INVESTIGATION OF CYTOTOXIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF MEDICINAL PLANTS USING ARTEMIA SALINA (BRINE SHrimp TEST)

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Abstract - Medicinal plants have been screened for their biological activity using Brine Shrimp (Artemia salina leach). Brine shrimp lethality test was used to investigate the cytotoxic activity of plant extracts. After 24 hour of treatment, the larvalcidal activity was assessed on the base of percentage of larval mortality. The bioactivity of hydroalcoholic extracts of different parts of medicinal plants, at different concentrations (1-5000 μg/mL), was evaluated with the shrimp lethality assay. Lethality concentration (LC50) was obtained using probit analysis. Out of the 17 plants tested, Veratrum album Bernh, Capsicum annuum L, Atropa belladonna L, Toxicodendron pubescens Mill and Berberis vulgaris L exhibited potent brine shrimp lethality with LC50 of 400, 420, 430, 610 and 612 μg/mL, respectively.

Keywords — Medicinal plants, cytotoxic effect, hydroalcoholic extracts, Brine Shrimp Test, lethality concentration determination.

I. INTRODUCTION

The purpose of this study was to investigate the cytotoxic effects of the hydroalcoholic extracts of medicinal plants using the brine shrimp test (BST) method as a broad measure of anti-tumor activity. Zani et al., (1995) used brine shrimp lethality assay as a prescreening system for anti-Trypanosoma cruzi activity. In a study, brine shrimp lethality test was employed to investigate the cytotoxic activity (Siriratorn et al., 2004). The investigation of active constituents from medicinal plants and extracts is mostly hindered by lack of an appropriate, easy, and quick screening method. There are various processes for bioassay that are employed in biochemical systems, isolated tissues and whole animals. These procedures can be expensive and quite complicated. Thus, a realistic method for common toxicity screening is necessary as an opening phase in the learning of bioactive plants. Brine shrimp, Artemia salina, is used for this purpose as an animal model (Solis et al., 1993; D'Souza et al., 2002). In a previous study, brine shrimp lethality test was employed to investigate the cytotoxic activity of Salvia triloba and Trigonella foenum graecum (Alkofahi et al., 1996). Cytotoxic activity in this screening is critical. Monitoring of lethality is one of the simplest biological responses. Monitoring criteria is either alive or dead. In this way, statistical analysis becomes easy. After 24 hours of exposure, the lethal concentration for 50% mortality is determined as the measure of toxicity of compound or extract. An increasing numbers of research papers and reviews, clearly indicate that medicinal plants exhibit a variety of therapeutic properties and provide health security to rural people in primary health care (Fatima and Robin, 2009; Kostova, 2005). The present study describes the cytotoxic activity of the hydro-alcoholic extract medicinal plants against brine shrimp (artemia salina) larvae.

II. MATERIAL AND METHODS

Some plants were collected from the Cholistan desert Bahawalpur and others were purchased from Bahawalpur market and authenticated at the Pharmacognosy Department of the Islamia University of Bahawalpur Pakistan. All plant material was dried in shade, powdered and kept (at 20°C) in closed plastic containers.

A. Preparation of Plant Extracts

Twenty grams of the fine powder of each plant sample were weighed and added into an Erlenmeyer flask containing 250 mL of hydro-alcoholic extract. The solution was covered and shaken every 30 min for about 6 hours, and then allowed to stand for about 48 hours at room temperature. Then, it was shaken and filtered using Whatman filter paper (No.1). After filtration, the solvent was removed by rotary evaporation under reduced pressure and the resulting extracts were tested for activity.

B. Method for Brine shrimp cytotoxicity assay

A hatching tray was taken and half (1/2) filled with seawater. Brine shrimp eggs were sprinkled in one half of the tray and covered that part of the tray with a lid. The other half part of the tray was kept open under luminescent light for 1-2 days at 30±3°C. The eggs hatched, and larvae were moved from the dark to the area of light through pores between the two halves of the tray. In each experiment, 0.5 ml of the plant extract was added in test tubes that contained 4.5 ml brine solution. The 2-3 days old larvae were taken by the help of Pasteur pipette and moved into the sample containing tubes. Incubation was done at 30±3°C for 24 hours under illumination. Experiments were conducted at different concentrations (1-5000 μg/mL) of the test substances in a set of three tubes per dose. Other tubes were supplemented with solvent serving as positive control and environmental test was done as negative control. After 24