ISSN: 2456-2572

APJPP 2016; Vol.1 (02): pp. 42-46 **Original Research Article**

ASIAN PACIFIC JOURNAL OF PHARMACY & PHYTOCHEMISTRY

Available online at http://apjpp.com

Received: 27-07-2016
Revised: 26-08-2016
Accepted: 28-08-2016

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ACUTE TOXICITY STUDY OF MANGIFERIN

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ABSTRACT

The aim of this work was to find the toxicity of isolated mangiferin from leaves of *Mangifera indica* L. var Alphonso by Brine shrimp lethality assay (BSLA) and Mutagenesis assay on *Drosophila melanogaster.* In brine shrimp lethality assay (BSLA), which based on the ability to kill laboratory cultured brine shrimp (*Artemia nauplii*), brine shrimp was treated with mangiferin of different concentration (5-40ppm). In mutagenesis method larvae was treated with mangiferin of various concentration (10, 25, 50 & 100mM) along with negative control (distilled water) and standard chemical mutagen (0.5% V/V formalin).In BSLA LC_{50} for mangiferin was 10ppm in 24hrs. It showed that mangiferin was safe and consistent with the reported data. In mutagenesis assay results of mangiferin at all concentration showed no significant morphological changes in the *D. melanogaster* on comparing formaldehyde exposed flies. Hence results revealed that mangiferin was not acutely toxic.

Key Words: *Mangifera indica*, Brine shrimp lethality assay, *Drosophila melanogaster.*

INTRODUCTION

The medicinal plants and traditional health systems solving the health care problems of the world is gaining increasing attention. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. *Mangifera indica* a species of *Anacardiaceae* family or locally known as *Mempelam, or Mangga* grows wild on tropics and subtropics. It is well known as a medicinal plant and economic product commonly used by all race in many Asian countries. All parts of the plant including the fruit, flower, leaves and stem are used in various ways to treat many health ailments and diseases [1,9]

The brine shrimp assay is a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials. The method is attractive because it is very simple, inexpensive and low toxin amount are sufficient to perform the test in the micro well scale.

Mutagenesis assays have been developed that are able to detect several genetic end point, it is important to know whether chemical is hazardous or safe. The eye and wing spot assays using ***Drosophila melanogaster*** was performed as they are sensitive *invivo* assay which are simple, much less, laborious, cheaper and at the same time more informative.

MATERIALS AND METHODS

Acute Toxicological Study Using Brine Shrimp Lethality Assay (BSLA)

Ten nauplli were drawn through a glass capillary and placed in each vial containing 4.5ml of brine solution. In each experiment, 0.5ml of the extract was added to 4.5 ml of brine solution and maintained at room temperature for 24 hrs under the light and surviving larvae were counted. Experiment was conducted along with control (vehicle treated), different concentrations of the mangiferin (5-50 ppm) in a set of three tubes per dose. Podophyllotoxin was used as a positive control in the bio assay [7, 10,11].

Statistical Analysis

The percentage lethality was calculated from the mean survival larvae of mangiferin treated tubes and control. LC_{50} values were obtained by best – fit line method.

Abbot's formula

$$\textbf{Corrected mortality (\%)} = \frac{\textbf{Test mortality (\%) - Control mortality (\%)}}{\textbf{100 - Control mortality (\%)}} \textbf{ x 100}$$

Assessment of Mutagenesis on *Drosophila Melanogaster*

If a mutagenic alteration takes place in one of the cells, the descendent cells will form a clone of mutant cells that can be detected as a spot in the adult tissue. Produced a loss of heterozygosis by several chromosome breakage mechanisms such as mitotic recombination, deletion, point mutation, chromosomal loss and abberration. About 75% of known human disease genes have a recognizable match in the genetic code of fruit flies and 50% of fly protein sequences have mammalian analogues. *Drosophila* is being used as genetic model for several human diseases including the neurodegenerative disorders Parkinson's, Huntington's and Alzheimer's disease. The fly is also being used to study mechanisms underlying aging and oxidative stress, immunity, diabetes and cancer as well as drug abuse [2,4,5]

Procedure

larvae from F1 generation of noramal flies were collected and washed with solution of 20% W/V sucrose and seeded in glass petridishes (20 larve/vial) containig 2 gm of banana mass with mangiferin of various concentration(10, 25, 50 & 100mM) along

with negative control of solvent alone (distilled water) and standard chemical mutagen (0.5% V/V formaldehyde).Larvae were fed on the above medium for six hours and transferred to fresh medium for the rest of their development. After eclosion, adult flies were collected and stored in70%V/V ethanol for the evaluation of the mutagenic effect. Morphological changes including eye color, spots in the wings, wing hairs, changes in the length and width of the wing, wing shape, abdomen length and total body length were observed under stereomicroscospe. All the experiments were carried out at room temperature (±12h day and night).The observation were tabulated and photograph of the compared flies were presented.

RESULTS

Table No.1 Effect of various concentrations of mangiferin on *artemia nauplii*

Concentration (ppm)	Number of Larvae released	Number of Larvae dead after 24hrs	Mortality (%)	Corrected (%) Mortality using Abbot's Formula
5	10	5	50	46.6
	10	5	50	
	10	4	40	
10	10	6	60	53.3
	10	5	50	
	10	5	50	
15	10	6	60	56.6
	10	6	60	
	10	5	50	
20	10	7	70	66.6
	10	6	60	
	10	7	70	
25	10	8	80	80.0
	10	8	80	
	10	8	80	
30	10	9	90	86.6
	10	9	90	
	10	8	80	
35	10	10	100	100
	10	10	100	
	10	10	100	
40	10	10	100	100
	10	10	100	
	10	10	100	
Control	10	-	-	-
	10	-	-	
	10	-	-	

Effect of Mutagenesis Of Mangiferin On *Drosophila Melanogaster*

It was observed that the mutagen formaldehyde produced change in eye colour from red to pink and reduction in the wing length but no changes where observed in the *D.melanogaster* treated with mangiferin.

Fig.1 Eggs and larvae of *d. Melanogaster* formaldehyde exposed *d. Melanogaster*

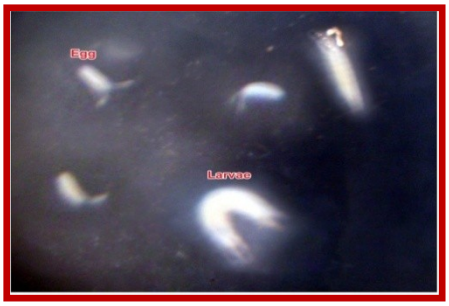

Table.No 2 Effect on mutagenesis of mangiferin on *drosophila melanogaster*

S. NO	Morphological	Normal Flies	StdFormal dehye Exposed Flies (0.5% V/V)	Mangiferin Treated Flies (mM)			
				10	25	50	100
1.	Eye Colour	Red	Dark pink	Red	Red	Red	Red
2.	Wing a) Shape b) Spots c) Hairs d) Length e) Width	Elliptical Present Present 100±2.8 43±1.5	Elliptical Present Present 83±1.3 35±2.1	Elliptical Present Present 95±1.5 43.8±1.43	Elliptical Present Present 96.33±0.88 40±1.25	Elliptical Present Present 97± 6 45±1.15	Elliptical Present Present 97±1.5 44±2.03
3.	Abdomen Length	42±1.25	42±2.1	43.6±1.2	41.3±0.74	43.6±0.66	42.33±1.4
4.	Total Body Length	96.6±2.02	95.8±1.9	94.47±0.88	95.67±0.43	96.33±0.63	94.34±1.73

DISCUSSION AND CONCLUSION

Brine shrimp lethality assay (BSLA) using free swimming hatched out *Artemia nauplii* which based on the ability to kill laboratory cultured brine shrimp. It was observed that 100% of mortality at 35ppm in the case of isolated mangiferin. LC_{50} for mangiferin was 10ppm in 24hrs. It showed that mangiferin was non toxic and safe and consistent with the reported data.

In mutation assay formaldehyde (0.5%v/v) selected as standard chemical mutagen. Mangiferin in the concentration (25, 50, 100mM) range was fed. After eclosion of the exposed flies from larvae stage, the phenotype changes, i.e., eye, color, wing hair, wing spot, wing shape, changes in the wing length and width, abdomen length and total body length were observed. Formaldehyde (0.5%v/v) produced visual mutations (eye color become pink from brown) and additionally there were noticeable changes in the wing length and width, but remaining factors unchanged when compared to normal flies. The results are tabulated (Table-2). The results observed were that the mangiferin at all concentration showed no significant morphological changes in the *D. melanogaster* on comparing formaldehyde exposed flies. This preliminary assay suggests its antimutagenic effect on *D. melanogaster.*

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