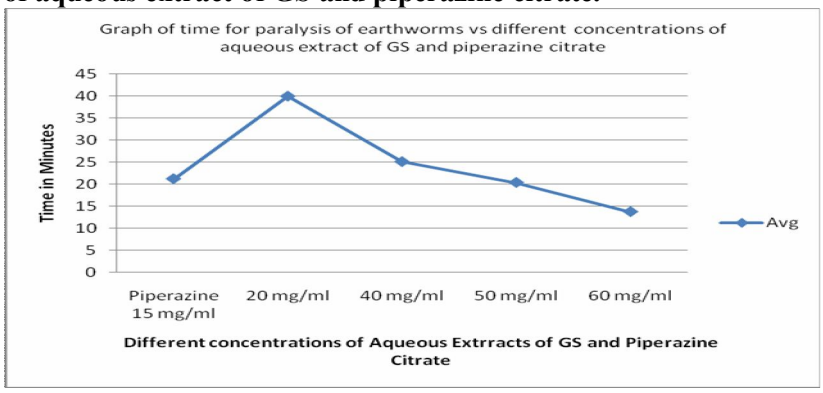
Graph no.1: Graph of time for paralysis of earthworms vs different concentrations of alcoholic extract of GS and piperazine citrate.



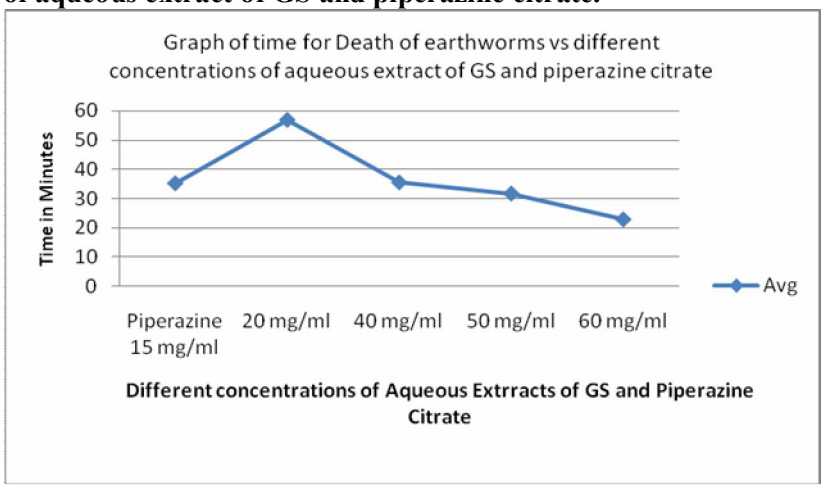
Graph no.2: Graph of time for death of earthworms vs different concentrations of alcoholic extract of GS and piperazine citrate.



Graph no.3: Graph of time for paralysis of earthworms vs different concentrations of aqueous extract of GS and piperazine citrate.



Graph no.4: Graph of time for death of earthworms vs different concentrations of aqueous extract of GS and piperazine citrate.



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