Pakistan Journal of Pharmacology
Vol.37, No.1, January, 2020, pp. 9-12

ORIGINAL ARTICLE

EVALUATION OF CYTO-TOXIC POTENTIAL OF AQUA DISTILLATE OF *ROSA DAMASCENA* MILL USING BRINE SHRIMP LETHALITY ASSAY

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ABSTRACT

Rosa damascena Mill. is commonly known as Gul-e-Muhammadi, Damascus rose, Persian Rose and Damask rose in native languages. It belongs to Rosaceae family and is well-known all over the world due to its distinctive aroma, visual beauty and unique flavour. The plant of *Rosa damascena* Mill is considered very healthy for human body due to presence of variety of organic and in-organic compounds such as minerals, vitamins, carotenoids, tannins, bio-flavonoids, phenolic compounds etc. Since assessment of cyto-toxic potential is a major initial step in the development of novel drugs, this study is designed to investigate the cyto-toxic potential of aqua distillate of *Rosa damascena* Mill using Brine Shrimp Lethality Assay.

Keywords: Natural product, cyto-toxic, Brine-shrimp, water distillation, aqua distillate

INTRODUCTION

The use of plant or plant based products as medicine is very old, probably as old as existence of human beings. The plant kingdom is considered as a rich source of bio-active compounds and a potential source for the discovery and development of novel drugs (Shakya, 2016). According to an estimate, more than 50,000 plants have been identified with medicinal properties (Msomi & Simelane, 2018). Today there is an extensive list of plants with known therapeutic activity (Osama, 2019). Despite of the magnificent advancement in allopathic medicine system, many developing countries still greatly rely on herbal remedies to cater their basic health care needs (Rasool *et al*., 2020). Many countries such as China, India, Egypt and South America are still using herbal remedies to treat different ailments (Khan & Ahmad, 2019). The use of natural products and herbal remedies is an effective, useful and economical way of treating different diseases (Abbas *et al*., 2019). Each plant is a chemical factory which is capable of synthesizing and producing a variety of unique and highly complex compounds. Up till now, a variety of drugs which are used clinically today have been isolated from plant/natural sources (Nwonu *et al*., 2019). Despite of the fact that herbal remedies are globally recognized and used in different ailments, still the safety of these therapies is a major concern and required vigorous investigation (Yang, 2020).

Rosa damascena Mill belongs to the family "King of flowers" i.e. Rosaceae. It is an ornamental plant widely cultivated all over the world specially China, Middle East, Europe, India and North America (Osama *et al*., 2020). It has been traditionally used as analgesic, astringent, intestinal and cardiac tonic (Nayebi *et al*., 2017). Different parts of Rose plant including fruit, flower, petals contains high content of biologically active compounds such as essential fatty acids, organic acids, fatty acids, sugars, vitamins, tannins, flavonoids etc. (Ruba *et al*., 2016). The flower of *Rosa damascena* Mill is a rich source of essential oil (Sadraei *et al*., 2013). It contains high content of fats, resins, tannins, tartaric acid, tannic acid, malic acid, volatile essential oils, quercetin glycoside, gallic acid and various flavonoids and possesses blood purifying properties (Achuthan *et al*., 2003). Globally it

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is commercially cultivated and harvested for the production of Rose water and Rose oil (Sadraei *et al*., 2013).

Aqua distillate of *Rosa damascena* Mill is prepared by water distillation process (Saffari *et al*., 2004). It is a good source of citronellol, linalool, nerol, eugenol, methyl-eugenol, phenyl acetic acid, geranial, geraniol, quercetin, ellagic acid and kaempferol etc. (Solimine *et al*., 2016; Verma *et al*., 2011; Lohani *et al*., 2013). This study is designed to investigate the cyto-toxic potential of aqua distillate of *Rosa damascena* Mill using Brain Shrimp Lethality Assay.

MATERIALS AND METHODS

Aqua distillation of rosa damascena mill

Aqua distillation was performed to obtain aqua distillate of *Rosa damascena* Mill flower. Fresh flowers of *Rosa damascena* Mill were purchased from local nursery which were identified and authenticated by Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi (Voucher no: RDF-01- 16/17). Petals were separated from flower and allowed to dry at room temperature. The distillation apparatus comprises of a stainless steel tank, a cohobation column, a condenser and a receiver. Dried petals with distilled water was added in the distillation apparatus in the ratio of 1: 2.5 kg of air dried rose petals along with 10 L of water was added in the distillation apparatus. Air vents were closed after complete removal of air and the apparatus was then operated as a closed system to distil the rose petals under maintained high temperature and pressure. The vapours were generated in cohobated column which were then condensed with circulating chilled water in a condenser and finally received in the receiver. The process of distillation was completed after collection of 2500 ml of distillate (Osama & Ikram, 2018; Babu *et al*., 2002).

Brine shrimp lethality assay

We performed Brine Shrimp lethality assay to determine the in-vitro cyto-toxic potential or LD_{50} of aqua distillate of *Rosa damascena* Mill flower. This test is developed for the monitoring of biologically active natural products and their toxicity. Bio-active compounds are toxic and fatal to brine shrimp larvae and this phenomenon is efficiently used to determine LD_{50} and cyto-toxic potential of natural products.

Brine shrimp eggs were sprayed on hatching tray with perforated partition and size 22 x 32 cm along with sea water which aids in hatching process and left for incubation period of 24 hours at room temperature. After 24 hours larvae were hatched from the eggs which were collected carefully for bio-assay.

Reference cytotoxic drug was etoposide. 3 stock solutions of test drug (aqua distillate of *Rosa damascena* Mill.) of different concentration i.e. 1000, 100 and 10µg/ml were prepared by diluting them with sea water and final volume was made to 5ml. By using Pasteur pipette, in each each vial 10 larvae were added and incubated for 24 hours at normal room temperature. After 24 hours, number of survivors and deaths were counted from each vial (Dokuparthi *et al*., 2018; Carballo *et al*., 2002; Mahmoudvand *et al*., 2017).

RESULTS AND DISCUSSION

Brine shrimp lethality assay of aqua distillate of rosa damascena mill flower

The results of in-vitro Brine shrimp lethality bioassay of our test drug i.e. aqua distillate of *Rosa damascena* Mill are represented in table 1.

According to the results, our test drug i.e. aqua distillate of *Rosa damascena* Mill did not show any cyto-toxicity to brine shrimps at 10, 100 and 1000 µg/mL concentrations.

In-vitro cyto-toxic potential or LD_{50} of aqua distillate of *Rosa damascena* Mill. was evaluated by Brine shrimp (*Artemia salina*) lethality assay. Cyto-toxicity assessment is the major initial step for development of novel drugs (Mahmoudvand *et al*., 2017).

Table 1: Brine Shrimp Lethality Assay of *Rosa damascena* Mill. (aqua distillate)

Concentration (µg/mL)	% Mortality *Rosa damascena* Mill. (aqua distillate)	% Mortality Standard cytotoxic drug (Etoposide)
10	6.66	46.66
100	10	
1000	16.66	

Brine shrimp lethality assay is a simple, rapid and in-expensive test developed for the monitoring of biologically active natural products and their toxicity. Bio-active products are usually toxic and fatal to brine shrimp larvae and this phenomenon is efficiently used to determine LD_{50} and cytotoxic potential of natural products (Dokuparthi *et al*., 2018). Our findings of in-vitro LD_{50}/ Brine shrimp lethality assay revealed no cyto-toxicity of aqua distillate of *Rosa damascena* Mill. at 1000, 100 and 10 µg/mL concentrations against standard cytotoxic drug etoposide. Presence of quercetin and kaempferol might be responsible for cyto-protective potential of *Rosa damascena* Mill. (aqua distillate). Presence of quercetin and kaempferol in *Rosa damascena* Mill. (aqua distillate) is reported by Solimine *et al* (2016). Studies by Devi & Shyamala (1999), Jeong *et al* (2005) and Nègre-salvayre & Salvayre (2005) reported cyto-protective effects of quercetin. Varshney *et al* (2017) reported cyto-protective potential of kaempferol. Hence these findings indicate strong safety profile of aqua distillate of *Rosa damascena* Mill.

CONCLUSION

In the light of above discussed findings, it is concluded that aqua distillate of *Rosa damascena* Mill. flower obtained by water distillation process does not possess any cyto-toxicity. However, in future more detailed in-vivo investigations are required to determine its cyto-protective effect in human body.

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