World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

EVALUATION OF ACUTE TOXICITY AND IN VITRO ANTI-ANTHELMINTIC ACTIVITY OF ANTHRAQUINONES AND DERIVATIVES FROM *PENTAS LANCEOLATA* (FORSSK.) DEFLERS LEAVES

Dr. Pragati Kumar[1]*, Ashish Kumar Sharma[2] and Prabodh Shukla[3]

[1]Pranveer Singh Institute of Technology, Bhaunti Kanpur, Uttar Pradesh.
[2]Nims Institute of Pharmacy, NIMS University Jaipur Rajasthan.
[3]Pharmacy College Saifai, Uttar Pradesh University of Medical sciences Saifai Etawah U.P.

Corresponding Author: Dr. Pragati Kumar
Pranveer Singh Institute of Technology, Bhaunti Kanpur, Uttar Pradesh.

Article Received on 30/04/2020 **Article Revised on 20/05/2020** **Article Accepted on 10/06/2020**

ABSTRACT

Background: Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. These having great importance for human tropical medicine and for veterinary medicine. **Objective:** The objective of the present research work had been done to evaluate the toxicity and *In vitro* Anti-Anthelmintic potential of anthraquinones and derivatives isolated from *Pentas lanceolata* (Forssk.) Deflers leaves. **Materials and Methods:** For the study of toxicity, the OECD guidelines were used. Experimental animals (Swiss albino mice), Six mice in each, were grouped into six groups; five experimental groups and one negative control. In studying the acute toxicity, 100, 250, 500, 1000, 1500 and 2000 mg/kg isolated compounds was given via i.p. route of drug administration. For acute toxicity, a single dose was given, and gross behavioural changes were recorded. For anthelmintic test Swiss albino mice, male and females weighted in medium of 25-30gm and naturally infected for *Syphucia obvelata* and *Aspiculuris tetraptera* were selected and test compounds was dissolved in normal saline water and given orally to individual groups of mice. The eggs of parasites (Both species were determined separately) were count out on the first, second, third and fifth day. On the seventh day, one mouse from each group was sacrificed and the intestine was observed to on the subject of the presence of parasites. **Results:** The acute toxicity study resulted that all four selected test Compounds are comparatively safe when administered through i.p. rout to mice. Any kinds of toxic symptoms as well as mortality did not represent by the Compounds up to the dose of 250 mg/kg body weight. For Anti-Anthelmintic activity, all four isolated Test compounds and Piprazine citrate as standard drug was studied on the laboratory animals and the study concluded that, the groups of animals administered isolated Test compounds but not in control group showed a significant decreased in no. of eggs/gram of faeces excreted after 1st day of treatment. Results showed significant differences (P-value<0.001) in the number of eggs excreted by the *S. Obvelata* and *A. Tetraptera* and in mice, administering the test compounds and control group. **Conclusion:** From the studies, it was proved that Anthraquinones and derivatives isolated from leaves of *Pentas lanceolata* are an effective Anti-anthelmintic drug with the dose of 250 mg/kg.

KEYWORDS: Anthraquinones, Anti-anthelmintic, Acute toxicity, Lethal Dose, *In-vitro.*

INTRODUCTION

The term ***Anthelmintic*** is used to explain the drugs used to treat infections of animals caused by parasitic worms. The parasitic worms include both flat worms like flukes and tapeworms and round worms. The parasites are of vast important for human and animals. The WHO estimated that two billion peoples are affected with the parasitic worm infections causing enhanced morbidity and mortality. More than half of the population of the world suffers from worm infections because the worms not killed by the drugs and the drugs not properly absorbed when given by oral route of drug administration. There is generally a wider margin of safety than with drugs for worm infections in other sites. Random use of imitation anthelmintics can lead to resistance of parasites. Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human.[1] The most of anthelmintics are restricted in their action between trematodes, cestodes, and nematodes. e.g. Praziquantel, used in the treatment of most of humans infected with trematodes or cestodes, act by disrupting calcium homeostasis, was inactive against nematodes.[2]

Mechanism of action of anthelmintic drugs: The anthelmintic drugs must be selectively toxic to the parasite. This is generally done either by inhibiting metabolic processes that are essential to the parasite but not crucial to the host, or by natural pharmacokinetic properties of the compound that essential for the parasite to be exposed to privileged concentrations of the anthelmintic than are the host cells. The pharmacological base of the treatment for helminths involves intervention with the reliability of parasite cells, neuromuscular harmonization, or protective mechanisms against host immunity, which direct to starvation, paralysis, and digestion of the parasite.[3,4]

Pentas lanceolata, also known as Egyptian Star, is native to tropical Africa and is commonly used as herbal medicine in Ethiopia, Uganda, Rwanda and Kenya. This is erect evergreen perennial shrub, 3 to 4 feet tall and is tinted all over most of the year with 3-inch-wide, dense clusters of long-tubed, star-shaped flowers available in white, pink, red, and lavender. The plants grow commonly during the all warm months. Leaves and stems are enclosed with fine hairs, and leaves have prominent veins on the undersides. Medicinally *Pentas lanceolata* is most popular plant and is used for the treatments of Lymphadenitis, in the treatment of Diarrhoea, to Treat Snake Bite, In the treatment of Malaria and to Treat Ascariasis when taken orally.[5-8]

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents used were of extra pure and analytical Grade, procured from Sigma Chemical Pvt Ltd, USA. All solvents were obtained from Fischer Scientific Ltd, India.

Isolation of Anthraquinones and derivatives

Anthraquinones and derivatives were isolated from the Methanolic extract of *Pentas lanceolata* leaves. Phytochemical, chromatographic and spectroscopic data and their interpretation resulted four bioactive compounds (Highest % Yields) named as β-primeveroside (Rubiadin-1-methyl ether-3-*O*-β-primeveroside) (Compound-A), Damnacanthol (3-hydroxy-2-hydroxymethyl-1-methoxy-9,10-anthraquinone) (Compound-B), Rubiadin (1,3-dihydroxy-2-methyl-9,10- anthraquinone) (Compound-C), and Lucidin-ω-methyl ether (1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone) (Compound-D).[9,10]

Acute Toxicity Study for isolated compounds

Animals

For the study healthy and fully mature Swiss albino mice of either sex having weight 25-30 gm were selected. The housing condition for the animals was maintained in polypropylene (32x24x16cm) cages. The fully dried and aseptic husk was used for bedding material for the animals and the conditions were fixed at room temperature (25±2^{o}c), humidity (55±5%) and 12hrs of light and dark cycle. Standard pelleted diet and water *ad libitum* were provided. The animals were randomly selected for grouping as experimental and control. Before study the ethical clearance was taken from Institutional Animal Ethics Committee (IACE) NIMS University, Jaipur-Rajasthan (NIMSUR/IAEC/CERT/2014/09/02).

Experimental Setup

For experimental protocol the animals were fasted before the study for whole day. On next day, the fasted animals were weighted individually, and dose of the drugs were calculated in reference to their body weight. Total 36 animals were selected and separated into six groups (n=6) for administration of each isolated compounds at dose 100, 250, 500, and 1000, mg/kg respectively. The test compound-A (Rubiadin-1-methyl ether-3-*O*-β-primeveroside, Rubiadin-1-methyl Ether) was diluted with distilled water and inject via intraperitoneal route of drug administration to six groups of mice at various doses (100, 250, 500, 1000, 1500 and 2000 mg/kg). After 24 hrs the mortality counted and LD50 was determined. The groups and dose were decided as following –

Group1: Given Compound-A of the dose 100 mg/kg body weight to animals, the normal activities observed from animals.

Group2: Given Compound-A of the dose 250 mg/kg body weight to animals. At this dose, primarily some animals were prickly but after 30-40 minutes the animals come in comfort zone.

Group3: Given Compound-A of dose 500 mg/kg body weight to animals. At this dose, primarily animals were prickly but after 1-2hrs they became normal.

Group4: Given Compound-A of dose 1000 mg/kg body weight to animals. 35 to 50% animals were died at this dose.

Group5: Given Compound-A of dose 1500 mg/kg body weight to animals. 65 to 80% animals were died at this dose.

Group6: Given Compound-A of dose 500 mg/kg body weight to animals. 80 to 100% animals were died at this dose.

After 30 minutes of dosing each animal was examined at regular intervals of the initial 24 hours for parameters like Alertness, Ocular action, motional action, ordinary habits, Pinna response and urination intervals etc. Before the calculation of Probits the correction was done in the data of % death for 0 and 100 as given -

Corrected percentage formula for 0 and 100% of the death: - For 0% mortality - 100(0.25/n) and for 100% mortality -100(n-0.25/n).[11]

Same experimental setup and Parameters were also observed for all compounds i.e. Compound-B, C and D. The LD50 were determined by consideration of Probit data.

Statistical analysis

The data are articulated as mean ± SD. Results were analysed statistically by using one-way ANOVA and by

Dunnet and Tukey's analysis. *P*-values < 0.05 considered as statistically significant.

In-vitro Anti-Anthelmintic activity

Animals

For anthelmintic test Swiss albino mice, male and females weighted in medium of 25-30gm and naturally infected for *Syphucia obvelata* and *Aspiculuris tetraptera* were selected separately. The housing conditions for the animals were individually in the cage of polypropylene (30x20x13cm), bedded with screen stark and stiff upon an absorbent paper, and standard conditions of light, room temperature and aeration.

Screening of Infection (*Sellotape tests)*

A narrow piece of translucent adhesive tape was pushed alongside the anus of the selected experimental animal and then positioned adhesive side down on a sparkling slide and observed under microscope of the 10x objective.[12-13]

Experimental Setup

For the experiment total 36 mice were used. The animals were divided into six groups (each group contains six Animals) of mice –

Group 1: treated with 250 mg/kg of Compound - A for 7 days. (Test Compounds – TC-A)

Group 2: treated with 250 mg/kg of Compound – B for 7 days. (TC-B)

Group 3: treated with 250 mg/kg of Compound – C for 7 days. (TC-C)

Group 4: treated with 250 mg/kg of Compound – D for 7 days. (TC-D)

Group 5: treated with 200 mg/kg Piperazine Citrate for 7 days as positive control.

Group 6: treated with 20 ml/kg normal saline as negative control.

Route of Administration

All these test compounds were dissolved in normal saline water and given orally to individual groups of mice.

Screening Methodology

The eggs of parasites (Both species were determined separately) were count out on the first, second, third and fifth day. On the seventh day, one mouse from each group was sacrificed and the intestine was observed for the presence of parasites. The M-stat analytical method was applied to determine the differences between the control and treated groups.[1,14-15]

RESULTS

Acute Toxicity

The compiled data of toxicity study was resulted that, after 24 hours of normal investigations at the dose of 250 mg/kg of isolated compounds there were no considerable changes represented by animals in behavioural outline like hesitant, diarrhoea, salivation, breathing, demolition in food ingestion, water intake, hair loss, sleeping habit, lassitude, agitation, or in body characters like colour of eyes, skin damages, weight of body, other damages, when compared to the control. The LD50 of selected four Compounds A, B, C and D in mice was calculated as 1000 mg/kg, 1500 mg/kg, 1000 mg/kg and 1000 mg/kg when given via ip. Rout of drug administration, correspondingly. The concluded data of the study are indicates in the Tables-1.

Table 1: Average Lethal doses of Test Compounds (n=06).

Group No.	Doses (mg/kg) of Compounds	Avg. Log Doses of Compounds	Avg. No. of Deaths	Avg. % Deaths	*Corrected %	Avg. Probits
1	50	1.74	0/6	0	4.18	3.06
2	250	2.42	0/6	0	4.15	3.09
3	500	2.68	2/6	24.34	25.34	4.58
4	1000	3.06	3/6	66.67	66.67	5.01
5	1500	3.20	5/6	83.34	83.34	5.94
6	2000	3.30	6/6	100	95.82	6.67

*****Corrected % Formula: For 0 and 100 % deaths

For 0% dead: 100(0.25/n), for 100% dead: 100(n-0.25/n)

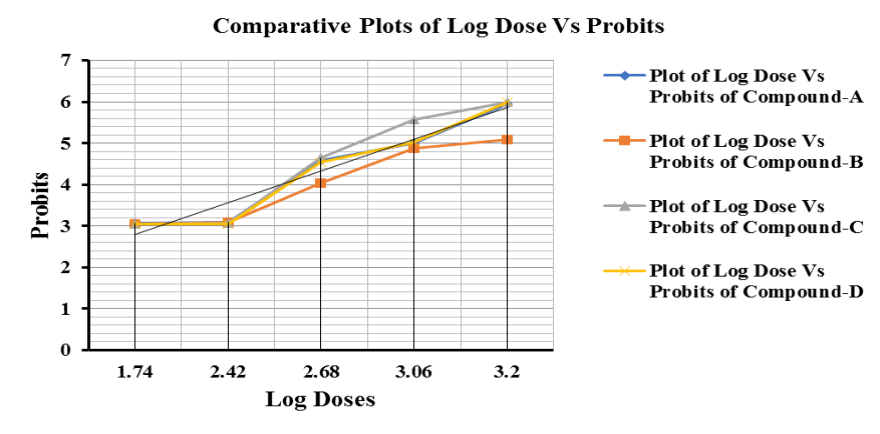

Fig. 1: Comparative curve of Log Dose Vs Probits for all Compounds.

***In-vitro* Anti-Anthelmintic activity**

All the isolated Test compounds and Piperazine citrate as standard drug was studied on the laboratory animals and the study concluded that, the groups of animals administered isolated Test compounds but not in control group showed a significant decreased in no. of eggs/gram of faeces excreted after 1st day of treatment. Results showed significant differences (P-value<0.001) in the number of eggs excreted by the *S. Obvelata* and *A. Tetraptera* and in mice, administering the test compounds and control group. The study showed a perceptible effect for Anthraquinone (test compounds) over time against parasites. So that on the 7th day, the number of parasite eggs in faeces was at the minimum count (P<0.001). The Mean and standard deviation of the different treatment groups is shown in Figure 4 & 5.

Table 2: Average Number of Eggs/gram of faeces, excreted by *Syphucia obvelata* at Day 0, 1, 3, 5 and 7 after drug treatment.

Treated Groups	Avg. No. of eggs count/gram of faeces (Days after) *S. obvelata*				
	0 day	1st day	3rd day	5th day	7th day
TC-A (250 mg/kg)	367 ± 03	287 ± 06	129 ± 05	55 ± 04	03 ± 01
TC-B (250 mg/kg)	389 ± 03	278 ± 02	152 ± 01	69 ± 02	00 ± 01
TC-C (250 mg/kg)	372 ± 03	211 ± 02	143 ± 02	78 ± 01	01 ± 01
TC-D (250 mg/kg)	398 ± 02	243 ± 02	168 ± 03	62 ± 02	00 ± 01
Piperazine citrate (200 mg/kg)	572 ± 07	293 ± 06	81 ± 03	03 ± 02	00 ± 01
Control (Normal Saline 20ml/kg)	627 ± 06	665 ± 08	673 ± 07	702 ± 09	717 ±11

The data represents Mean ± SEM, n=6/groups, the significant difference considered as **(P- value < 0.001).**

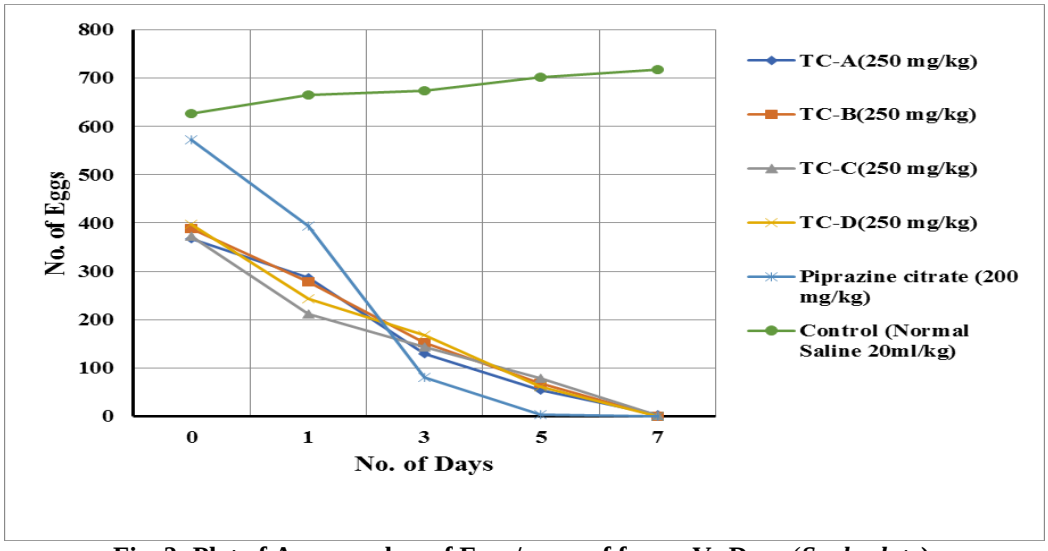

Fig. 2: Plot of Avg. number of Eggs/gram of faeces Vs Days (*S. obvelata*).

Table 3: Average Number of Eggs/gram of faeces, excreted by *Aspiculuris tetraptera* at Day 0, 1, 3, 5 and 7 after drug treatment.

Treated Groups	Avg. No. of eggs count/gram of faeces (Days after) *A. tetraptera*				
	0 day	1st day	3rd day	5th day	7th day
TC-A (250 mg/kg)	211 ± 03	108 ± 02	51 ± 02	16 ± 01	01 ± 01
TC-B (250 mg/kg)	234 ± 02	119 ± 02	62 ± 01	19 ± 02	00 ± 01
TC-C (250 mg/kg)	224 ± 03	129 ± 03	59 ± 02	23 ± 01	00 ± 01
TC-D (250 mg/kg)	247 ± 02	154 ± 02	67 ± 03	34 ± 02	01 ± 01
Piperazine citrate (200 mg/kg)	578 ± 06	301 ± 05	59 ± 02	02 ± 01	00 ± 01
Control (Normal Saline 2ml/kg)	639 ± 03	678 ± 05	698 ± 06	721 ± 07	748 ±08

The data represents Mean ± SEM, n=6/groups, the significant difference considered as **(P- value < 0.001).**

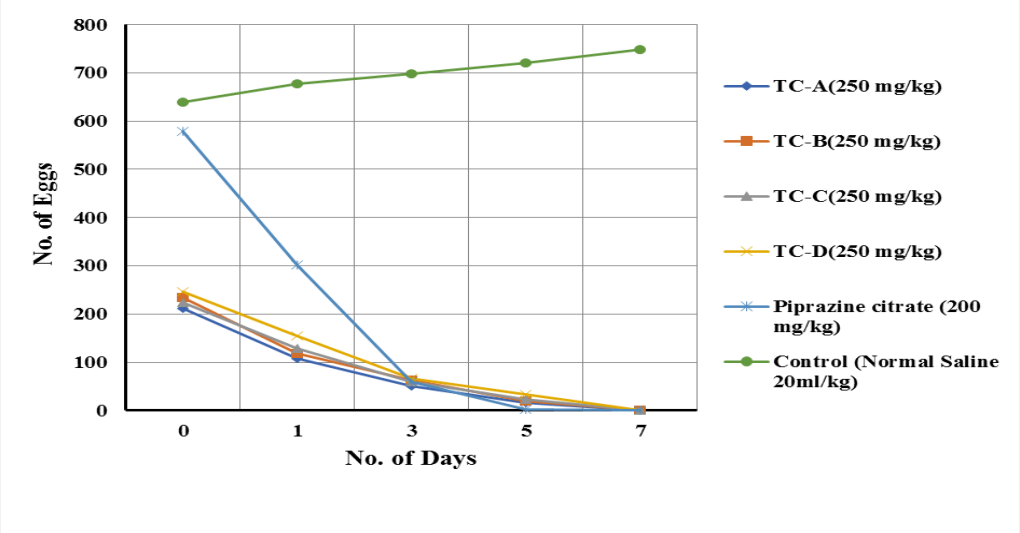

Fig. 3: Plot of Avg. number of Eggs/gram of faeces Vs Days (*A. tetraptera*)

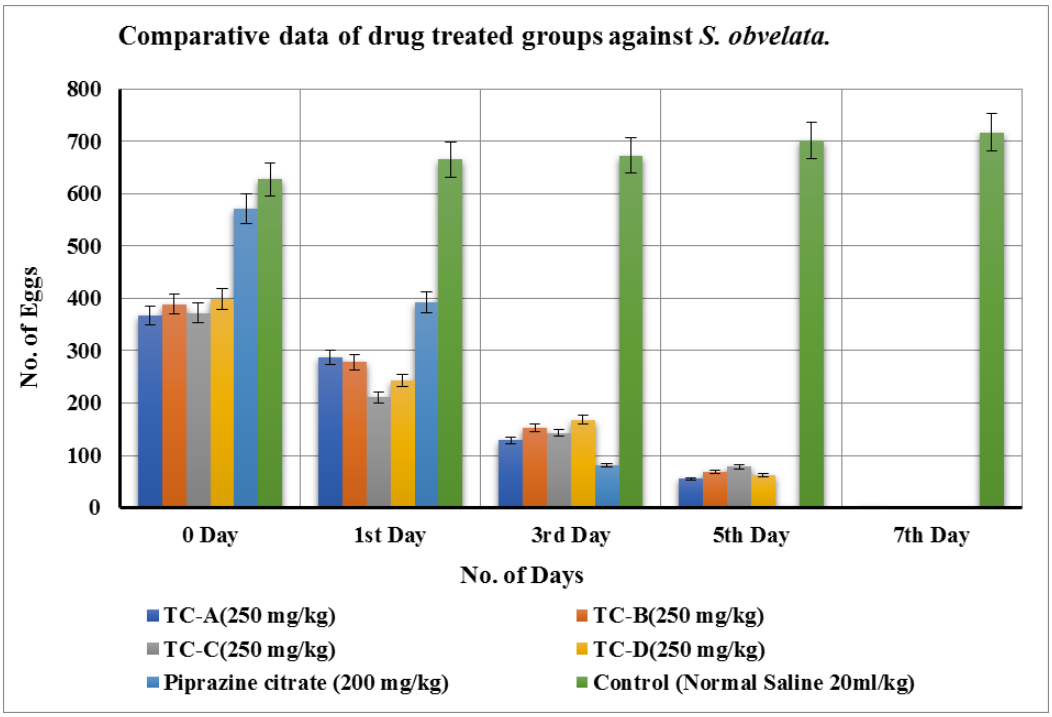

Fig. 4: Comparative chart of the drug treated groups against *S. obvelata.*

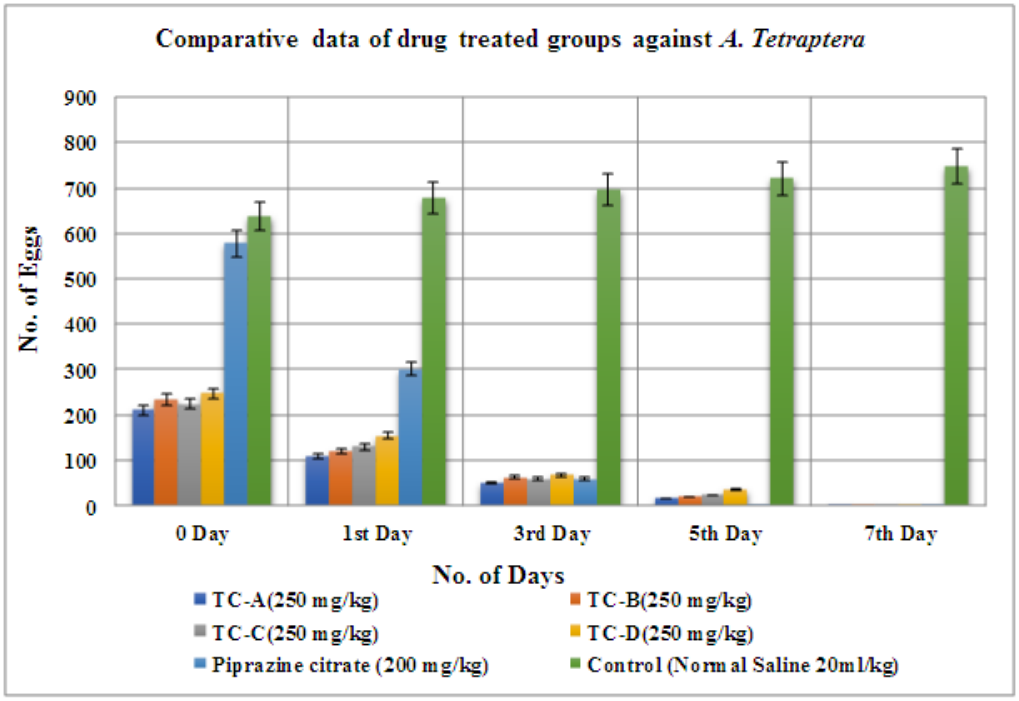

Fig. 5: Comparative chart of the drug treated groups against *A. tetraptera*

DISCUSSION

In the present study, the toxicological profiles of isolated compounds suggested by Miller and Tainter method administering the isolated test compounds by i.p. rout of drug administration. For the determination of lethal dose, primarily least tolerated dose (100% death) and highest tolerated dose (0% death) were resolute by hit and trial process. Before the study an ethical clearance was mandatory for the acute dose calculation that was provided by institutional animal ethical committee, NIMS University. For the study healthy swiss albino mice of both sexes were used that were divided into six group containing six animals in each group. The test animals were treated with test compounds at the primary dose of 100, 250, 500, 1000, 1500 and 2000 mg/kg body weight. After the dose administration each group of animals were observed in reference to their different characteristic profiles like behaviour of the animals etc and find out that **'No death'** was noticed up to 250 mg/kg body weight (i.p.) dose, while, 100% death was caused at the dose of 1500-2000 mg/kg (i.p.) dose of the test compounds. By the curve plotted between log dose and Probits values the LD50 of the test compounds was calculated as 1000 mg/kg for compound-A, 1500mg/kg for Compound-B, 1000 mg/kg for Compound-C and 1000 mg/kg for Compound-D. One-tenth of these doses were given as the restorative dose for the assessment of Anti-anthelmintic potential.

For the assessment of *In vitro* anthelmintic effect, Piperazine citrate was used as a standard drug for the study. For the experimental, total 36 mice were selected of the groups of six animals in each and all the isolated Test compounds and Piperazine citrate as standard drug was examined for the result on digestive tract parasites of mice. The results of the study represented that the groups of animals that were treated with the standard drug or test compounds in comparison to control group without any treatment, the total eggs of the parasite count excreted in each gram of faeces was reduced considerably after next day of drug as well as test compounds treatment. There was a considerable variation **(P-value<0.001)** in the number of eggs excreted by the ***S. Obvelata*** and ***A. Tetraptera*** in naturally infected mice when compare to control group. The results of the study concluded a perceptible action of isolated anthraquinones (**test compounds**) over time against parasites. The study was continued till 7th day of the drug and test compounds treatment and find out that the number of eggs of parasites in faeces was at the least quantity **(P<0.001)**.

CONCLUSION

The Results obtains from this research work emphasize that the anthraquinones and derivatives from *Pentas lanceolata* leaves extracts was not toxic to the mice at the tested doses and poses significant anti-anthelmintic activity. Hence in conclusion utilization or increased consumption of this plant products will be advantageous to mankind and it will contribute to the prevention of helminthic infections. Further investigations on health promoting aspects in animal models in future will be made.

ACKNOWLEDGMENT

The authors would like to thank the Management of NIMS University, Jaipur Rajasthan for providing the necessary facilities to carry out this research work.

AUTHORS CONTRIBUTIONS

Dr. Ashish Kumar Sharma gave a substantial contribution by executing the experimental work in the laboratories, drafted the manuscript and extensively revised to improve the quality of the manuscript. Conception, the design of the study and supervision of the work were done by Dr. Prabodh Shukla.

CONFLICT OF INTERESTS

We declare that there were no conflicts of interest.

REFERENCES

1. Singh D, Swarnkar CP, Khan FA, Anthelminthic resistance in gastrointestinal nematodes in livestock in India. J Vet Parasit, 2002; 16: 115-130.
2. Greenberg RM. Ca^{2+} signalling, voltage-gated Ca^{2+} channels and praziquantel in flatworm neuromusculature. Parasitol, 2005; *131*: 97-108.
3. Martin RJ. Gamma-Aminobutyric acid- and piperazine-activated single-channel currents from *Ascaris suum* body muscle. Br. J. Pharmacol, 1985; *84:* 445-461.
4. Kwa MS, Veenstra JG, Roos MH. Benzimidazole resistance in *Haemonchus contortus* is correlated with a conserved mutation at amino acid 200 in beta-tubulin isotype. Mol. Biochem. Parasitol, 1994; *63*: 299-303.
5. Mirutse G, Zemede A, Zerihun W. Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study, J. Ethnopharmacol, 2009; 1016: 09-1016.
6. Tilahun T, Mirutse G, Zemede A, Zerihun W. Medicinal plant knowledge of the Bench ethnic group of Ethiopia: an ethnobotanical investigation, J Ethnobiol Ethnomed, 2009; 5: 34.
7. Taddeo A, and Claude K. Qualitative (phytochemical) analysis and antifungal activity of Pentas decora, a plant used traditionally to treat skin fungal infections in Western Uganda, Research in Pharmaceutical Biotechnology, 2011; 3(7): 75-84.
8. Endale M, Alao JP, Akala HM, Rono NK. Antiplasmodial quinones from Pentas longiflora and Pentas lanceolata. Planta Med, 2012; 78(1): 31-5.
9. Khandelwal KR, Pawar AP, Kokhale SB. Practical Pharmacognosy, Pune: Nirali Pakashan, 1995; 144-146.
10. Kumar Pragati, Sharma AK, Shukla P. Physiochemical screening of marker anthraquinones and derivatives from the *Pentas lanceolata* (forss. K.) Deflers leaves extract, European journal of pharmaceutical and medical research, 2017; 4(5): 439-446.
11. Kulkarni SK. Hand Book of Pharmacology, Vallabh Prakashan, New Delhi, 2nd edition, 1993; 49-51.
12. Jacobs AH, Enterobiasis in children. Incidence, symptomology, and diagnosis, with a simplified Scotch cellulose tape technique. *Journal oj Pediatrics*, 1942; 21: 497-503.
13. Graham CF, A device for the diagnosis of *Enterobius* infection. *American Journal oj Tropical Medicine*. 1941; 21: 159-161.
14. Chan KF, Life cycle studies on the nematode *Syphacia obvelata* Am. J. Hyg, 1952; 56: 14-21.
15. Steward JS, Anthelmintic studies: A controlled critical entero-nemacidal test. Parasitology, 1955; 45: 231-241.