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Research Article

IN-VITRO CYTOTOXIC STUDY OF MOULLAVA *SPICATA* (DALZ.) NICOLSON LEAF EXTRACT.

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Abstract: ***Objectives:*** The aim of present study is to evaluate cytotoxic and genotoxic activity of *Moullava spicata* (Dalz.) Nicolson. ***Materials and Methods:*** The aqueous extract of this plant subjected to genotoxic and cytotoxic study using yeast viability test, hemolytic assay and Allium test. ***Result:*** The extract showed dose dependent effect on yeast viability and hemolysis of erythrocytes. The Allium test showed significant reduction of root length ($p > 0.01$) and number ($p > 0.001$) indicated cytotoxic effect of plant extract. Microscopic observations showed cytotoxic effects such as ghost cell, membrane damage or rupture, apoptotic bodies and the extent damage was increased significantly after 96 hours treatment. ***Conclusion:*** The study showed that the leaf extract of the *M. spicata* contains various biologically active compounds and possess cytotoxic effects.

Key**words:** *Moullava spicata* (Dalz.) Nicolson; Allium test; Hemolysis; Cytotoxicity

INTRODUCTION:

Plants are the source of different constituents which are directly or indirectly used in traditional medicine since many years. Many modern drugs have natural product origin. As their active ingredients and chemical structure discovered major share goes to plant constituents, which directly or indirectly derived from the plants [1].

However it has been reported that many plants used as food or in traditional medicine have mutagenic, cytotoxic and genotoxic effects [2, 3, 4]. These are giving awareness for randomly utilizing traditional medicinal plant. Studies also showed long term exposure to traditional medicines contain plant product is associated with increase in the rates of morbidity and mortality. Sinha, K.C and Upadhyay S.N reported antifertility effect in Neem [5, 6]. *Moullava spicata* (Dalzell) Nicolson is belonging to the family Fabaceae (Sub family: Caesalpiniaceae) is an endemic, endangered medicinal plant of Western Ghats [7]. Traditionally leaves of this plant used to treat chicken pox and lactating women. Root and bark of this plant are used to heal diabetic wound [8], pneumonia and skin diseases [9].Though many reports available on the medicinal properties of this plant, no systematic study has done on its toxicity.

This is the first report focused on cytotoxic effect of the plant. The aim of present study is to evaluate cytotoxic and genotoxic activity of the water extract of *M. spicata* leaves.

MATERIALS AND METHODS:

The leaves of *M. spicata* were collected from the place Chanthala, Kollamogaru village (Sullia talluk, Dakshina Kannada district) located in Western Ghats region during the month of February 2012.The identity of plant was authenticated in the Department of Botany,FMKMC college, Madikeri,Karnataka.

Preparation of aqueous extract: The leaves were washed with tap water and deionized water. Then leaves were dried in room temperature for a week, powdered and stored at 4^0C until use. For preparation of extract 250g of dried powder was added to 500ml of deionized water and kept in incubation to at 65^0C for 24 hours with continues agitation. Extract was filtered using muslin cloth and then Whatman No.1 filter paper. Filtrate was concentrated to 100ml and consider as a stock (100%).

Qualitative Analysis Phytochemicals: The extract was tested for the presence of phytochemicals like saponins, phenolic compounds, flavonoids, glycosides, tannin, steroids and terpenoids. [10, 11]

Yeast viability study: was studied by viable count method using methylene blue stain, described by Mills. [12] Four concentrations (1%, 2%, 4% and 8%) of the leaf extract were prepared from the stock using phosphate buffer. 200µl of each concentration of this extract was taken in eppendorf tube along with 50 µl of 1% yeast suspension. 200µl deionized water was maintained as control. This was incubated at 37^0C for 30 minutes in an incubator shaker. Then stained with 0.2% methylene blue and number of viability of the yeast cell was determined by using haemocytometer. 576.1±20.1 cells were analyzed in each of the slide and percentage of the viable cells was determined (n=2) [12].

Determination of cytotoxicity by Hemolysis assay: This assay was performed as per the method described by Malagoli with slight modification. The erythrocytes were

collected from the peripheral blood and then washed three times with 0.85% NaCl saline solution. After each washing cells were centrifuged 150g for 5 minutes, supernatant was discarded. Finally 2% erythrocyte suspension was prepared using 0.85% sodium chloride saline [13, 14, 15].

Four concentrations (1%, 2%, 4% and 8%) of leaf extracts were prepared in phosphate buffer. 200µl of these extract were taken in separate test tubes and volume was made up to 2000µl using buffer saline. Tubes were containing distilled water served as control. To this 200µl of erythrocyte were added. After 30 minutes of incubation at 37^0C liberated hemoglobin was estimated at 405 nm and percentage of hemolysis was determined (n=2). The percentage of hemolysis was calculated using the formula,

$$H\% = A_t / A_\alpha \times 100$$

A_t =Absorbance before hemolysis; A_α =Absorbance after hemolysis

Allium test: The Genotoxicity of *M. spicata* leaf extract was tested as per the method of Sehgal R with slight modifications. The onion bulbs were commercially obtained from Madikeri town, Karnataka. The bulbs were divided into four groups (5 each). Two groups were maintained as control and another two as test. Bulbs were grown in dark in moist condition until the root had grown to approximately 0.2-0.5cm in length. Then the base of each bulbs were immersed in the extract at 5%, 10%, 20%, 40% concentrations and number of the roots were counted and length of the roots were measured for 96 hours [16, 17].

The slide preparation for microscopic observation was followed the method described by Sharma and Sharma (1980). After 48 and 96 hours of treatment, roots were used for microscopic observation. Roots were stained with standard acetocarmine and slides were observed under microscope. Of 400 cells were analyzed by examining the chromosomal aberration and cell death (n=2) [18].

RESULT AND DISCUSSION:

The phytochemical analysis of *M. spicata* aqueous extract revealed the presence of saponins, phenolic compounds, flavonoids, glycosides, tannins and terpenoids except steroids as listed in table 1. Phytochemicals exhibit different biological and pharmacological activity including cytotoxic or genotoxic or cytoprotective or antigenotoxic effects [4].

Table.1: Phytochemical analysis of the *M. spicata* aqueous extract. [- not detected; + detected; ++ detected (medium); +++ detected (high range)].

Name of the test	Aqueous extract
Saponins	++
Phenolic compounds	+++
Flavonoids	+++
Glycosides	+
Carbohydrates	+++
Steroids	-
Tannins	++
Terpenoids	+

We used Baker's yeast for cell viability study. Aqueous extract of *M. spicata* showed significant (p>0.001) inhibition to cell viability. The dose dependent decreasing of percentage of viability was observed. *Saccharomycis cerviceae* is used widely as a model organism for studying toxic compounds such as genotoxicity and Cytotoxicity[19]. However cellular viability following DNA damage has been used in field of genotoxicity, toxicology, carcinogenesis and cancer therapy.

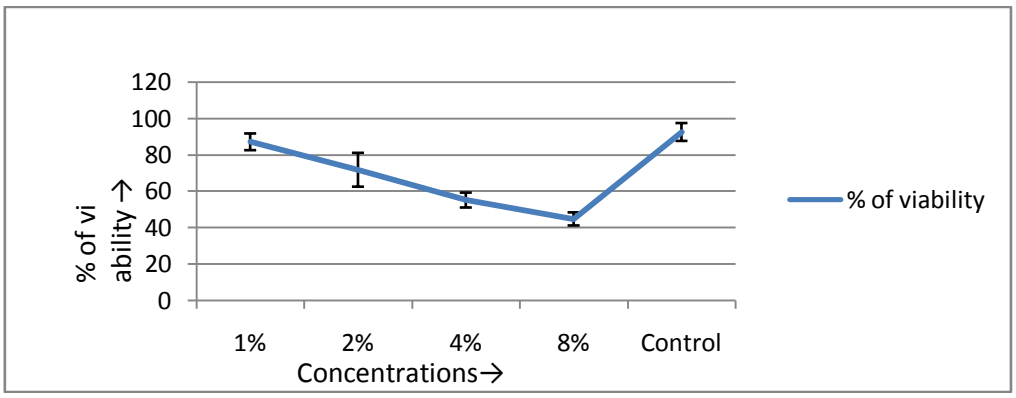

Figure 1: Yeast viable cell count showing reduction of percentage of the viability. P>0.0016

Hemolysis assay is an extremely sensitive method for cytotoxic studies with wide range of phytochemicals [20]. We observed significant hemolytic activity of the aqueous extract. The hemolysis was high at higher concentration of the extract tested. Recent report showed plant with flavonoids rich and phenolic compounds have cytotoxic properties [21, 22]. In addition phytochemical analysis showed detectable amount of flavonoids and phenolic contents in extract.

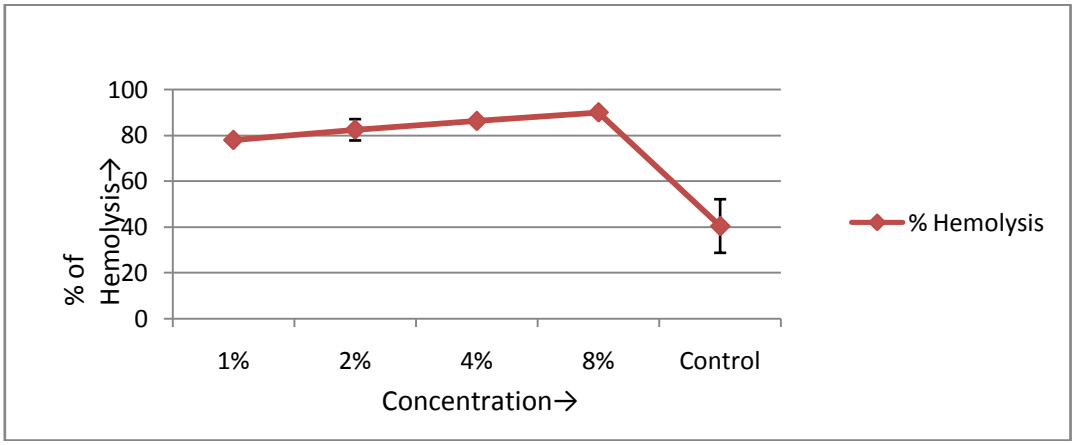

Figure 2: Percentage of hemolysis in different concentrations of extract. P >0.001

The effect of *M. spicata* leaf extract on root number and root length are shown in table no.3. The concentration dependent decrease of root number and root length was observed. The roots treated with concentration 10%, 20% and 40% appeared slight brown in colour. This reduction of root number and root length indicates root growth inhibition [23]. The root morphology of the control was normal but colour changes of the roots were observed in higher concentrations of the extract. Fiskesjo (1993) reported colour change of the root is due to cell death and leads to cytotoxicity [24].

Table No. 2: Reduction of root Length and Number.

98 hours Treatments			
	Concentrations	**Numbers of roots**	**Length of roots (cm)**
Test	5%	18.50±3.5	3.505±0.5
	10%	18±1.4	2.950±0.07
	20%	12±1.4	1.380±0.1
	40%	9.50±0.7	1.050±0.2
Control	Water	20±1.4	3.180±0.3
		P >0.01	P >0.001

The microscopic observation of root cell exposed to plant extract showed chromosomal and cellular damages such as double nucleus, membrane rupture or damage, ghost cells, apoptotic bodies. The extract showed concentration dependent increase of cellular abnormalities. However compared to 46 and 96 hours of treatment, slides which observed at 96 hours showed high significant number of cellular damages. However except double nucleus other chromosomal aberrations are not found in any concentration of the aqueous extract. The formation of double nucleus is due to the prevention of cytokines or cell plate formation [25].

At present study the higher number of the membrane damage, ghost cell formation and apoptotic bodies was observed. Ghost cells are the dead cells, nucleus and cytoplasmic structures are not stainable but outline remain visible. Celik and Aslanturk have been reported *Inula viscosa* leaf extract showed various number of ghost cell frequency leading the cell death. The leaf extracts cause nucleus damage and prevention of cytoplasmic structure leading to ghost cell formation. The higher concentration of stress, toxins, chemical, heavy metals caused cell death in plants but commonly stress condition cause cell death [4].

Table No.:3: Effect aqueous extract on cell abnormalities and double nucleus formation of *Allium cepa.* *non significance; Double Nucleus (DN); Membrane Rupture (MR); Ghost Cells (GC); Apoptotic Bodies (AB)

48hours							96hours				
Leaf extract	**No. of cells**	**DN**	**MR**	**GC**	**A B**	**Average No. of CA**	**DN**	**MR**	**GC**	**AB**	**Average No. of CA**
5%	400	-	1.50±0.7	0.0	0.50±0.7	0.0	-	7.50±0.7	9.0±2.8	1.50±0.7	0.25±00
10%	400	-	10.50±2.1	5.50±3.5	3.0±1.4	0.25±0.3	0.50±0.7	9.50±0.7	15.0±2.8	6.000±1.4	0.48±0.3
20%	400	-	10.50±0.7	10.0±4.2	9.0±2.8	0.25±0.3		14.50±4.9	21.50±3.5	14.00±1.4	2.11±0.1
40%	400	-	12.0±2.8	13.5±6.3	14.0±4.2	0.75±0.3	1.50±0.7	19.50±3.5	36.0±9.8	19.0±2.8	2.52±0.3
Control	400	-	0.50±0.7	0	1.5±2.1	0.00	-	1.500±0.7	1.50±2.1	2.50±3.5	00
		-	P>0.002	P > 0.05	P >0.01	P>0.2*	P>0.07*	P>0.0086	P>0.007	P> 0.0020	P>0.0003

CONCLUSION:

From a drug development perspective, it may sometimes be important to subject chemicals or herbal products for genotoxi as well as cytotoxic studies to establish its benefits. The present study has shown that aqueous extract of the *M. spicata* induced cytotoxicity and significant DNA damage. This suggests that components in these extracts might interact directly with the DNA. However, further studies are needed in other test systems including animal model, Cell lines for detecting biological, biochemical effects.

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