Algerian Journal of Natural Products 5:1 **(2017)** 417- 426

OPEN ACCESS

Online ISSN: 2353-0391

Algerian Journal of Natural Products

www.univ-bejaia.dz/ajnp

Type of the Paper (Article)

Assessment of Biological Activity on *Cirsium arvense* L.

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Received: 19/01/2017 /Accepted:08/07/2017 DOI: https://doi.org/10.5281/zenodo.824566

Abstract: This study was aimed at evaluating antibacterial, cytotoxic, anthelmintic and anti-hyperglycemic activity of aerial part of *Cirsium arvense*. Antibacterial activity was determined by disc diffusion method at the concentration of 200, 250 and 500 µg/disc. Cytotoxic and anthelmintic activity was carried out according to Meyer and Gade method with slight modification respectively. The anti-hyperglycemic activity was determined by the method followed by Djilani. The extract showed dose dependent antibacterial activity against the experimental bacterial strains except *Staphylococcus epidermidi*, *Streptococcus agalactiae* and *Enterococcus faecalis*. In brine shrimp lethality bioassay, it showed LC_{50} at 51 µg/ml that is comparable to that of standard drug, vincristine sulphate (LC_{50} 0.44 µg/ml). Also it showed dose dependent anthelmintic activity. It did not show anti-hyperglycemic activity. Our findings provide evidence that potential bioactive compounds are responsible for biological activities and further studies are required to isolate these compounds.

Keywords: *Cirsium arvense; Antibacterial; Cytotoxicity; Anthlemintic; Anti-hyperglycemic.*

I. Introduction

We evaluated phytochemical test, antioxidant activity and analyzed phenolic compounds using HPLC-DAD system of *C. arvense* extract [1]. The experimental results indicated that ethanol extract of *C. arvense* contained a moderate concentration of rutin hydrate, and quercetin (30.41 and 39.99 mg/100 gm of dry extract, respectively). Traditionally *C. arvense* has been used to treat many diseases. It is useful as a health promoting tonic, diuretic and astringent. Its leaves can be chewed to relieve the pain of toothaches, cancer sores and sore throats as they have anti-inflammatory properties. It can be a source of fiber, vitamins and minerals. The juice of the roots is effective at killing intestinal parasites and reduces the symptoms of poison ivy and gastritis. *C. arvense* has been used traditionally for different purposes and contains important polyphenols in moderate amount. So, as part of our ongoing research, here we evaluated some pharmacological activities (antibacterial, cytotoxic, anthelmintic and anti-hyperglycemic) to find out responsible compounds for the aforementioned activities.

II. Experimental Section

II.1. Chemicals and reagents

Ethanol and Dimethyl sulfoxide (DMSO) were obtained from Merck (Darmstadt, Germany). All types of analytical grade solvents and reagents were obtained from Sigma Chemical Co. Ltd., (St. Louis, MO, USA).

II.2. Reference drugs

Kanamycin, Albendazole and Vincristine sulfate were purchased from Incepta Pharmaceuticals Ltd., Bangladesh.

II.3. Microorganisms

Nine pathogenic bacterial strains (five Gram positive bacterial strains namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pyogens*, *Streptococcus agalactiae*, *Enterococcus faecalis* and four Gram negative *Shigella flexneri*, *Shigella sonnei*, *Shigella boydigius*, *Escherichia coli*) were used in this experiment. The experiment was conducted in Microbiology Laboratory of Pharmacy Discipline, Khulna University.

II.4. Experimental animals

Swiss-albino mice were kept at the animal house of Pharmacy Discipline, Khulna University for adaptation after collection under standard laboratory conditions (relative humidity of 56% - 60%, room temperature of 25 ± 2 °C and 12 hours light and dark cycle). Animals were fed with ICDDR; B formulated rodent food and allowed free access to water. All experiments using mice were carried on an isolated and noiseless condition and following the guidelines of the Animal Ethics Committee.

II.5. Plant materials and extraction

The aerial part of *Cirsium arvense* was collected in 2012 at the daytime from Khulna, Bangladesh. The crude extract was prepared as described in Hossain ML et al. 2016.

II.6. Antibacterial Activity

Antimicrobial activity test was determined by disc diffusion method [2, 3]. Sample at 200, 250 and 500 µg/disc, Kanamycin at 30 µg/disc and ethanol at 10 µl/disc as negative control was applied. The growth media was prepared in aseptic condition and poured into petridishes.

II.7. Brine Shrimp Lethality Bioassay (Cytotoxic activity)

Cytotoxic activity test of different fractions was carried out according to the Meyer method [4]. For *Artemia salina* leach (brine shrimp eggs) was used as the test organism. The eggs of the brine shrimp were collected from an aquarium shop (Khulna, Bangladesh) and hatched in artificial seawater (3.8 % NaCl solution) for 48 hr to get mature shrimp called nauplii.

II.8. Anthelmintic Activity

Anthelmintic activity of the plant extract was investigated on live parasites of *Paramphistomum cervi* of cattle collected from freshly slaughtered cattle at local abattoirs by the method of Gade DR [5] with slight modification. After cleaning, parasites were stored in 0.9 % phosphate buffered saline (PBS) of pH 7.4 prepared with 8.01 gm NaCl, 0.20 gm KCl, 1.78 gm Na_2HPO_4 and 0.27 gm KH_2PO_4 in 1 litre of distilled water at 37 ± 1°C. The parasites were divided into different groups consisting of four parasites in each group. Extract at the concentration of 25, 50 and 75 mg/ml and reference standard Albendazole at the concentrations of 15 mg/ml of 10 ml in PBS were prepared and transferred to Petridishes. Control group was treated with 0.1 % tween-80 in PBS. Four parasites were placed in each petridish and observed.

II.9. Anti-hyperglycemic Activity (Oral Glucose Tolerance Test)

The Anti-hyperglycemic activity (Oral Glucose Tolerance Test) of sample was determined by the method followed by Djilani [6] using Swiss-albino mice (*Musmus culus*) aged 4-5 weeks as experimental animal. Different concentrations of sample (200, 250 and 500 mg/kg body weight) were used and compared with standard, Glibenclamide at the dose of 5 mg/kg body weight to determine the anti-hyperglycemic activity.

II.10. Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 5 statistical package (Graph Pad Software, USA). The data were analyzed by one way analysis of variance (ANOVA). All the results were expressed as mean ± SE for triplicate determinations.

III. Results and Discussion

Plant is a biosynthetic laboratory. The plants contain several non-nutritive chemicals known as phytochemical constituents. Among these constituents phenolic compounds, flavonoids, tannins and alkaloids are the most valuable for therapeutic activity. So, identification of the nature of the compounds present in extracts is essential to evaluate the biological activity of the extract. It is already reported that the polyphenolic compounds, as like phenolic acids, flavonoids and tannins, commonly found in different plants and exert multiple biological response, including antioxidant, antimicrobial, anthelmintic, antidiarrhoeal activity [7-11]. So, the result of the present study can correlate with the previous study. Phenolic compounds, secondary plant metabolites abundantly found in both edible and non-edible plants possess biological properties of antioxidant, anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, cardiovascular protection, improvement of the endothelial function, as well as inhibition of oxidative damage of DNA [12], angiogenesis and cell proliferation activity [13].

Tannins have been reported to possess anticarcinogenic and antimutagenic potentials as well as antimicrobial properties. The phytochemicals of flavonoids, polyphenols and tannins, quinones, terpenoids and essential oils and alkaloids show antimicrobial activity [9]. Flavonoids showed antimicrobial activity by complexing with cell wall and also binds to adhesions. Tannins may protect plants from invasion of pathogenic microorganism due to their antimicrobial and antifungal properties. Polyphenols and tannins exert antimicrobial activity through following mechanism binds to adhesions, enzyme inhibition and substrate deprivation, complex with cell wall, membrane disruption and metal ion complexation. Alkaloids exert antimicrobial activity through intercalating into cell wall and DNA of parasites [9]. *C. arvense* was effective against gram positive and gram negative bacteria in dose dependent manner and showed more effectiveness against gram negative bacteria compared to gram positive bacteria.

The ethanol extract of *C. arvense* showed antibacterial activity against the experimental bacterial strains except *Staphylococcus epidermidi*, *Streptococcus agalactiae* and *Enterococcus faecalis*. At the dose of 200 µg/disc, the extract showed maximum zone of inhibition (5.6 mm) against *Escherichia coli*. At the dose of 250 and 500 µg/disc, it showed maximum activity against *Escherichia coli* and *Staphylococcus pyogens* respectively (Table 1 and Figure 1).

Table 1. *In vitro Antibacterial Activity of C. arvense extract*

Bacterial Strains	Diameter of Zone of Inhibition in mm ± S.E.			
	Extract (200 µg /disc)	Extract (250 µg /disc)	Extract (500 µg /disc)	Kanamycin (30 µg/disc)
Staphylococcus pyogens	5.2 ± 0.84	7.0 ± 0.92	13.6 ± 0.89	30.0 ± 0.18
Staphylococcus aureus	4.5 ± 0.87	6.6 ± 0.82	11.1 ± 0.91	30.0 ± 0.21
Staphylococcus epidermidis	0	0	0	26.3 ± 0.12
Shigella boydigius	3.8 ± 0.94	5.2 ± 0.83	9.9 ± 0.88	17.6 ± 0.22
Shigella sonnei	4.1 ± 0.86	6.2 ± 0.96	10.4 ± 0.87	21.3 ± 0.19
Streptococcus agalactiae	0	0	0	31.0 ± 0.14
Escherichia coli	5.6 ± 0.78	8.8 ± 0.85	13.3 ± 0.87	20.8 ± 0.17
Enterococcus faecalis	0	0	0	30.0 ± 0.18
Shigella flexineri	3.8 ± 0.94	6.1 ± 0.91	12.5 ± 0.84	23.3 ± 0.14

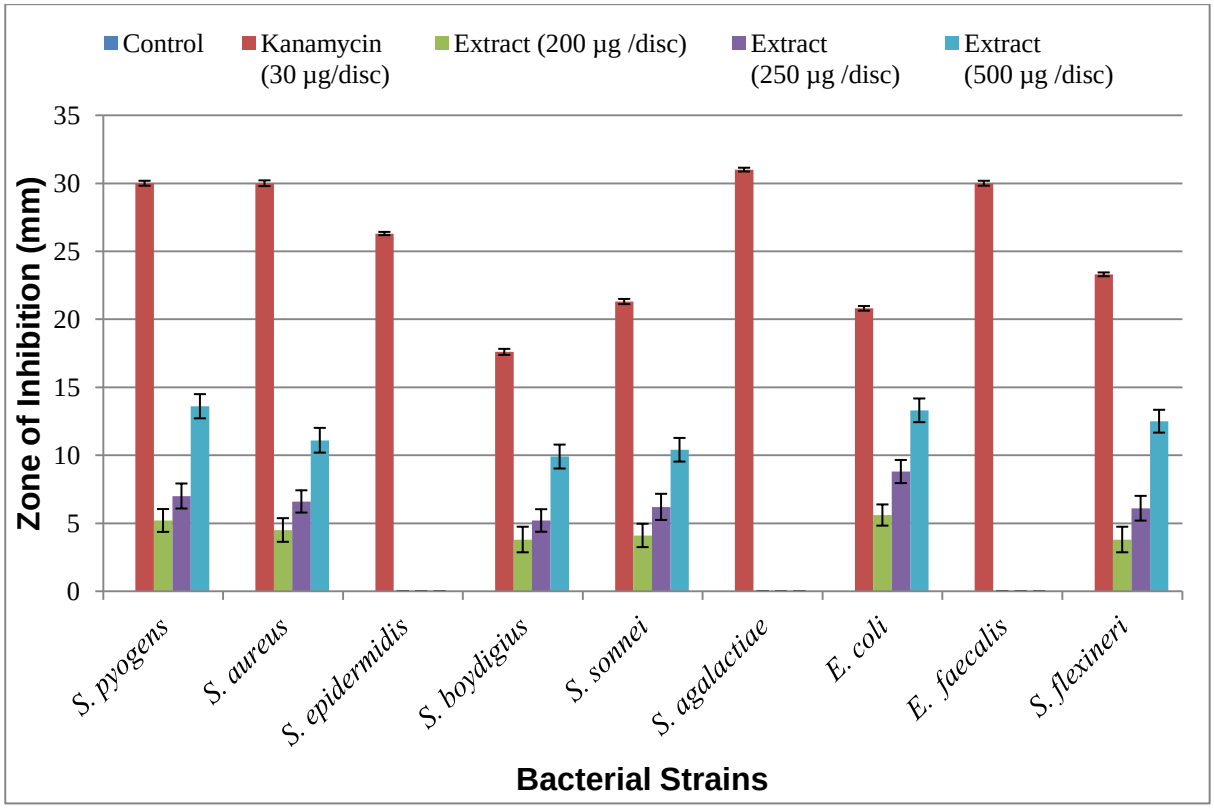


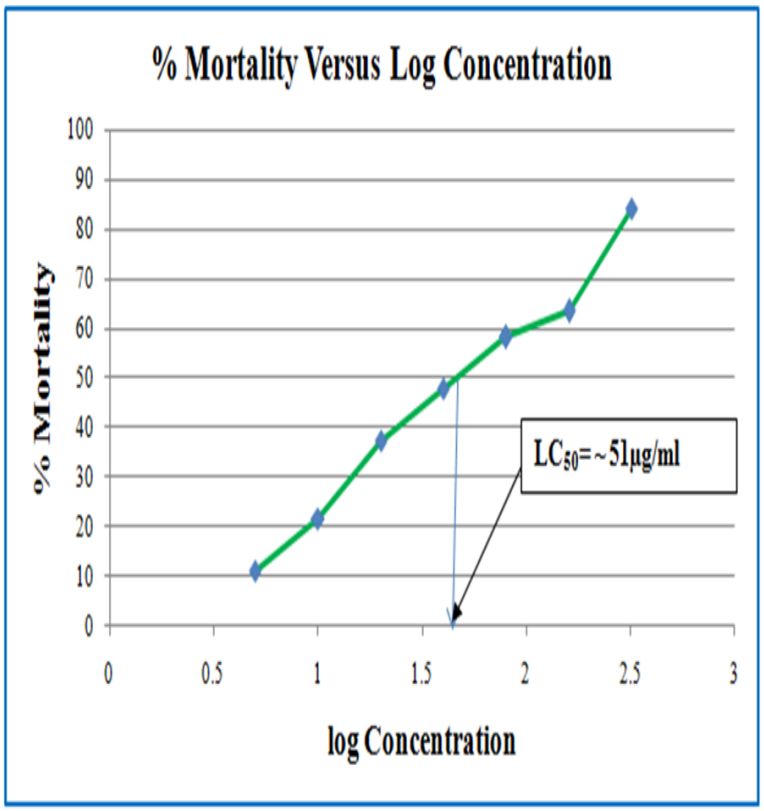
Figure 1. *Antibacterial activity of C. arvense extract*

It can be concluded that extract showed dose dependent antibacterial activity against different bacterial stains. Further investigation can be carried out using pure compounds of this extract.

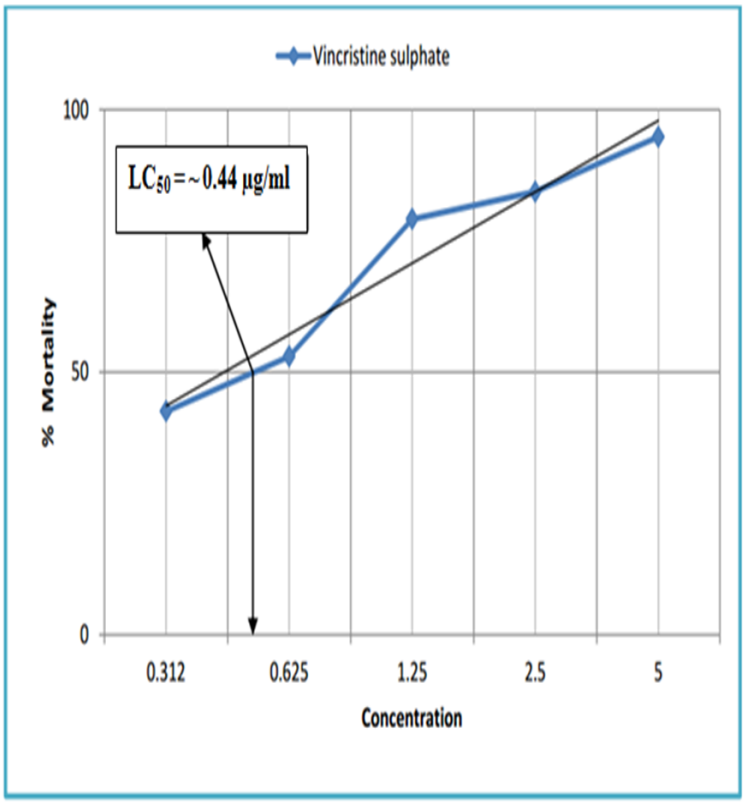
The brine shrimp test (BST) represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. In Brine Shrimp Lethality bioassay, the crude extract showed lethality indicating the biological activity of the compound present in the extract. This indicates a further investigation of any possible novel compounds in the aerial part of *C. arvense* being extracted with ethanol for their structures and functional aspects as anticancer agents. The lethal concentration LC_{50} of the test samples after 24 hours was obtained by a plot of percentage of the shrimps killed against the sample concentration (toxicant concentration) and the best fit line was obtained from the curve data by means of regression analysis. The LC_{50} produced by the extract as well as Vincristine sulphate as standard were calculated by probit analysis software LdP (LdP Line software, USA) and was found to be 51 µg/ml for *C. arvense* extract whereas 0.44 µg/ml for vincristine sulphate (Table 2, Figure 2).

Table 2. *Result of Brine shrimp lethality bioassay of extract*

Conc. of extract (µg/ml)	Log Conc.	No. of alive shrimp in Test-1	No. of alive shrimp in Test-2	No. of alive shrimp in Test-3	Avg. no. of alive shrimp (Extract) ± S.E.	Avg. No. of alive shrimp (Control)	% Mortality
5	0.699	9	8	8	8.3 ± 0.33	9.57	13.27
10	1	8	7	7	7.3 ± 0.33		23.72
20	1.301	6	6	5	6.3 ± 0.33		34.17
40	1.602	5	5	4	5.3 ± 0.33		44.62
80	1.903	4	4	4	4 ± 0		58.20
160	2.204	3	4	3	3.3 ± 0.33		65.52
320	2.505	1	2	1	1.3 ± 0.33		86.42



(a)



(b)

Figure 2. *Graphical representation of Lethality test for (a) Vincristine sulphate (b) C. arvense extract*

In anthelmintic activity test, obtained result indicate that the higher concentration of each plant extract produced paralytic effect much earlier and the time to death was shorter. The experimental result obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic.

The time of paralysis was recorded when no movement was observed unless shaken vigorously. The death time was recorded after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50°C) or subjected to external stimuli. Anthelmintic activity was expressed as the time required for paralysis and death of parasites as compared to control (Figure 3). The extract showed significant ($P < 0.005$) and dose dependent anthelmintic activity and revealed 75, 62 and 48 minutes for death time at the doses of 25, 50 and 75 mg/ml respectively whereas standard Albendazole (15 mg/ml) showed 26 minutes (Table 3). The extract revealed anthelmintic activity in a concentration dependent manner which is comparable with standard drug, Albendazole.

Table 3. *Anthelmintic activity of C. arvense extract*

Treatments Concentration (mg/ml)	Worm No.	Time taken for paralysis (min.)	Mean time of paralysis (min.) ± S.E.	Time taken for death (min.)	Mean time of death in (min.) ± S.E.
Control 0.1 %Tween-80 in PBS	C1	---	---	---	---
	C2	---			
	C3	---			
	C4	---			
Standard Albendazole 15 mg/ml	S1	16.25	18.01 ± 0.84	24.32	26.37 ± 0.91
	S2	18.45		27.55	
	S3	20.11		28.22	
	S4	17.21		25.39	
Extract 25 mg/ml	T1	59.36	57.36 ± 0.99	76.29	75.36 ± 0.87
	T2	58.21		73.42	
	T3	54.68		77.25	
	T4	57.17		74.46	
Extract 50 mg/ml	T1	45.46	47.48 ± 0.92	62.15	62.09 ± 0.88
	T2	47.41		64.47	
	T3	48.53		60.28	
	T4	44.5		61.44	
Extract 75 mg/ml	T1	37.32	36.75 ± 0.98	49.56	47.985 ± 0.66
	T2	34.15		47.88	
	T3	36.72		48.23	
	T4	38.84		46.27	

n = 5; *p < 0.05 Compared to control

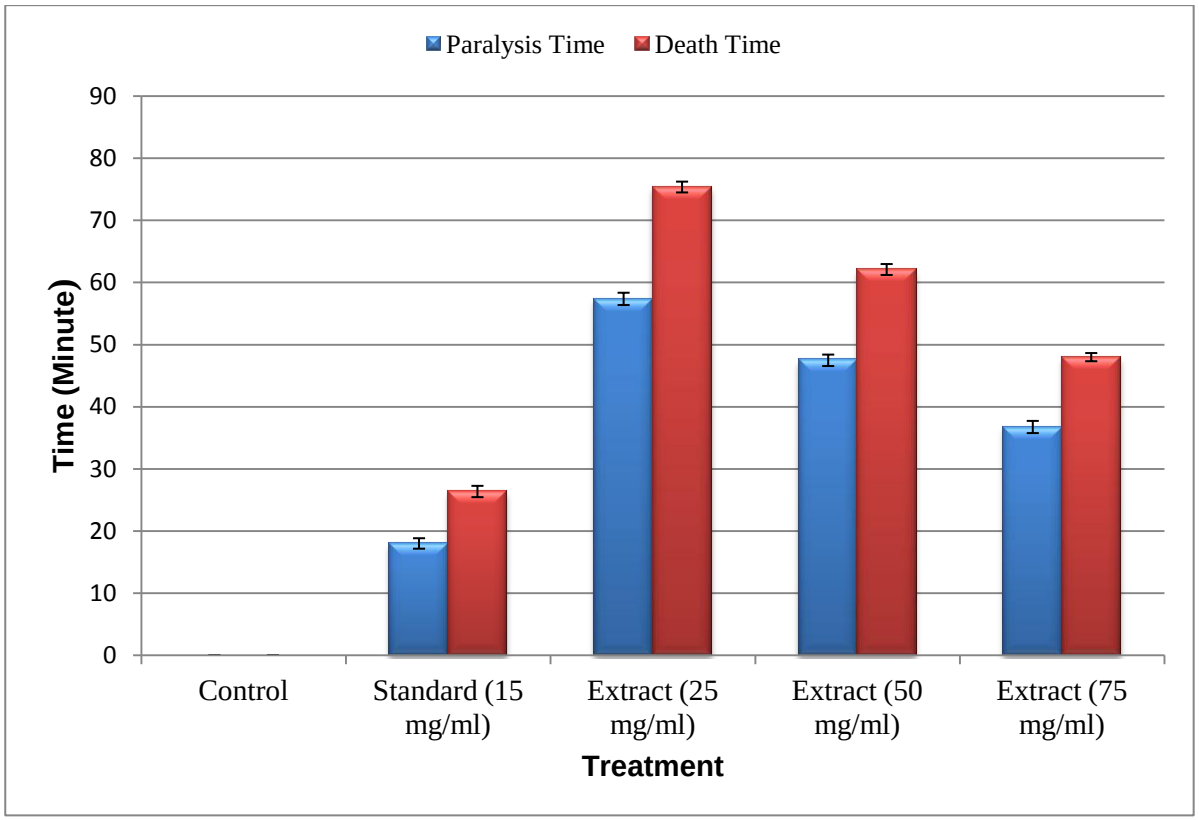


Figure 3. *Anthelmintic activity of C. arvense extract*

Upon literature review, I found that no anti-hyperglycemic activity test had been performed on *C. arvense*. So, I performed this test to identify whether it has anti-hyperglycemic activity. The extract did not show anti-hyperglycemic activity (Table 4 and Figure 4).

Table 4. *Effect of C. arvense extract on OGTT in mice*

Treatment Group	Dose (per kg Body Weight)	Blood Glucose Level (mM/L) ± S.E.			
		Zero hour	At 1st hour	At 2nd hour	At 3rd hour
Control	10 ml	5.20 ± 0.57	17.85 ± 0.53	7.92 ± 0.55	5.51 ± 0.61
Standard	5 mg	5.35 ± 0.56	12.25 ± 0.50	6.10 ± 0.51	4.13 ± 0.48
Sample	200 mg	5.42 ± 0.66	27.35 ± 0.68	17.56 ± 0.76	11.12 ± 0.88
Sample	250 mg	5.48 ± 0.63	25.24 ± 0.75	15.42 ± 0.77	10.30 ± 0.84
Sample	500 mg	5.65 ± 0.74	19.61 ± 0.82	10.90 ± 0.73	6.10 ± 0.81

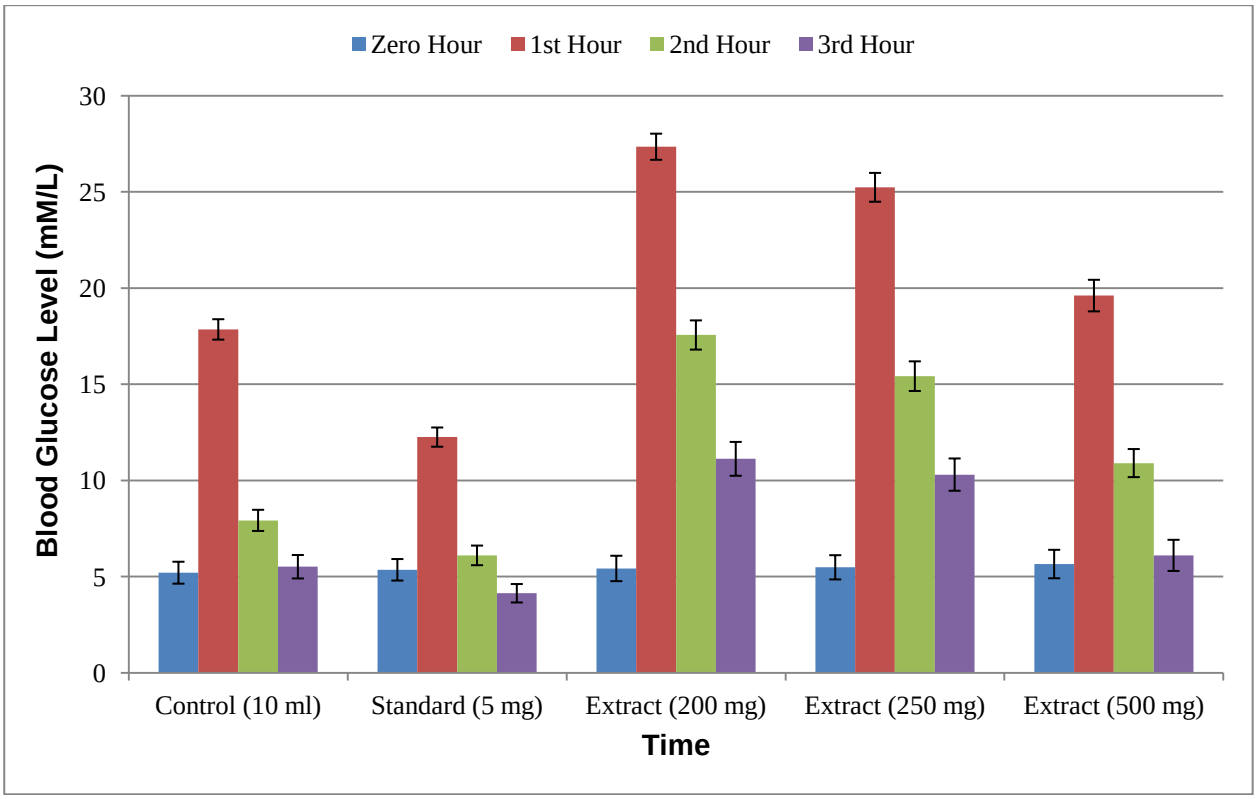


Figure 4. *Effect of C. arvense on OGTT in mice*

P-coumaric acid, rutin hydrate, quercetin and kaemferol were identified by Hossain ML et al. 2016 in ethanol extract of *C. arvense* using HPLC-DAD system. These phenolic compounds might be responsible for the aforementioned activities and also provide strong evidence with the traditional uses of this plant. Pharmacological evaluation of the plant extract provides the strong evidence of the existence of antibacterial, cytotoxic and anthelmintic activities.

IV. Conclusion

The present study was designed to assess antibacterial, cytotoxic, anthelmintic and anti-hyperglycemic activity of *Cirsium arvense* extract. It can be concluded that ethanol extract of *Cirsium arvense* is very useful and effective and may be potential source of novel bioactive compounds. For this reason, the extract of the plants should be studied further to isolate and purify the active compounds.

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Please cite this Article as:

Md. Lokman Hossain, A.S.M. Monjur-Al-Hossain, Subarna Saha and Samir Kumar Sadhu, Assessment of Biological Activity on Cirsium arvense L. , *Algerian J. Nat. Products,* **5**:**1** (201**7**) 417- 426.

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Online ISSN: 2353-0391
Editor in chief: Prof. Kamel BELHAMEL

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DOI:https://doi.org/10.5281/zenodo.824566	