ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY
Research Article

An Experimental Study of Analgesic Activity and Cytotoxic Potential of Methanolic *Moringa oleifera* Stem Bark Extracts in Animal Models

Tamanna Sharmin Tonny*, Masum Rana, Saleha Akter and Sumaiya Alam Ria

Department of Pharmacy, Primeasia University, Bangladesh

***Corresponding Author**: Tamanna Sharmin Tonny, Department of Pharmacy, Primeasia University, Dhaka, Bangladesh.

Received: July 15, 2018; **Published:** October 29, 2018

Abstract

Moringa oleifera is a well-known medicinal plant containing abundant natural nutraceuticals and phytochemical having therapeutic value, can be used against a wide range of acute and chronic ailments. In this present study, different doses of Methanolic *Moringa oleifera* stem bark extract were evaluated for their analgesic activity and cytotoxic potential by Tail immersion method and Brine shrimp lethality bioassay respectively. In the Tail immersion method, after 90 minute of experimentation, the Methanolic *Moringa oleifera* stem bark extract (MMOSBE)s produced analgesia in a dose dependent manner and when used in the dose 200 mg/kg produced significant 9.9 second response time compared to around 12.03 second of response time exhibited by the standard drug Diclofenac sodium. The MMOSBEs have shown excellent cytotoxic potential having LC50 value 0.33 µg/ml compared with the standard drug, Vincristine Sulphate having LC_{50} value of 0.21 µg/ml. The results indicate that Methanolic stem bark extract of *Moringa oleifera* has moderate to high analgesic potential and great cytotoxic activity which can be further investigated to develop a potentially safe pain relieving anticancer agent.

Keywords: *Moringa oleifera; Analgesia; Cytotoxic Potential*

Abbreviations

LC_{50}: Lethal Concentration 50; MMOSBE: Methanolic *Moringa oleifera* Stem Bark Extract

Introduction

Therapeutic values of medicinal plants have emphasized their immense importance as mean of treatment since human civilization throughout the world. Since dawn of ages, Nature has been the main source of therapeutic agents and numerous modern active drug molecules have been isolated from natural sources. Much of these isolations were done based on their time honored traditional uses as medicines. Basically it can be said that the, the health care structure of the world is based on this traditional, nature based medicine system, as about 80% of the earth's population depend mainly on natural medicines for their essential health management [1]. The World Health Organization (WHO,1977) has defined "a medicinal plant' as any plant, containing one or more parts which comprises therapeutic substituent [2]. There has been a rampant surge in the use of traditional medicines, in the recent few decades. They have gained immense popularity in both developing and developed countries due to their negligible side effects owing to their natural origin compared to synthetic drugs, as well as for the classical and enriching causes [3].

The genus *Moringa* of family Moringaceae contain about 13 species of *Moringa* trees. Among them, the most extensively used species *Moringa oleifera* is known as "*Moringa*" [4]. It is widely distributed in South Asia, where it is indigenous in the Himalayan Foothills from Northeastern Pakistan to Northern West Bengal, India. But, later it has been introduced to make it a natural habitat in other parts of world such as, Pakistan, other parts of India, Bangladesh, Afghanistan, Sri Lanka, Southeast Asia, West Asia, The Peninsula of Arab, East and West Africa, Southern Florida, West Indies, Mexico, Peru, Paraguay and even Brazil [5].

Moringa oleifera is a best known medicinal plant containing plentiful natural sources of Vitamin A, B, C, D, E and K [6]. Various important minerals like Calcium, Copper, Iron, Potassium, Magnesium, Manganese and Zinc. Abundance of more than 40 natural anti-oxidants, has made them useful as medicinal plant since 150 B.C., whereas they were used by ancient kings and queens to improve their mental prudence as well as skin condition [7]. Phytochemicals in the simplest form of words, can be defined as the chemicals produced by the plants. In general they are not compulsory nutrition for human body, but they do have important effect on health or on flavor taste etc. of the plants. A wide range of special phytochemical components can be identified in *Moringa* species. This plant family has ample general compounds like simple sugar, rhamnose and also contain fair share of unique compound like glucosinolates and isothiocyanates [8]. As for example, particular constituents of *Moringa* preparations that have been attributed to have hypotensive, anticancer, and antibacterial activity include 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy)benzyl isothiocyanate [1], 4-(a-L-rhamnopyranosyloxy) benzyl isothiocyanate [2], niazimicin [3], pterygospermin [4], benzyl isothiocyanate [5] and 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate [6]. With all these unique components, *Moringa* family is also plentiful with lots of vitamins and minerals as well as comparatively common phytochemicals for example the carotenoids (β-carotene or pro-vitamin A) [9].

Moringa contains a sumptuous mixture of amino acids, antioxidants, nutrients, anti-inflammatory properties necessary for nourishment and therapeutic purpose. *Moringa* is often called as the "Miracle Tree" and also "Mother's Best Friend". To treat malnutrition, Moringa has been upgraded as a substitute to imported food supplies by the World Health Organization, since 1998 [10]. Moringa contains a sumptuous mixture of amino acids, antioxidants, nutrients, anti-inflammatory properties necessary for nourishment and therapeutic purpose. *Moringa* is often called as the "Miracle Tree" and also "Mother's Best Friend". To treat malnutrition, *Moringa* has been upgraded as a substitute to imported food supplies by the World Health Organization, since 1998 [10].

Traditional medicines used in various cultures throughout the world from ancient history, contains Moringa for treating various ailments like skin infections, anemia, anxiety, asthma, blood impurities, bronchitis, chest congestion, cholera, conjunctivitis, cough, diarrhea, ear and eye infections, High blood pressure, joint pain, psoriasis, respiratory conditions, sore throat, muscle pain, tuberculosis, intestinal worms, lactation, diabetes and hysteria. It also has potential to alleviate malnutrition, common weakness, menopausal symptoms, lactating mothers, depression and osteoporosis like serious complications [11]. This research work has been done to study the ability of this unique plant to reduce algesia by tail immersion method and cytotoxicity of the extract by Brine shrimp Lethality Assay.

Materials and Methods

Material Selection

The fresh bark sample of the plant *Moringa oleifera* Lam. was selected and collected for biological investigation.

Collection of Sample and Identification

The bark samples of *Moringa oleifera* were collected during the month of April and May 2016, from the adjoining area of Mirpur, Dhaka, Bangladesh. The authentication of the plant barks and leaves were done from the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. After identification by the experts of the Bangladesh National Herbarium, the sample was given an Accession number which was DACB 43200.

Preparation of plant material

The process of shade drying was used for around 20 days to dry the bark samples. Suitable grinder was used to grind the bark into dry coarse powder weighing, 160g. The powdered sample was then stored in an airtight container and kept in a cool, dark and dry place for further use in analytical procedures.

Preparation of plant Extract

The dried bark powder was soaked in the solvent Methanol in a clean flat bottomed glass container. The container was sealed and preserved for a period of 10 days in a cool dark place, where regular shaking and stirring were done. First filtration by a piece of clean,

white cotton material was done to separate the mixture. Then Whatman filter paper (no. 02) was used for final filtration. The extraction procedure to obtain the crude extract was done with the help of a Rotary evaporator. The percentage of yield was determined using the mass of the extract which was previously calculated. The Formula used for calculation is:

Yield (%) = Mass of crude extract x 100/Total mass of dry powder

= {(31x 100)/76}%

= 40.79%

Determination of analgesic activity by tail immersion method

The investigation for analgesic activity of the crude extract of *Moringa oleifera* was conducted using the Tail Immersion Method using albino mice. The analgesic activity of the raw extract was compared to the pain inhibitory effect shown by the standard Non-Steroidal Anti Inflammatory Drug (NSAID) used, which was Diclofenac Na.

Materials and Reagents

Materials and Reagents	Source
Diclofenac Na	Gift from Square Pharmaceutical Ltd.
Tween-80 (as suspending agent)	BDH Chemicals Ltd
Normal saline solution	Beximco Infusion Ltd.
Sterile disposable syringe	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electronic and digital balance	Denver Instruments, M220/USA
Stop Watch	Bangladesh

Animals used for Experimentation

For the experiment,12 albino mice aged 5 - 6 weeks, weighing about 25 - 30 gm were use, which were purchased from the animal research center of Jahangirnagar University, Savar, Dhaka. They were kept in individual cages and appropriate environmental conditions were maintained such as, Temperature (25.0oC ± 3.0oC), Humidity 35% to 60% and alternatively 12 hours of light and dark cycle. Every day cages were cleaned for removal of excretes to ensure a fresh environment.

Dividing the animals into groups for the analgesic activity

The 12 experimental mice used in Tail Immersion Method were selected randomly and divided into four groups, represented by group I (control group), group II (Standard group, where Diclofenac Na was used as the drug), group-III (dose 100 mg/kg) and group-IV (dose 200 mg/kg), whereas each group consisted 3 mice to study the method accordingly. Each group was tended to a particular treatment, such as control, Standard and doses of extracts of *Moringa oleifera* respectively.

Identifying the test animals

Before the commencement of the experimentation, the weight of each mouse was taken and the doses of the test materials and standard drug were adjusted accordingly. To achieve the exact interpretation of the treatment, individual mouse was required to be identified or marked. For this purpose, the Mice were marked on their tail to individualize and identified as M-1 = Mice 1, M-2 = Mice 2, M-3 = Mice 3 accordingly.

Preparation and administration of doses of plant extracts and active drug

Preparation of Saline water

Sodium Chloride weighing 4.5 gm was taken and diluted up to 1000 ml to prepare the Saline water.

Preparation of Standard drug (Diclofenac Na)

To prepare the standard drug solution of Diclofenac Na at the dose of 10 mg/kg body weight, required amount of drug was weighed according to body weight and dissolved in distilled water. Each mouse was treated orally by 1 ml of this standard Diclofenac Na solution. In The mice used in the experiment weighed in average about 30 gm.

Preparation of drug doses obtained from crude extract

To study the analgesic activity of the extract, it was necessary to administer Methanolic *Moringa oleifera* bark extract into the mice according to their body weight. Two doses, 100 mg/kg and 200 mg/kg were studied. For this purpose, the doses were measured, to which 10 ml of distilled water were added. The extract and suspending agent were mixed thoroughly which was then made up to the final volume of each suspension. Each mouse was orally treated with 1 ml of this suspension.

Analgesic activity determination by tail immersion method

In this process, 3 cm of the tail of the experimental mouse was introduced in hot water at a temperature of 55 ± 0.5oC. Within a few minutes, the mice showed reaction by withdrawing their tail. The index of response latency was recorded as the time duration from placement and shaking/licking of the paw or jumping. The time of response was recorded by a Stopwatch. First of all, the initial response of each animal was recorded followed by the determination of later reaction time respectively. Substances having potential of reducing analgesia is supposed to increase the time duration between the placement and shaking/licking of the paw or jumping within a definite time limit and compared to the control group.

Working procedure of tail immersion test

At the time of zero hour, Group-I, Group-II and Group III-IV were treated orally with Distilled water, Diclofenac sodium and test samples accordingly by the help of a feeding needle. After the completion of the feeding episodes, about 3 cm of the tail of each mouse was introduced in hot water at a temperature of 55oC.After introduction, mice reacted by withdrawing the tail within few minutes. For proper time measurement, stop watch was used to observe each mouse of all groups individually. The index of response latency was recorded as the time interval from placement and jumping/movement of the tail.

Statistical Analysis

The results of the Tail immersion method were represented as mean± SD and the statistical analysis of the results was carried out using One-way ANOVA. The difference between the means was tested with Post Hocs by SPSS software, whereas values of $p < 0.05$ were considered statistically significant.

Lethality bioassay test

To check the bioassay and cytotoxicity of the bioactive compounds, Brine shrimp Lethality bioassay method is widely used [12]. For screening, *Artemia salina* nauplii was used as the living zoological sample which had been known as an effective carrier used for lethality screening. An aquarium shop from Dhaka, Bangladesh was used for collection of the eggs of Brine shrimp (*Artemia salina*)and hatched in a tank maintained at temperature around 37oC and with continuous oxygen supply. It took around two days to hatch and get the nauplii to be matured. Required amount of extract was dissolved in definite volume of pure dimethyl sulfoxide (DMSO) to prepare the sample stock solution. Each vial was treated with Four milliliter of saline water equivalent to sea water. Then vials were used to transfer specific volumes of sample solution and series dilution were done from 400 µg/ml, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 to 0.78125 µg/ml to obtain the final sample concentrations.

Same amount of DMSO compared to sample vials were taken in control vials. 10 living nauplii were intubated into each of the vials by Pasteur pipette. The vials were observed after 24 hr and the number of viable nauplii in each vial was counted. The number of survived nauplii was used to calculate the percentage of Brine shrimp Lethality in case of each extract concentration. Vincristine sulphate was taken as the standard cytotoxic drug [13].

Results and Discussion

Analgesic activity of *Moringa oleifera* by tail immersion method

Analgesic activity of the stem bark extract of the plant *Moringa oleifera* was investigated in animal model (mice) using Tail immersion method. To study the analgesic potential Swiss albino mice were used. The extract was taken in two different doses of 100 mg/kg body wt and 200 mg/kg body wt respectively as samples for conducting the research. The results of the investigation is shown in the following table 1.

Group	Treatment	Reaction time (in sec) after administration of drugs at different time (minutes)											
		0 min	Mean ± SD	Sig	30 min	Mean ± SD	Sig	60 min	Mean ± SD	Sig	90 min	Mean ± SD	Sig
1.	Normal Control (10 mg/ kg body wt.)	1.51s	1.64 ± 2.02s	.044	4.06s	4.21 ± 2.02s	.044	4.01s	4.36 ± 2.02s	.113	4.74s	3.02 ± 2.02s	.023
		2.01s		.113	5.01s		.558	4.04s		.558	2.20s		.692
		1.40s		.023	3.58s		.692	5.05s		.337	2.12s		.337
2.	Diclofenac Na (10 mg/ kg body wt.)	9.70s	6.47 ± 1.32s	.004	9.60s	9.55 ± 1.32s	.004	18.01s	27.34 ± 1.32s	.548	7.60s	12.03 ± 1.32s	.025
		3.31s		.548	11.06s		.009	40.00s		.009	10.90s		.237
		6.40s		.025	8.00s		.237	24.00s		.066	17.60s		.066
3.	MMOSB extract (100 mg/kg body wt.)	7.20s	5.23 ± 5.04s	.003	7.30s	4.96 ± 5.04s	.003	5.80s	4.67 ± 5.04s	.957	6.50s	4.03 ± 5.04s	.066
		5.00s		.957	4.39s		.003	4.80s		.003	3.60s		.066
		3.50s		.066	3.45s		.066	3.40s		.072	2.00s		.072
4.	MMOSB extract (200 mg/kg body wt.)	6.31s	7.30 ± 2.76s	.011	8.88s	7.41 ± 2.76s	.011	8.24s	18.09 ± 2.76s	.255	9.24s	9.91 ± 2.76s	.007
		4.63s		.255	4.31s		.075	18.94s		.075	6.40s		.784
		10.96s		.007	9.03s		.784	27.10s		.048	14.10s		.048

Table 1: *Evaluation of analgesic potential of the crude methanolic extract of Moringa oleifera by tail immersion method.*

** MMOSB extract=Methanolic Moringa oleifera stem bark extract*

***The mean difference is significant at the .05 level.*

From the result of the study it was observed the Methanolic stem bark extract of *Moringa oleifera* has a noticeable analgesic potential. The increase of time duration in the reaction time was dose dependent though there were significant differences among the group of mice receiving different doses of extracts. The time duration among responses increased at a regular basis after 0 minute, 30 minutes, 60 minutes but surprisingly in case of all the treatments after 90 minutes of administration, the reaction time decreased. This can be studied further by increasing the experimentation time. After 60 minutes of administration, Methanol extract (200 mg/kg) showed best analgesic potential, where average response time was 18.09s than the methanol extract of (100 mg/kg) dose of the stem bark sample of *Moringa oleifera* showing response time of, 4.67s. The findings of the evaluation are represented in the figure 1-3.

The figure shows that, after 90 minutes of administration of the crude Methanolic bark extract of Moringa oleifera at 200 mg/kg body weight exhibit an average 9.9 second response time compared to the standard analgesic drug Diclofenac Sodium which create around 12.03 second of response time, which is quite satisfactory.

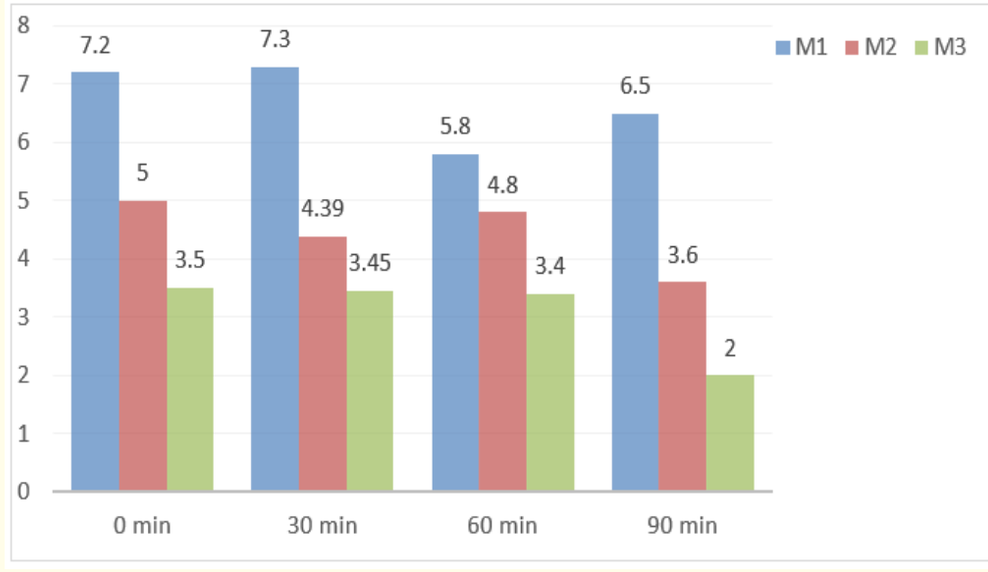

Figure 1: *Analgesic activity of methanolic crude bark extract of Moringa oleifera at dose (100 mg/kg) body weight.*

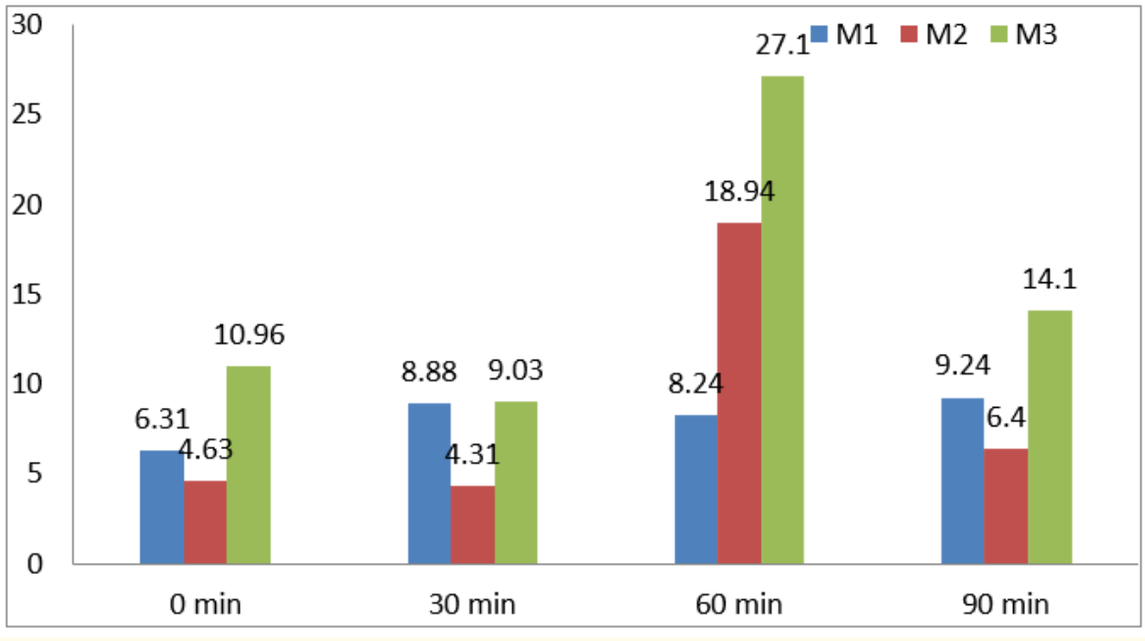

Figure 2: *Analgesic activity of methanolic crude bark extract of Moringa oleifera at dose (200 mg/kg) body weight.*

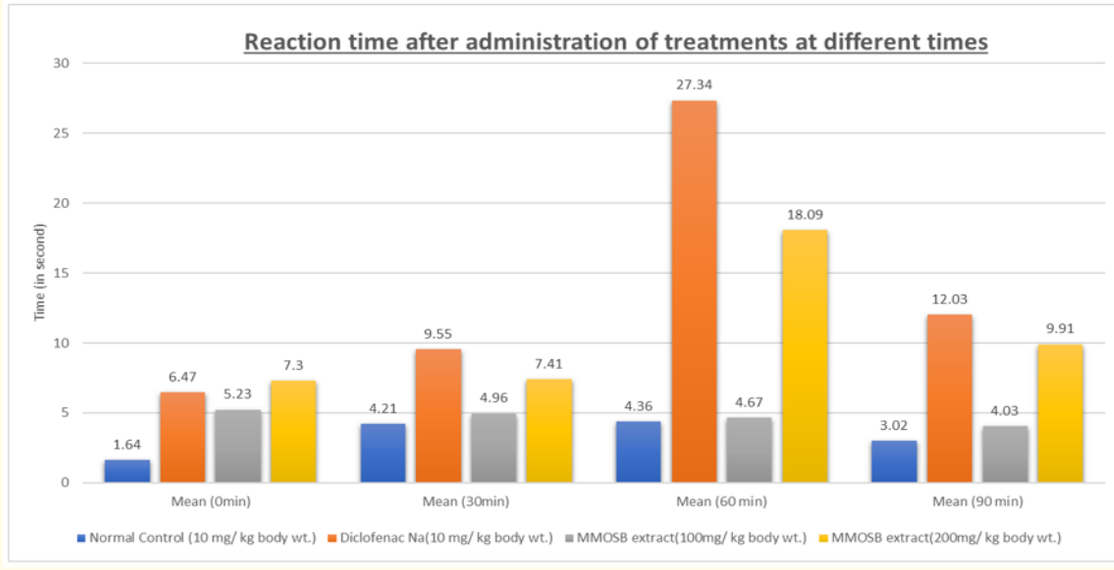

Figure 3: *Average response time of mice's after 90 minutes of experimentation by tail immersion method.*

Brine shrimp lethality test

The Brine Shrimp Lethality Bioassay (BSLA) has been used extensively to examine the toxicity of the crude extracts towards the nauplii of brine shrimp, which can also predict the potential toxicity of the test samples.

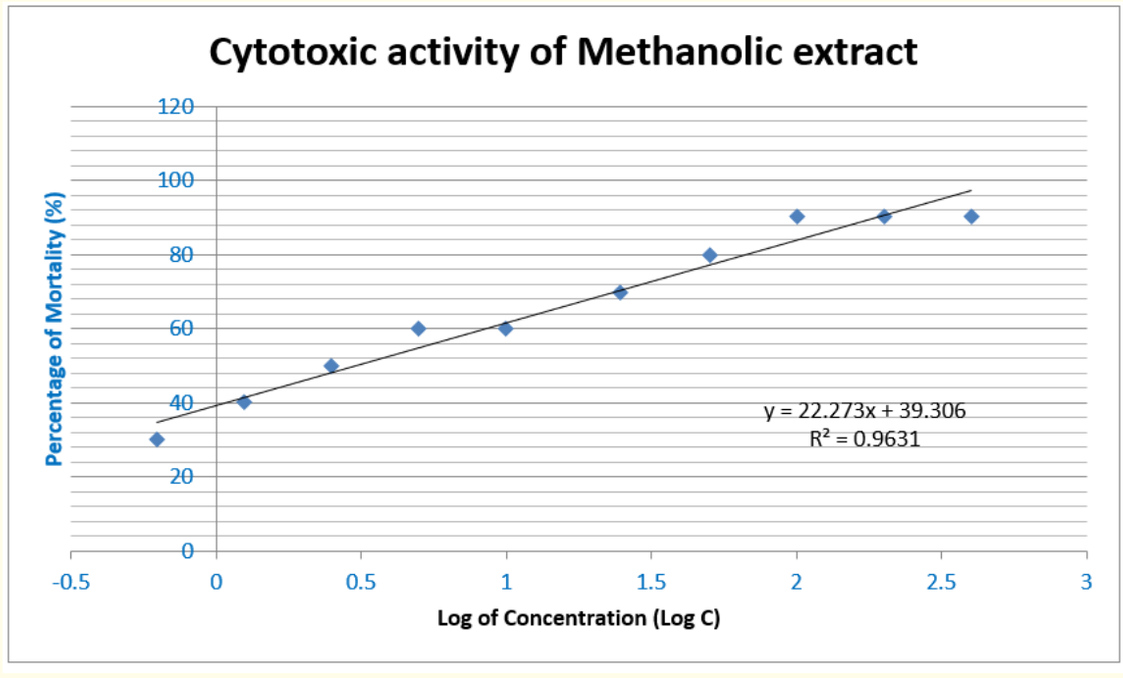

Figure 4: *Cytotoxic activity of Methanolic bark extract of Moringa oleifera at different doses.*

**y=Regression equation;*R2= Coefficient of determination*

Test Samples	Concentration (µg/ml)	Log of Conc. (Log C)	Total number of Nauplii taken in each Test Tube	Number of dead Nauplii (after 24h)			Average number of dead Nauplii	Percentage of Mortality (%)	LC_{50} value (µg/ml)
				Test Tube 1	Test Tube 2	Test Tube 3			
Control	Distilled Water	----	10	0	0	0	0	00	----
Methanolic *Moringa oleifera* extract	400	2.602	10	9	8	9	9	90	0.33
	200	2.301	10	9	9	8	9	90	
	100	2.000	10	8	9	9	9	90	
	50	1.698	10	8	7	8	8	80	
	25	1.397	10	7	7	8	7	70	
	10	1.000	10	6	6	7	6	60	
	5	0.698	10	6	5	6	6	60	
	2.5	0.397	10	5	6	5	5	50	
	1.25	0.096	10	5	4	4	4	40	
	0.625	-0.204	10	4	3	3	3	30	
	20	1.301	10	9	10	9	9	90	
	10	1.000	10	9	8	9	9	90	
	5	0.698	10	8	8	7	8	80	
	2.5	0.397	10	7	7	8	7	70	
	1.25	0.096	10	7	7	6	7	70	
	0.625	-0.204	10	6	5	6	6	60	
	0.3125	-0.505	10	5	5	6	5	50	
	0.15625	-0.806	10	5	4	5	5	50	
	0.078125	-1.107	10	4	4	3	4	40	

Table 2: *Cytotoxic activity of Moringa oleifera extracts in comparison to Vincristine sulphate against Brine shrimp nauplii.*

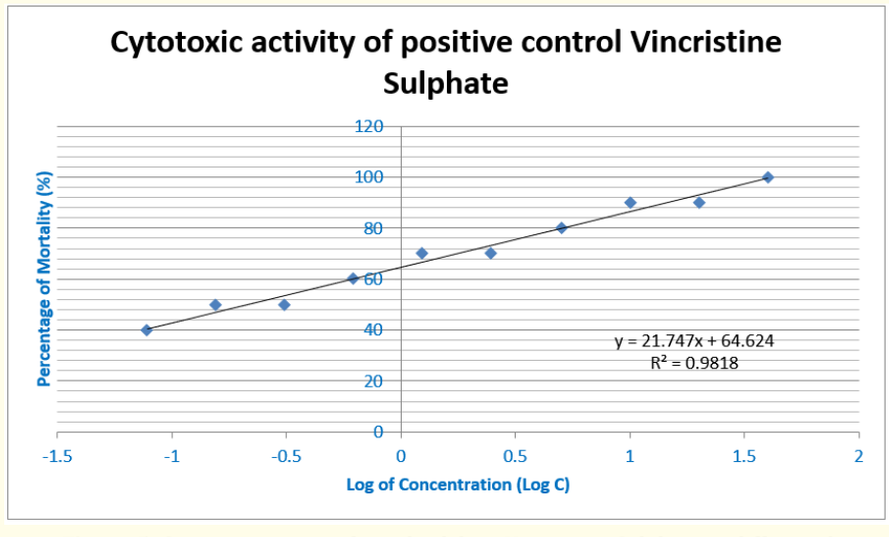

Figure 5: *Cytotoxic activity of standard drug Vincristine Sulphate at different doses.*

**y=Regression equation; *R2= Coefficient of determination*

The 24 hour Nauplii count was used to obtain dose-response data which were formed into a straight line by the help of a logit transformation and the value of LC50 was calculated from the best fit line procured by Linear regression analysis [14]. Maximum mortalities were recorded at a concentration of 400 µg/ml, while the least mortality were found at the concentration of the Methanolic plant extract at 0.625 µg/ml. No mortality was found for the negative control group which indicated that the results were only happened because of the activity of test examples. The Methanolic *Moringa oleifera* stem bark extracts have shown excellent cytotoxic potential having LC50 value 0.33 µg/ml compared with the standard drug, Vincristine Sulphate having LC_{50} value of 0.21 µg/ml. For this reason, as previously suggested association between cytotoxicity and activity opposed to Brine shrimp nauplii by scientists, the significant cytotoxic potential showed by the Methanolic stem bark extract in this experiment can be chosen for advanced cell line assay.

Conclusion

Aboriginal therapeutic plants possess the most significant place in the safe economic drug development process. From the analgesic activity study by Tail immersion test, the Methanolic *Moringa oleifera* extract treatment (200 mg/kg) showed better analgesic potential than the dose (100 mg/kg), which was satisfactory even compared to the standard drug treatment. Furthermore, the results shown by *Moringa* extract in Brine shrimp lethality bioassay has suggested a remarkable cytotoxic, antitumor and anticancer potential. Considering this high cytotoxic activity, the plant extract can be used to reduce algesia however the dose should be adjusted in range between moderate to minimum level according to body weight. Therefore, this study indicates that there is a scope for further investigation of this plant extract in order to identify, isolate, purify and characterize the active compounds responsible for the analgesic property and antitumor potential as well as to establish a safe dosage regimen for them.

Acknowledgements

The department of Pharmacy. Primeasia University is highly appreciated for provision of resource materials, facilities and extraction of the plant material required to conduct the investigation.

Bibliography

1. Murugan M., *et al*. "Evaluation of phytochemical analysis and antibacterial activity of Bauhinia purpurea L. and Hiptage benghalensis L. Kurz". *Journal of Applied Pharmaceutical Science* 1.9 (2011): 157-160.

2. Soetan KO., *et al*. "The need for bioactivity-safety evaluation and conservation of medicinal plants - A review". *Journal of Medicinal Plants Research* 3.5 (2009): 324-328.

3. Oyewo EB., *et al*. "Blood glucose and lipid reducing activities of the oral administration of aqueous leaf extract of Moringa oleifera in Wistar rats". *Journal of Natural Sciences Research* 3.6 (2013): 92-99.

4. Nasir E Ali. "Flora of West Pakistan: an annotated catalogue of the vascular plants of west Pakistan and Kashmir". Karachi, Pakistan: Fakhri Printing Press (1972): 1028.

5. Mahmood KT., *et al*. "Moringa oleifera: a natural gift-A review Khawaja". *Journal of Pharmaceutical Sciences and Research* 2.11 (2010): 775-781.

6. Anwar F., *et al*. "Moringa oleifera: A Food Plant with Multiple Medicinal Uses". *Phytotherapy Research* 21.1 (2007): 17-25.

7. Dixit S., *et al*. "Medicinal properties of moringa oleifera: a review". *International Journal of Education and Science Research* 3.2 (2016): 173-185.

8. Fahey JW., *et al*. "The chemical diversity and distribution of glucosinolates and isothiocyanates among plants". *Phytochemistry* 56.1 (2001): 5-51.

9. Fahey JW. "Moringa oleifera: A Review of the Medical evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1". *Trees for Life Journal* 1 (2005): 5.

10. Ashfaq M., *et al*. "Moringa: A Miracle Plant for Agro-forestry". *Journal of Agriculture and Social Sciences* (2012).

11. Mishra G., *et al*. "Traditional uses, phytochemistry and pharmacological properties of Moringa oleifera plant: An overview". *Scholars Research Library Der Pharmacia Lettre* 3.2 (2011): 141-164.

12. Meyer BN., *et al*. "Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents". *Journal of Medicinal Plant Research: Plant Medica* 45.5 (1982): 31-34.

13. Fatima UK., *et al*. "Comparative Anti-microbial activity and brine shrimp lethality bioassay of different parts of the plant". *Journal of Applied Pharmaceutical Science* 2.12 (2012): 85-88.

14. Hafner EE and Heiner Noack E. Arzneim-Forsch 27 (1977): 1871.

Volume 6 Issue 11 November 2018