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Antioxidant and antiapoptotic activities of *Calotropis procera* latex on Catfish (*Clarias gariepinus*) exposed to toxic 4-nonylphenol

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ABSTRACT

Calotropis procera L. is known as medicinal plant. The Phytochemical analyzes of its latex revealed that it possessed antioxidants, namely terpenes, phenolic compounds and cardenolides, flavonoids and saponins, while tannins, alkaloids and resin were absent in moderate to high concentration. In the present study, the role of latex of *Calotropis procera* as antioxidant and antiapoptotic was reported. To carry out this aim, fishes were exposed to 100 $\mu g\, l^{-1}$ 4-nonylphenol as chemical pollutant. The enzymes, superoxidase dismutase, catalase, acetlycholinstrase (AchE), glutathione s-transferase, cortisol, G6PDH) and apoptotic cells increased significantly ($p < 0.05$) accompanied by irregular disturbance of (Na^+, K^+) ions in the presence of 4-nonylphenol. On the other hand, these enzymes, ions, and apoptotic cells decreased normally and significantly ($p < 0.05$) in the presence of latex. Total phenol content, total capacity antioxidant, reducing power decrease significantly ($p < 0.05$) in the presence of 4-nonylphenol and increase normally in the presence of latex. Latex was used for the first time to protect catfish after 4-nonylphenol exposure. Our study confirms that crude latex of *Calotropis procera* possessed antioxidant and antiapoptotic activities against the toxicity of 4-Nonylphenol.

1. Introduction

Pollution of water is one of the major problems in Egypt (Abdel-Shafy and Aly, 2002). Chemical pollution appears to be due to increase of industrialization. 4-nonylphenol (NP) is widely used in the manufacture of non-ionic surfactants, lubricants, stabilizer polymers, insecticides and herbicides (Gao et al., 2000; Soares et al., 2008). It has been found in aquatic environment, particularly in river water and it is toxic, soluble, stable and persistent in the environment (Rivero et al., 2008). It has sublethal effects including reduced fertility, irregular heart beat and loss of movement (Cox, 2003). The adverse effects of this compound are most probably due to its bioaccumulation in fish (Soares et al., 2008). The hematotoxic, biochemical, hormonal, histopathological, embryotoxic and genotoxic effects of 4-nonylphenol in catfish were reported (Mekkawy et al., 2011; Sayed et al., 2012a; Sayed et al., 2013; Sayed et al., 2012c; Sayed et al., 2012b; Sayed et al., 2011). African catfish (*Clarias gariepinus*) has been used as an excellent model for toxicological studies since it has a well-documented biology, used as human diet and extensively use in aquaculture (Mahmoud et al., 2009; Mekkawy et al., 2010).

Scientific evidence suggests that under oxidative stress conditions, oxygen radicals such as superoxide anion (O^{2-}), hydroxyl radical (OH) and peroxyl radicals (H_2O_2) are produced in biological systems. These oxygen radicals termed also as Reactive Oxygen Species (ROS) produces oxidative damage to cellular components such as proteins, lipids and DNA leading to cytotoxicity and cell death (Prabha and Vasantha, 2011).

Calotropis procera Ait. (Family Asclepiadaceae) is an ayurvedic plant with important medicinal properties. It is found in most parts of the world with a warm climate in dry, sandy and alkaline soils. It is an erect, tall, large, highly branched and perennial shrub or small tree that grows to a height of 5.4 m, with milky latex (Ramos et al., 2007). Latex is a natural plant polymer secreted by highly specialized cells known as laticifers. It is milky fluid secreted by ducts of laticiferous tissue (Hagel et al., 2008) and

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mainly flowed inside laticifers including roots, stems, leaves and fruits of all flowering plants (Pickard, 2008). It is an emulsion-like sticky material that exudes from various plant parts after having a small tissue injury. Latex is a complex mixture of secondary metabolites (Santos and Van Ree, 2011). It contains many phytochemicals such as alkaloids, sterols, fatty acids, starches, sugars, oils, tannins, resins, gums, and many enzymatic proteins such as proteases, chitenases, lipases, peptidases, esterase, peroxidases, papain, hevein, lectins and diversity of allergens (Heli et al., 2008; Santos and Van Ree, 2011). Latex contains antioxidants, namely superoxide dismutase (SOD), catalase and glutathione (Kumar et al., 2005). Latex also contains glycosides, tannins, phytosterols, flavonoids, acetogenins and saponins (Edman, 1983) which show diverse biological activities against bacteria, fungi, viruses, protozoans, nematodes, insects (Ramos et al., 2006), cancer tumor cell lines and anti-proliferative (Khairnar et al., 2012) and anti-inflammatory activities as well (Mesquita et al., 2011). Methanolic extract of *C. procera* latex provides protection against inflammation and oxidative stress in Freund's complete adjuvant-induced monoarthritis in rats (Kumar and Roy, 2009). The present investigation aims to assess the biological activity of *C. procera* latex as antioxidant and antiapoptotic agent by using catfish (*C. gariepinus*) as animal model as surrogate for humans.

2. Materials and methods

2.1. Collection of plant latex

The plant latex was collected into sterile containers by pressing the surface of fresh leaves of *C. procera* under aseptic technique during June and July 2014 at the university of Al-Azhar, Assiut, Egypt. The containers were cotton-plugged and stored at 4 °C until required.

2.2. Phytochemical analyzes of Calotropis procera latex

Latex sample of *C. procera* was analyzed for phytochemical composition by qualitative methods (Mahajan and Badgujar, 2008; Marinova et al., 2005) as follow:

2.2.1. Test for alkaloids

Few drops of dilute HCL and 0.5 ml Wagner's reagent (1 g iodine+2 g KI in 300 ml H2O) was added to a portion of the latex. A brown flocculent precipitate indicates the presence of alkaloid.

2.2.2. Test for phenolic compounds

Phenolic compounds in the latex were detected by mixing a portion of the latex with few drops of diluted Folin Ciocalteu reagent (mixture of phosphomolybdate and phosphotungstate) and aqueous sodium carbonate solution. The mixture was allowed to stand for 10 min and formation of gray color indicates the presence of phenolic groups.

2.2.3. Test for flavonoids

Two methods were applied for the qualitative detection of flavonoids. (i) A portion of latex sample was dissolved in 10% Hcl, and then a small amount of Zinc powder was added. Appearance of effervescences with pink color indicates the presence of flavonoids. (ii) Latex was dissolved in concentrated H_2So_4, and the formation of intense color indicates the presence of flavonoids

2.2.4. Test for terpenoids

A red to purple color formation indicates the presence of terpenoids, when a chloroform soluble portion of latex was treated with an equal volume of concentrated H_2So_4.

2.2.5. Test for tannins

A portion of latex was mixed with few drops of 0.1% ferric chloride. The development of brownish green coloration indicates the presence of tannins.

2.2.6. Test for saponins

A half ml of latex was dissolved in 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth as evidence for the presence of saponins.

2.2.7. Test for resin

One ml of latex was treated with few drops of acetic anhydride solution followed by one ml of conc. H_2So_4. Resins give colouration ranging from orange to yellow.

2.2.8. Test for cardiac glycosides (Keller Kelliani's test)

5 ml of latex was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1 ml concentrated sulfuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

2.3. African catfish (Clarias gariepinus)

Adult males of African catfish (*C. gariepinus*) were collected from the River Nile in July 2013. Fish were fed with a commercial fish food twice a day and kept at approximately 28 °C with 12 h light: 12 h dark cycle and held in a closed recirculating systems containing experimental medium (300 L water) for 12 months to acclimatize to laboratory condition before the experiment. In each closed recirculating systems 6 fishes were kept. During the acclimation period 20% of the water in each recirculating system was replaced daily and were fed 5% body weight twice a day with commercial pellets. Fish average length was about 34.5 ± 1.32 cm and weight about 432.2 ± 24.14 g. The water temperature, pH, dissolved oxygen (DO) concentrations and electrical conductivity (EC) were measured daily and their means ± SD were 28.2 ± 0.08 °C, 6.7 ± 1.1, 6.5 ± 0.89 mg l^{-1} and 260 ± 0.2 μmho cm^{-1}, respectively).

2.4. Experimental design

In the start of experiment, we determined LD % of latex on fish, and found that 8 ml/60 l.

Prior to experiments, the fish were determined to be free of external parasites (AFS-FHS, 2003). Fish were divided into three groups (12 fish per group) as (4 fishes in each replicate) and fed 5% body weight twice a day with commercial pellets as follows:

2.4.1. ***Control group*** *(without any treatment)*

Fish were kept in 60 l of de-chlorinated water. Water was changed once daily.

2.4.2. 4-nonylphenol group

Fish exposed to 100 μg l^{-1} 4-nonylphenol (Mekkawy et al., 2011). Water was changed daily and tank was supplied by water with 4-nonylphenol at the same concentration daily.

2.4.3. Latex and 4-nonylphenol group

Fish exposed to 4-nonylphenol (100 μg l^{-1}) and 7 ml of *Calotropis* latex for two weeks, water was changed daily and the tank was supplied by water with *Calotropis* latex and 4-nonylphenol at the same concentration daily for two weeks in triplicates for each group. The doses selected for this study were based on the current guidelines and recommendations of bulk chemicals (OECD, 2012). The experimental media water was changed each day and the