Antihemolytic activity of Solanum virginianum
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ABSTRACT

Aim: The aim of the study was to find the antihemolytic activity of Solanum virginianum. Background: Medicinal plant S. virginianum L is belong to the family Solanaceae, commonly known as yellow berried nightshade. Recent scientific studies have emphasized the possible use of S. virginianum in modern medicine. Hemolysis is the rupturing (lysis) of red blood cells (erythrocytes) and the release of their contents (cytoplasm) into surrounding fluid (e.g., blood plasma). The antihemolytic activity of the plant extract is determined using the antihemolysis formula and spectrophotometric method.

Materials and Methods: The study deals with the antihemolysis potential of S. virginianum ethanol extract. An erythrocytes suspension is created from a “A” positive patient and a hemolytic activity is performed by an in vitro procedure. Antihemolytic potential of the extract was inspected by spectrophotometric procedure checked by addition of the plant and compared with the standard. The level of percentage hemolysis by the extracts was calculated according to the following formula: % of hemolysis = At-An/Ac-An x 100. Results: The results of this study conclude that the ethanol extracts from the plant of S. virginianum are non/toxic to the human erythrocytes. Conclusion: The test tube with the strongest concentration of the plant extract had a hemolytic action whereas the test tube with the weakest plant extract had an antihemolytic action. Through the experiment, the hemolytic activity of the S. virginianum plant extract significantly increased in a dose-dependent manner.

KEY WORDS: Antihemolytic activity, Aqueous extract, Spectrophotometric method, Solanum virginianum

INTRODUCTION

Magical properties of herbals are mainly due to the presence of many secondary metabolites of a different property. The herbals provide crude drugs for the common man. The therapeutic potentiality of herbals lies in this phytochemicals that produce marked physiological action on the human body. Ethnopharmacological information is broadly considered an effective method in the discovery of new anti-infective molecules from plants. Many phytochemicals represent adaptive traits that have undergone radical changes during evolution to provide defense in plants against environmental[1] and biotic stress.[2] Defense strategy includes toxicity, antifeedant, microbicidal, antimetastatic, and antinutrients.

Medicinal plants are the rich source of medicinally important compounds, and since ancient time, plants and plant-derived products are used as medicine in the traditional and folk medicinal system. Initially, the herbal drugs were used in the form of dried powder, gums, extracts, or formulations of more than one plant products. Advanced scientific techniques brought a revaluation in the herbal medicine industry, and all focus is concentrate on active principles (bioactive molecule). However, a lot of processing is required to develop a drug from natural sources. Toxicity of the active molecule is a key factor during drug designing, and hemolytic activity represents a useful starting point in this regard, it provides the primary information on the interaction between molecules and biological entities at the cellular level. Hemolytic activity of any compounds is an indicator of general cytotoxicity toward normal healthy cells.[3] Usually, saponins (a group of phytochemical) present in the plants showed hemolytic activity by creating changes in the erythrocyte membrane. In vitro, hemolytic assay by spectroscopic method provides an easy and effective method for the quantitative measurement of hemolysis. This method provides the evaluation of the effect of different concentrations of biomolecules on human erythrocytes.

Solanum xanthocarpum used by the local people as folk medicines in treating throat infections and other...
inflammatory problems. The fruits are known for several medicinal uses such as anthelmintic, antipyretic, laxative, anti-inflammatory, anti-asthmatic, and aphrodisiac activities. The fruit paste is applied externally to the affected area for treating pimples and swellings. The various parts of the plant are reputed in indigenous Hindu Medicine to have high medicinal value in various diseases such as cough, asthma, fever, and heart disease. The plant extract of Solanum xanthocarpum also possesses insecticidal and molluscicidal properties. Its fruits are eaten as an anthelmintic and for indigestion. Its root is an expectorant, used in Ayurvedic medicine for cough, asthma, and chest pain. It has been used in Ayurveda for a variety of therapeutic purposes.

Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. Several medicinal plants have been used as a dietary adjunct and in the treatment of numerous diseases without proper knowledge of their function. Although phytotherapy continues to be used in several countries, few plants have received scientific or medical scrutiny.

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extract. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, and herbology and phytotherapy medical herbalism is today a sophisticated system of natural medicine using plant extracts and herbs to help treat physical and mental disorders. Medical herbalism is the modern version of traditional herbal medicine which has been used throughout the world for thousands of years. Herbalists use concentrated whole plant extracts, in the form of tinctures, infusions, salads, creams, and pills, as part of a holistic treatment plan to address the underlying causes of your condition. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. In many cases, substances such as alkaloids serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds. About 80% of individuals from developed countries use traditional medicines which have bioactive compounds derived from medicinal plants; hence, such plants should be investigated further for a better understanding of their properties, safety, efficacy, and efficiency.

Solanum species are used, traditionally, to treat various disorders such as pain, inflammation fever, and enteric diseases. In addition, it shows much potential such as antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, antipyretic, microbicidal, cytotoxicity, anticonvulsant, antifulerogenic, and also against sexually transmitted diseases. In western countries, 80% of the people still apply traditional ethnic medicines obtained from natural herbs. In India, >75% of the indigenous people using medicinal plants as curative.

**MATERIALS AND METHODS**

**Collection of Sample**

Fresh samples of S. virginianum leaves, stem, berries, and flower, were collected from Anna Siddha Hospital Herbal Garden, Anna Nagar, Chennai, Tamil Nadu. The plants were identified by botanist and they identified as S. virginianum. The collected samples were brought to the laboratory, washed thoroughly in running tap water to remove adherent particles if any and used for further analysis.

**Processing of Plant**

The leaves of the plants were collected and washed thoroughly in tap water followed by distilled water. The leaves were shade dried at room temperature. Dried leaves were uniformly grinded using a mechanical grinder.

The leaves powder was extracted in distilled water. 10 g of plant powder was extracted in distilled water using a Soxhlet extractor. The extract was concentrated using rotary evaporator and dried using lyophilizer. The dried extract was collect in airtight container and stored at 4°C.

**Preparation of Erythrocytes Suspension**

About 5 ml of blood was collected from a healthy individual (blood Group A positive) in a tube containing heparin. The blood was centrifuged at 1500 rpm for 3 min in a laboratory centrifuge. Plasma (supernatant) was discarded and the pellet was washed 3 times with sterile phosphate buffer saline solution (pH 7.2 ± 0.2) by centrifugation at 1500 rpm for 5 min. The cells were resuspended in normal saline to 0.5%.

**Hemolytic Activity**

*In vitro* hemolytic activity was performed by spectrophotometer method (Yang et al., 2005). A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (125, 250, 500, and 1000 μg/ml concentrations in phosphate buffer saline). The mixtures were incubated for 30 min at 37°C in an incubator. The mixture was centrifuged at 1500 rpm for 10 min in a laboratory centrifuge. The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as minimal and maximal hemolytic controls. Each experiment was performed in triplicates at each concentration.
The level of percentage hemolysis by the extracts was calculated according to the following formula:

\[ \% \text{Hemolysis} = \frac{A_n - A_t}{A_c - A_n} \times 100 \]

Here:
- \( A_n \) is absorbance of the control (saline control)
- \( A_c \) is the absorbance of the control (water control)
- \( A_t \) is the absorbance of the test sample.

**Statistical Analysis**
All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically using Microsoft Excel 2007.\(^{16}\)

**RESULTS**
The results of this study conclude that the ethanol extracts from the plant of *S. virginianum*, are non/less toxic to the human erythrocytes.

**DISCUSSION**
Herbal drugs secured significance in recent years due to their genuine efficacy as phytomedicines. The lead molecule or principles present in these herbal products act either as templates or precursor for synthetic drugs. The spectrum of alkaloids isolated from herbas depends on many factors, such as the nature of tissue, the extraction period, temperature, and the polarity of the solvent system. Alkaloids have been proved to have multiple biological effects, including antihemolytic potential.\(^{17}\)

Initially, the blood was separated into serum and RBC, and it was made into a pellet. It was found that various phytochemical were present in high proportion alkaloids, terpenoids, glycosides, flavonoids, saponins, coumarins, tannin, proteins, and amino acids that could rupture the RBC wall. The test tube with the strongest concentration of the plant extract had a hemolytic action whereas the test tube with the weakest plant extract had an antihemolytic action. The aqueous extract was used so that it could yield better results due to the increase in its polarity.

Hemolysis is usually connected with free radical damage and its inhibition by antioxidants is an effective measure. The protective activity can be explained by the scavenging effect by caulophyllumine-A against free radicals induced membrane lipid peroxidation. Many researchers provided evidence that secondary metabolites provide stability in erythrocyte membranes through scavenging the free radicals and ROSs or as a source of membrane stabilization. In fact, a positive concentration, there is a steady increase in hemolytic activity. The optical density was also obtained to prove the increase in hemolytic activity. Hence, if the plant extract concentration is low, we can analyze that there is less hemolytic activity and can be used in drugs provided the concentration is low.
correlation has been noticed between phytochemicals and antioxidant activity in many plant species. Erythrocytic cells are target components of oxidative burst due to the presence of membrane polyunsaturated fatty acids and also coupled with oxygen transport to hemoglobin molecules. Antihemolytic activity by phytochemicals has been reported.

CONCLUSION
Since ancient time, plants products been utilized for the treatment of various health problems. Plants are one of the most important sources of drug discovery and development. The plants used in this study have been excessively used in traditional medicine to cure a variety of diseases. The test tube with the strongest concentration of the plant extract had a hemolytic action whereas the test tube with the weakest plant extract had an antihemolytic action. Through the experiment, the hemolytic activity of the S. virginianum plant extract significantly increased in a dose-dependent manner.

REFERENCES

Source of support: Nil; Conflict of interest: None Declared