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TOXICITY STUDY OF *URENA LOBATA* USING *ALLIUM SATIVUM*: A NEW EUKARYOTIC BIOMONITORING TEST SYSTEM

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Abstract

Urena lobata (L.) has diverse biological activities. A recent study suggests that its leaf extract exerts acute toxicity on three different phases of Zebra fish, where the authors calculated the median lethal concentration (LC_{50}) value ranges between 2,548 and 8,748 g/L. This study aims to re-check its toxic effects on a new eukaryotic test model named the *Allium sativum* toxicity test model. For this, the ethanolic leaf extract of *Urena lobata* (ELEUL) was tested at 2, 4, 8, 16, and 32 mg/mL on the cloves of *A. sativum* at 24, 48, and 72 h exposure times (ET) using copper sulphate ($CuSO_4$) as a reference standard. Manual observation of root number and length profiles was considered in this study. The results suggest that ELEUL significantly ($p< 0.05$) and concentration-dependently reduced the number and length of roots of the test system in comparison to the control group. However, the ELEUL exerted more toxic effects at 72 h ET on the test system, and it showed an adaptive capacity at 48 h inspect of 24 h ET. The LC_{50} value was obtained between 4 and 8 mg/mL. Taken together, the ELEUL exerted toxic effects on the root meristems of *A. sativum* cloves, and this new model was sensitive like other popularly used toxicogenetic biomonitoring systems, like the *A. cepa* test model. Therefore, *A. sativum* might be another hopeful plant-based toxicogenetic test model.

Keywords: *Allium sativum*; eukaryotic system, biomonitoring model, toxicity study

Introduction

It is evident that *Urena lobata* (L.) (Family: Malvaceae) is used to treat many diseases empirically (Islam and Uddin, 2017; Roespandi et al., 2018). To date, a number of pre-clinical studies have proven its efficacy (Babu et al., 2016). However, the safety profile of this hopeful medicinal plant has yet to be evaluated. One study reports that the plant at 100, 200, and 300 mg/kg (p.o.) caused alteration of biochemical and morphological organization of the rat liver significantly with repeated and increased use of the aqueous root extract (Mshelia et al., 2013). A seminar presentation suggests that an acute toxicity study of the leaf extract (decoction method) of the plant exerted a significant toxic effect on the embryo, juvenile, and adult phases of zebra fish (*Danio rerio*) (Roespandi et al., 2018).

Many species of the Allium genus are quite important due to their economic and health benefits (Fredotović et al., 2020). For example, *Allium cepa* and *A. sativum* have many health benefits. It is to be noted that *A. cepa* is popularly used as a test model for toxicogenetic studies (e.g., toxicity, cytotoxicity, genotoxicity, mutagenicity) of varieties of test substances (e.g., crude extracts or their fractions, isolated compounds, laboratory synthetic derivatives, drugs and chemicals, environmental pollutants, etc.) in the laboratory due to its availability, economy, sensitivity, etc. (Levan, 1938; Fiskesjö, 1985; Cresencio et al., 2017). Its results can be related to animal-based higher eukaryotic test systems as a eukaryotic test model. Therefore, it is also used as an environmental toxicogenetic biomonitoring system (Fiskesjö, 1985). However, the *A. cepa* model has some limitations, such as onions not being preserved for a long time. It requires a whole bulb, whereas *A. sativum* only requires cloves, so the *A. sativum* model may be less expensive than the *A. cepa* model. Each garlic bulb produces enough cloves and even more roots than an onion bulb. Therefore, a single *A. sativum* clove can provide an adequate number of meristems rather than a

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whole onion bulb. This study re-evaluates the toxicity of ethanolic leaf extract of *U. lobata* by using a new eukaryotic plant-based test model called the *A. sativum* toxicity test system.

Materials and Methods

Collection and identification of Urena lobata

Fresh *U. lobata* leaves were collected from the hilly areas of Foy's Lake, Chittagong, Bangladesh in October, 2010 and the plant was identified by a taxonomist at the Forest Research Institute, Chattagram, Bangladesh (Voucher specimen: BFRIH-7011).

Extraction

Collected plant materials (leaves) were washed with running tap water and air-dried thoroughly. Then, it is followed by shade-drying at a temperature not exceeding 45° C and grinding into coarse powder with a suitable mechanical grinder. Approximately 125 g of leaf powder was soaked in 500 mL of absolute ethanol at room temperature for seven days with occasional shaking and stirring. The extract was filtered off first by using a surgical cotton plug and then filtered by using Whatman filter paper no. 1. A rotary evaporator was used to concentrate the filtrate at a lower temperature and pressure. This yielded 7.06 g (5.65%) of extractive *U. lobata*. The extract was preserved in an amber-colored glass vial until the test commenced.

Collection of test systems

For this study, fresh large-size garlic (*A. sativum*) was purchased from the local market in Gopalganj district (Bangladesh).

Standard

The standard, copper sulphate ($CuSO_4$), was purchased from Merck India Ltd.

Preparation of test samples and controls

The ELEUL at 2, 4, 8, 16, and 32 mg/mL was tested to determine the toxic effects on the above-mentioned test system. For this, the required amount of extract was soaked in distilled water for 12 hours prior to adding the samples to the test marked tubes. The highest concentration (32 mg/mL) was diluted to get 16 to 2 mg/mL concentrations. Distilled water and $CuSO_4$ (0.6 μg/mL) were used as control (vehicle) and positive control (PC), respectively.

Toxicity test (A. sativum test)

General steps involved in this study

Step-I: Dried outer layers were removed carefully, and only large cloves were collected.
Step-II: The outer peels of each clove were removed carefully.
Step-III: The old region in the budding parenchyma was removed by using a sharp incisor and a small spheroid laceration was made. This facilitated root growth (RG) in the cloves.
Step-IV: The cloves were washed with running tap water for 5 minutes and their root portions were then soaked in the test/controls by using toothpicks.
For this study, plastic containers (capacity: 15-20 mL) were used, and the study was conducted for up to 72 hours at room temperature in a dark place. The number and root length were measured every 24 h. The root length was measured in cm. Five cloves were used for each concentration/sample.

Calculation

%Inhibition of RG = [(RL Control - RL Test sample) ÷ RL Control] × 100
%RG = 100 - %Inhibition of RG
%Adaptive capacity = [(RG Highest ET - RG Lowest ET) ÷ RGHighest ET] × 100
N.B. – RG was determined inspect of respective exposure time (ET). RG and RL are mean root growth and root length, respectively.

Statistical analysis

Values are mean ± standard error mean (SEM) and were analyzed by using Graph Pad Prism software (version: 6.0); analysis of variance (ANOVA) followed by Tukey post test considering $p< 0.05$ with a 95% confidence level.

Results

ELEUL concentration-dependently reduced the RL/RG at all ETs. The highest percentage of RG inhibition was seen at 72 h by 32 mg/mL and the lowest at 48 h by 2 mg/mL. The LC_{50} value was between 4 and 8 mg/mL (Table 1).

Table 1. Effects of ethanolic leaf extract of *Urena lobata* and controls on *Allium sativum* root meristems at 24, 48 and 72 h

Treatments		Root length (cm)			%Inhibition of root growth		
		24 h	*48 h*	*72 h*	*24 h*	*48 h*	*72 h*
Control		14.08 ± 2.11	28.19 ± 3.11	53.08 ± 2.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$CuSO_4$ (0.6 µg/mL)		3.18 ± 0.34*	7.08 ± 1.57*	20.13 ± 2.58*	77.41 ± 0.34*	74.88 ± 1.57*	62.08 ± 2.58*
ELEUL (mg/mL)	2	11.08 ± 2.08*	27.13 ± 1.24*	39.08 ± 2.08*	27.08 ± 2.08*	03.76 ± 1.24*	26.28 ± 2.08*
	4	9.12 ± 1.28*	22.21 ± 2.91*	31.12 ± 1.28*	35.23 ± 1.28*	21.21 ± 2.91*	41.37 ± 1.28*
	8	6.86 ± 1.91*	12.08 ± 1.04*	20.86 ± 1.91*	51.28 ± 1.91*	57.15 ± 1.04*	60.70 ± 1.91*
	16	4.60 ± 0.68*	10.11 ± 1.05*	12.60 ± 0.68*	67.33 ± 0.68*	64.14 ± 1.05*	72.49 ± 0.68*
	32	3.48 ± 1.07*	7.58 ± 1.68*	10.08 ± 1.07*	75.28 ± 1.07*	73.11 ± 1.68*	81.13 ± 1.07*

*Values are mean ± standard error mean (SEM) (n = 5); *p< 0.05 when compared to the vehicle group; ANOVA followed by Tukey post test, considering p< 0.05 at 95% confidence level; Control: Distilled water; $CuSO_4$: Positive control; ELEUL: Ethanolic leaf extract of Urena lobata.*

Figure 1 suggests that the PC ($CuSO_4$) increased the adaptation capacity (DNA damage repair capacity or resistance towards toxic response of the toxic agents) at 72 h inspect of 24 h ET. Its adaptive capacity at 72 h inspect of 48 h was better than that seen at 48 h inspect of 24 h ET. On the other hand, ELEUL showed a concentration-dependent adaptation capacity at 48 h inspect of 24 h ET, where the adaptation power was gradually decreased with decreasing the test concentration. ELEUL only at 2 mg/mL showed a negligible adaptation capacity (1.09%) at 72 h inspect of 24 h ET, while it remained non-responsive at 8-32 mg/mL at the same ET inspect of 24 h ET. ELEUL did not show adaptive capacity at 72 h inspect of 48 h ET.

Discussion

A. cepa is widely used in toxicogenetic studies (Fiskesjö, 1985). However, some general facts, such as storage facilities and cost, can be reduced in the case of the *A. sativum* test. It may be due to garlic (*A. sativum*) remaining fresh for a longer time than the onion (*A. cepa*). On the other hand, only one clove of garlic is necessary to replace a whole onion bulb. Furthermore, each garlic bulb produces enough cloves, and each clove produces more roots than a whole onion bulb.

Toxic substances clearly disrupt cellular events (Bhattacharya et al., 2012; Adeyemo and Farinmade, 2013). It is due to these substances accumulating in the roots that can cause chromosomal aberrations (e.g., C-mitosis, chromosomal bridges, chromosomal tack, and micronuclei formation). The ultimate result is RG inhibition (Qin et al., 2015). Accumulated toxicants in the meristems impair the microtubule organizations by inhibiting RG in a plant-based eukaryotic test model. Therefore, a substance having toxic and cytotoxic effects can elongate the cell cycle differentiation phase (Fusconi et al., 2006), increase apical meristematic activity (Webster and Macleod, 1996), and inhibit protein synthesis in root tips (Seth et al., 2007). Copper (Cu), a toxic agent, and works in this manner (Achary and Panda, 2010). In this study, both $CuSO_4$ and ELEUL significantly reduced the RG profile in *A. sativum* in comparison to the vehicle. The ELEUL concentration-dependently decreased in the RG profile in *A. sativum* suggests that this test model is a sensitive model like the *A. cepa* test model. Moreover, the number of roots was also reduced at a higher concentration of ELEUL. Additionally, ELEUL also showed an RG reducing effect at 48 h inspect of 24 h of ET, suggesting the system's adaptation capacity on the 2nd day of test concentrations of ELEUL.

It seems this test system reduced the toxic effects due to the damage preventive capacity of it on day 2 (48 h), probably by distressing the adaptive response pathways and/or cellular damage repairing capacity in this test system (Achary and Panda, 2010; Panda and Achary, 2014). However, low concentrations of ELEUL showed more adaptive capacity than the higher concentrations. It may be due to its genomic protection capacity at low concentration regardless of exposure time (Panda and Achary, 2014). Roespandi et al. (2018) suggested that *U. lobata* leaf extract might have teratogenic effects as it had more toxic effects on the embryo than in the juvenile and adult phases of *D. rerio*. Thus, the meristematic regions of the test system in this current study might be attracted by the toxic effects of the ELEUL. Moreover, the LC_{50} value obtained in this study is also consistent with the previous studies (Mshelia et al., 2013; Roespandi et al., 2018).

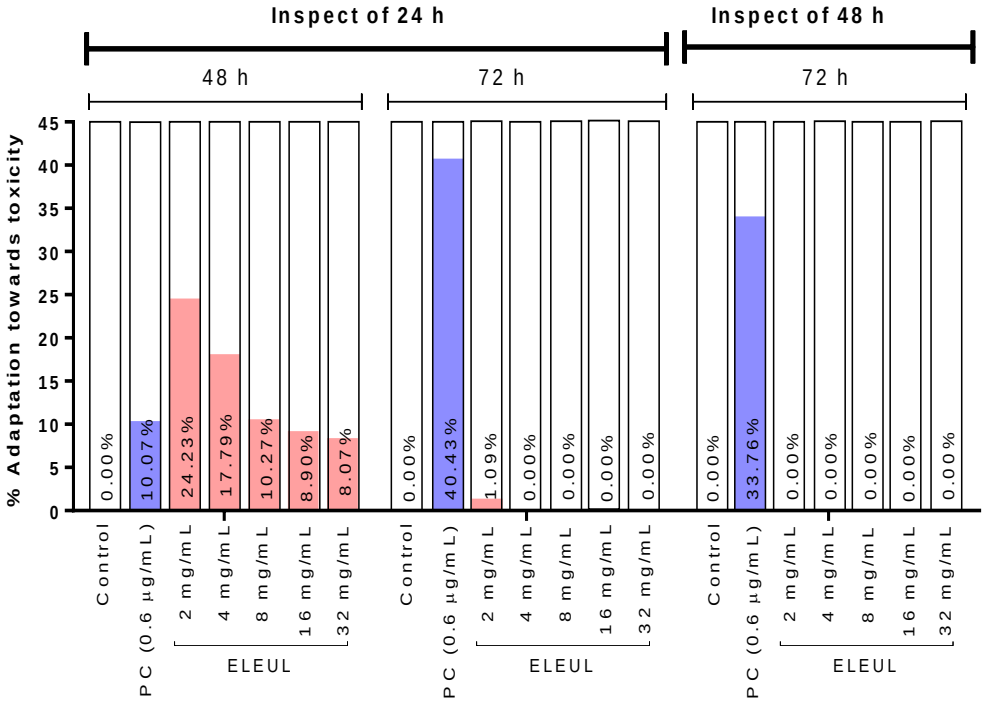

Figure 1. Percentage adaptive capacity of ethanolic leaf extract of *Urena lobata* and controls on *Allium sativum* root meristems [Values are percentage (%) (n = 5); Negative values are considered to have no adaptive capacity and have been omitted from the table; Control: Distilled water; $CuSO_4$: Positive control; ELEUL: Ethanolic leaf extract of *Urena lobata*; ET: Exposure time]

Aerial parts of *U. lobata* contain alkaloids, flavonoids, phytosterols (e.g., stigmasterol, β-sitosterol, daucosterol, 7α-methoxysitosterol, 7β-hydroxysitosterol, 7α-hydroxysitosterol, ergosterol peroxide), flavonoid and cardiac glycosides, tannins, terpenoids, saponins, triglycerides, and phenolic and phenolic glycosides (Fagbohun et al., 2012; Shrestha et al., 2016; Aimé et al., 2020). To date, a number of important phytoconstituents have been isolated from this medicinal plant. These include imperatorin, kaempferol-3-O-β-D-glucopyranoside, tiliroside, benzoic acid, protocatechuic acid, monordicophenoide A, glycerol, β-adenosine, and L-tryptophan (Shrestha et al., 2016). Many compounds of *U. lobata* have cytotoxic effects on eukaryotic systems. For example, stigmasterol is evident to a decrease in RNA content in G1 and G2 phases of human intestinal (Caco-2) cells (Alemany et al., 2012), while β-sitosterol induces apoptosis through activating caspase-3 and -9 in hepatocellular (Huh7 and HepG2)

cells (Vo et al., 2020). Therefore, the toxic effects of ELEUL in this study might be in agreement with the cytotoxic effects of its other compounds present in the plant.

Conclusion

The ELEUL concentration-dependently decreased the RG profile of *A. sativum*. An adaptive power of 48 h was seen in a comparison of 24 h of ET in this test system. The root number and length of *A. sativum* were also effectively inhibited by the ELEUL. It seems this test system also showed an almost similar response to the widely used test model *A. cepa*. The toxic effects were significant ($p< 0.05$) at 95% confidence intervals when compared to the vehicle group. Therefore, *A. sativum* can be incorporated into the toxicogenetic analysis of various substances in different areas of toxicological research. This study will be able to provide supportive information to the future pre-clinical and clinical settings on this hopeful medicinal plant's toxicogenetic effects on higher eukaryotic models, and *A. sativum* might be a hopeful toxicogenetic test system.

The study was conducted in a home-setting environment; therefore, it suffers from a well-defined laboratory setup. Physical observation of the RG profile was the basis of this study in comparison to the vehicle RG as a basement. No microscopic observation was done. Other suitable methods, such as alkaline comet assay, micronucleus test, and so on, should be investigated to determine their feasibility in comparison to the commonly used *A. cepa* test.

Conflict of interest

None declared.

References

Achary, V. M. M., & Panda, B. B. (2010). Aluminium-induced DNA damage and adaptive response to genotoxic stress in plant cells are mediated through reactive oxygen intermediates. *Mutagenesis*, *25*, 201-209.

Adeyemo, O. A., & Farinmade, A. E. (2013). Genotoxic and cytotoxic effects of food flavor enhancer, monosodium glutamate (MSG) using *Allium cepa* assay. *African Journal of Biotechnology*, *12*, 1459-1466.

Aimé, A. O. V., Carinne, K. F., Hortense, G. K., Anatole, P. C., Véronique, P. B. (2020). Phytochemical screening and in vitro antimicrobial and antioxidant activity of *Urena lobata* and *Emilia coccinea* methanolic stems extracts. Global Scientific Journals, 8, 1423-1442.

Alemany, L., Laparra, J. M., Barberá, R., & Alegría. A. (2012). Evaluation of the cytotoxic effect of 7keto-stigmasterol and 7keto-cholesterol in human intestinal (Caco-2) cells. *Food and Chemical Toxicology*, *50*, 3106-3113.

Babu, S. S., Madhuri, D. B., & Ali, S. L. (2016). A pharmacological review of *Urena lobata* plant. Asian Journal of Pharmacy and Clinical Research, *9*(2), 20-22.

Bhattacharya, S., De Sarkar, N., Banerjee, P., Banerjee, S., Mukherjee, S., Chattopadhyay, D., *et al.* (2012). Effects of Arsenic toxicity on germination, Seedling growth and Peroxidase activity in *Cicer arietinum. International Journal of Agriculture and Food Science*, 2, 131-137.

Cresencio, C. Jr., Abelada, J. J. Z., Apostado, R. R. Q., Hernando, B. J. H., Lador, J. E. C., Obenza, O. L. P., et al. (2017). *Allium cepa* test: An evaluation of genotoxicity. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *7*, 12-19.

Fagbohun, E.D., Asare, R. R., & Egbebi, A. O. (2012). Chemical composition and antimicrobial activities of *Urena lobata* L. (Malvaceae). *Journal of Medicinal Plants*, 6, 2256-2260.

Fiskesjö, G. (1985). The *Allium* test as a standard in environmental monitoring. *Hereditas*, *102*(1), 99-112.

Fredotović, Ž., Soldo, B., Šprung, M., Marijanović, Z., Jerković, I., & Puizina, J. (2020). Comparison of Organosulfur and Amino Acid Composition between Triploid Onion *Allium cornutum* Clementi ex Visiani,

1842, and Common Onion *Allium cepa* L., and Evidences for Antiproliferative Activity of Their Extracts. *Plants (Basel)*, *9*(1), 98.

Fusconi, A., Repetto, O., Bona, E., Massa, N., Gallo, C., Dumas-Gaudot, E., & Berta, G. (2006). Effect of cadmium on meristem activity and nucleus ploidy in roots of *Pisum sativum* L. cv, Frisson seedlings. *Environmental and Experimental Botany*, 58, 253-260.

Islam, M. T., & Uddin, M. A. (2017). A revision on *Urena lobata* L. International Journal of Medicine, *5*(1), 126-131.

Levan, A. (1938). The effect of colchicine on root mitoses in *Allium*. *Hereditas*, *24*(4), 471-486.

Mshelia, I. Y., Dalori, B. M., Hamman, L. L., & Garba, S. H. (2013). Effect of the aqueous root extract of *Urena lobata* (Linn) on the liver of albino rat. *Research Journal of Applied Sciences*, *Engineering and Technology*, *5*(1), 1-6.

Panda, B. B., & Achary, M. M. (2014). Mitogen-activated protein kinase signal transduction and DNA repair network are involved in aluminum-induced DNA damage and adaptive response in root cells of *Allium cepa* L. Frontiers in Plant Science, *5*, 1-10.

Qin, R., Wang, C., Chen, D., Björn, L. O., & Li, S. (2015). Copper-induced root growth inhibition of *Allium cepa* var. *agrogarum* L. involves disturbances in cell division and DNA damage. *Environmental Toxicology and Chemistry*, *34*, 1045-1055.

Roespandi, Y. P., Aini, N., & Widodo, M. A. (2018). Acute toxicity of *Urena lobata* leaf extract on embryo, juvenile and adult of zebra fish (*Danio rerio*). Poster session, PO3-13-35, WCP2018.

Seth, C. S., Chaturvedi, P. K., & Misra, V. (2007). Toxic effect of arsenate and cadmium alone and in combination on Giant Duckweed (*Spirodela polyrrhiza* L.) in response to its accumulation. *Environmental Toxicology*, 22, 539-549. Shrestha, S., Park, JH., Cho, JG. Lee, D.-Y., Jeong, R.-H., Han, J.-T., Cho, S. K., Lee, D.-S., & Nam-In Baek, N.-I. (2016). Phytochemical Constituents of the *Urena lobata* Fruit. *Chemistry of Natural Compounds*, *52*, 178-180.

Vo, T. K., Ta, Q. T. H., Chu, Q. T., Nguyen, T. T., & Vo, V. G. (2020). Anti-Hepatocellular-Cancer Activity Exerted by β-Sitosterol and β-Sitosterol-Glucoside from *Indigofera zollingeriana* Miq. *Molecules*, *25*, p. 3021.

Webster, P. L., & Macleod, R. D. (1996). The root apical meristem and its margin. In: Waishel Y, Eshel A, Kafkafi U (eds) Plant roots. The hidden half, 2nd edn. Marcel Dekker, New York, pp. 51-76.