In Vitro Evaluation of Antidiabetic, Antiinflammatory And Antihaemolytic Efficacy of The Hydroethanolic Leaf Extract of *Cordia Sebestena*

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Abstract- *Cordia sebestena. L is also known as Geiger tree. It is a rounded, evergreen belongs to Boraginaceae family. It grows up to 25 with equal spread. In the present study, hydroethanolic extract of Cordia sebestena leaves were used to evaluate in vitro antidiabetic, antiinflammatory and antihaemolytic activities. In vitro antidiabetic activity by alpha amylase inhibitory activity, anti-inflammatory activity by inhibition of Protein denaturation, Proteinase inhibitory activity and antihaemolytic activity by hyposaline induced haemolysis. Hydroethanolic extract showed an effective pharmacological activity in all assays when compared with their respective standards.*

Keywords- *Cordia sebestena* leaves, *In vitro*, antidiabetic activity, anti-inflammatory activity, antihaemolytic activity.

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that affects the metabolism of carbohydrate, fat and protein. It includes a group of metabolic disease characterized by hyperglycemia in which blood sugar level are elevated either because the pancreas do not secrete enough insulin or body cells which do not respond properly to the insulin secreted by pancreas. The effect of diabetes mellitus include long term complications like heart disease, stroke, dysfunction and failure of various organs (Keerthana *et al.,* 2013) and it is one of the most common endocrine metabolic disorder which causes the various microvascular and macrovascular complications. Nearly 2.8% of the world's population affected by diabetes and it is expected to increase up to 5.4% in the year of 2025 (Patel *et al.,* 2011).

Inflammation is the response of living tissues to injury and it is caused by a variety of stimuli including physical damage, ultra violet radiation, microbial invasion and immune reactions. Inflammation involves an increase of blood supply to the affected region by means of vasodilatations (Habibur *et al.,* 2012). The body response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions and body response to inactive or destroy the invading organism to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemicals mediators from injured tissue and migrating cells (Chandra *et al.,* 2012).

In established medicines there are several records for treating people suffering from pain and inflammation with phytochemicals. In recent times, focus on plant research has increased and Non Steroid Anti-inflammatory Drugs (NSAIDs) constitute one of the most widely used drugs (Sreena and Sujith, 2016). Long term use of NSAIDs is also associated with side effects such as stomach bleeding, allergic reactions, kidney problems and heart problems (Sembulingam and Prema, 2012).

Haemolytic activity of any compounds is an measure of general cytotoxicity towards normal healthy cells. Normally saponins present in the plants showed haemolytic activity by creating changes in the erythrocyte membrane. *In vitro* haemolytic assay by spectroscopic method provides an easy and effective method for the quantitative measurement of haemolysis. This method provides the assessment of the effect of different concentration of biomolecules on the human erythrocytes (Kumar *et al.,* 2011).

Cordia sebestena L is also known as Geiger tree belonging to the family Boraginaceae. It grows up to 25 feet in tropical as well as sub tropical countries. Many compounds originally isolated from *Cordia* species have been reported as presenting several biological activities such as antifungal, larvicidal, anti androgenic etc. Syrup of the bark, flowers or fruit is taken for cough and bronchial ailments (Renata *et al.,* 2005). But no report was found regarding antidiabetic, antiinflammatory and antihaemolytic

activity of *Cordia sebestena* till date. In the Present study investigation attempts have been made to find out the antidiabetic, antiinflammatory and antihaemolytic properties of *Cordia sebestena* by alpha amylase, protein denaturation, proteinase inhibitory, and hyposaline induced haemolysis of hydroethanolic extract respectively.

II. MATERIALS AND METHODS

Collection of Plant material

The leaves of Cordia *sebestena* L were collected from Coimbatore, Tamil Nadu in India and the leaves of *Cordia sebestena* L were authenticated by Dr. C. Murugan, Scientist 'D' & Head of office, Botanical Survey Of India, Southern Regional Center, Coimbatore (Authentication No.BSI/SRC/5/23/2017/Tech).

Preparation of extract

The collected plant leaves were shade dried, powdered and 10g of each leaves were mixed with different solvents such as aqueous, hydroethanol, ethyl acetate, chloroform and petroleum ether separately in a round bottom flask and kept air tight for 72 hours and was shaken frequently for uniform mixing and distribution of powdered sample. Then the solution is filtered through a Whatman No. l filter paper and the solvent present in the filter were evaporated to dryness. Finally crude from of crystals was stored in refrigerator for further use.

Determination of *In vitro* antidiabetic activity

In vitro antidiabetic activity of *Cordia sebestena* was performed by alpha -amylase inhibitory assay by DNSA method. To 100 µl of different concentration of (100, 200, 300, 400 and 500 µg/ml) of plant extracts and standard drug acarbose was taken and added 200µl of alpha amylase and the mixture was incubated at 37ºC for 20 min. To the reaction mixture 100µl (1%) starch solution was added and incubated at 37ºC for 10 min. The reaction was stopped by adding 200µl of DNSA and kept in a boiling water bath for 5 minutes. The reaction mixture diluted with 2.2 ml of water and absorbance was read at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200µL in distilled water. Control, representing 100% of enzyme activity was prepared in a similar manner without extract. The experiments were repeated thrice using the same protocol (Shai *et al.,* 2010).
Percentage inhibition (I %) was calculated by,

I % = (Ac-As)/Ac X 100

where Ac is the absorbance of the control and As is the absorbance of the sample .

Determination of In *vitro* antiinflammatory activity
Inhibition of Protein Denaturation Method

In vitro antiinflammatory activity of *Cordia sebestena* was performed by inhibition of protein denaturation method. The reaction mixture 3ml contained, 50µl of different concentration of (100, 200, 300, 400 and 500 µg/ml) plant extract and standard drug diclofenac sodium was taken and added 450µl BSA to all the above test tubes. For the control tests, 50µl of distilled water was taken instead of test solution. The test tubes were incubated at 37°C for 20 minutes and then heated at 57°C for 3 minutes. After cooling the test tubes, 2.5ml phosphate buffer saline (PH 6.3) was added to each tube. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660nm (Satyendra *et al.,* 2012).

% Protein Denaturation Inhibition (I %) = (Ac-As)/Ac X 100

where Ac is the absorbance of the control and As is the absorbance of the sample.

Proteinase Inhibitory Activity

In vitro antiinflammatory activity of *Cordia sebestena* was performed by proteinase inhibitory method. To 1ml of different concentration of (100, 200, 300, 400 and 500 µg/ml) of plant extract and standard drug diclofenac sodium was taken and then 1ml of Tris HCl buffer was added. The reaction mixture was incubated at 37°C for 5 min and then 1ml of casein was added. The mixture was incubated for an additional 20 min and then 2 ml perchloric acid was added to terminate the reaction. The reaction mixture was centrifuged and the absorbance of the supernatant was read at 210nm against buffer as blank. The experiment was performed in triplicate (Sakat *et al.,* 2010). The percentage of inhibition of proteinase inhibitory activity was calculated

I % = (O.D control – O.D sample) X 100/ O.D control

Determination of *In vitro* antihaemolytic activity
Hypotonic solution - induced haemolysis method

This method was done by method of Shinde *et al.,* (1999). 5ml of whole blood of a healthy person in heparinised tube was collected. The blood was centrifuged at 3000rpm for 10 minutes. Supernatant be detached with RBCs

were washed three times with sodium chloride isotonic solution, through centrifugation using the similar volume as supernatant. Finally, RBCs were resuspended in the same volume of isotonic buffer solution. 0.5 ml of RBCs suspension was mixed with 5ml of hypotonic solution is containing 0.5ml of different concentration of (100, 200, 300, 400 and 500 µg/ml) of plant extracts and standard drug (ascorbic acid) was taken. The control sample was prepared by 0.5 ml suspension mixed with hypotonic buffered saline. The mixture was incubated for 10 min at room temperature, centrifuged at 3000rpm for 10min and the optical density of supernatant was measured at 540nm. The percentage of inhibition of Haemolytic activity was calculated,

Percentage inhibition (I %) = OD of control – (OD of extract/ OD of control) x100

Statistical Analysis

The results was expressed as the mean±SD for three replicates. Linear regression analysis was used to calculate IC50 value.

III. RESULTS AND DISCUSSION

***In vitro* antidiabetic activity**
Alpha -amylase inhibitory assay

Alpha amylase is an enzyme that hydrolyses alpha-bonds of alpha-linked polysaccharide such as starch to give in high levels of glucose and maltose. α-amylase inhibitors combine to α-bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide (Nair *et al.*, 2013). The comparison of the α-amylase inhibitory activity of the hydroethanolic extract of *Cordia sebestena* with that of standard drug acarbose are given in figure 1. The hydroethanolic extract of *Cordia sebestena* shows a maximum alpha amylase inhibitory activity of 76.51 ± 2.25 at 500 µg/ml whereas for standard (acarbose) was found to be 86.37 ± 2.89 at 500 µg/ml. The IC50 value of the hydroethanolic extract of *Cordia sebestena* and acarbose were found to be 270.67µg/ml and 222.72µg/ml respectively. This result are in similar to report by Keerthana *et al.*, (2013), who revealed that the *in vitro* amylase inhibitory activities of the methanol extract of *T. Sinensis* increase with increasing concentration.

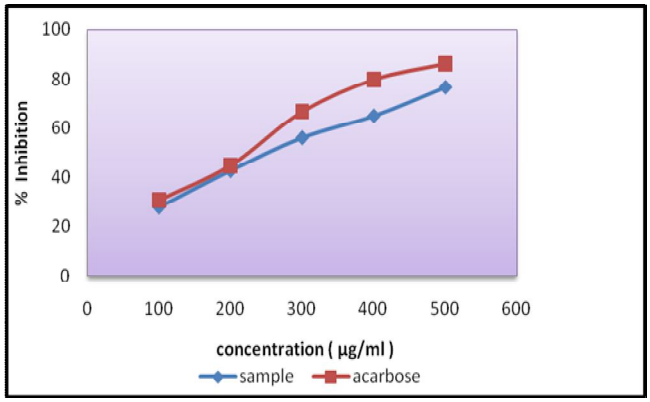

Figure 1: alpha amylase inhibitory activity of *Cordia sebestena*

***In vitro* antiinflammatory activity**
Inhibition of Protein denaturation

BSA denaturation method was selected for the *in vitro* assessment of antiinflammatory property. BSA assay seeks to eliminate the use of live specimens as far as possible in the drug development process (Moore *et al.*, 2010). The comparison of the inhibition of protein denaturation activity of hydroethanolic extract of *Cordia sebestena* with that of standard drug Diclofenac sodium was shown in figure 2. The hydroethanolic extract of *Cordia sebestena* shows a maximum inhibition protein denaturation activity of 90.97 ± 2.78 at 500µg/ml whereas for standard (Diclofenac sodium) it was found to be 95.40 ± 1.98 at 500µg/ml. The IC50 value of the hydroethanolic extract of *Cordia sebestena* and diclofenac sodium were found to be 169.53µg/ml and 120.39 µg/ml respectively. This result are in line with previous report by Nagaharika *et al.*, 2013, who revealed that the antiinflammatory activity of leaves of *Jatropha gossypifolia* increased with increasing concentration.

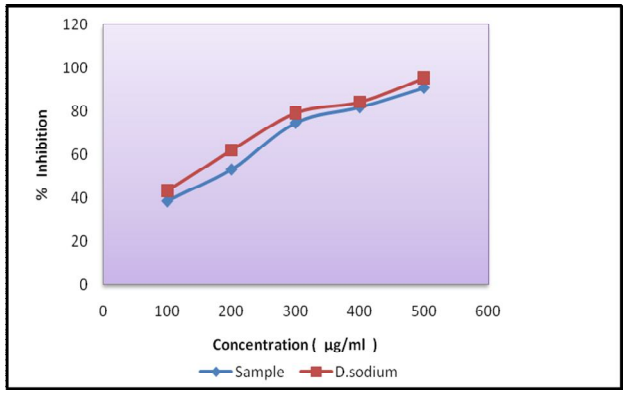

Figure 2: Inhibition of Protein denaturation activity of *Cordia sebestena*

Proteinase inhibitory activity

Neutrophils are most important source for proteinases which carries in their lysosomal granules and are involved in inflammatory reactions. It was already reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Govindappa *et al.*, 2011). The comparison of the proteinase inhibitory activity of hydroethanolic extract of *Cordia sebestena* with that of standard drug diclofenac sodium was shown in figure 3. The hydroethanolic extract of *Cordia sebestena* shows a maximum proteinase inhibitory activity of 85.49 ± 1.38 at 500µg/ml whereas for standard (diclofenac sodium) it was found to be 89.07 ± 1.39 at 500µg/ml. The IC50 value of the hydroethanolic extract of *Cordia sebestena* and diclofenac sodium were found to be 240.17µg/ml and 224.79µg/ml respectively. This result are in accordance with previous report by Nagaharika *et al.*, 2013, who revealed the antiinflammatory activity of leaves of *Jatropha gossypifolia* increased with increasing concentration.

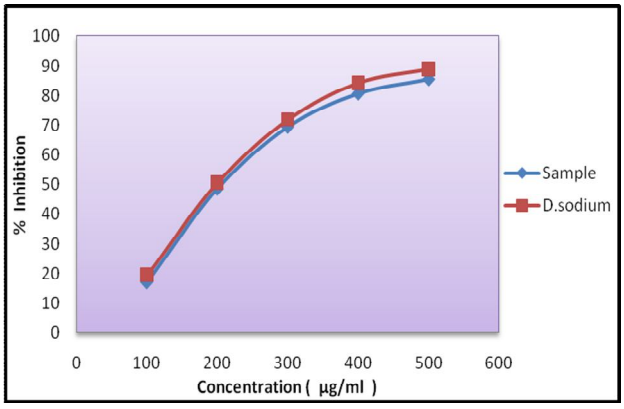

Figure 3: Proteinase inhibitory activity of *Cordia sebestena*

***In vitro* antihaemolytic activity**

Hypotonic solution - induced haemolysis activity

The study of erythrocytes is a good model for direct indication of toxicity of injects able formulation and membrane, thus extensively used for oxidative stress studies. Exposure of red blood cell to hypotonic medium leads to membrane lysis and oxidation of haemoglobin which results in secondary damage through free radical induced lipid peroxidation (Hallowe and Whiteman, 2004). The comparison of the haemolysis activity of hydroethanolic extract of *Cordia sebestena* with that of standard drug ascorbic acid was shown in figure 4. The hydroethanolic extract of *Cordia sebestena* shows a maximum haemolysis activity of 81.16 ± 0.94 at 500µg/ml whereas for standard (ascorbic acid) it was found to be 85.26 ± 0.89 at 500µg/ml. The IC50 value of the hydroethanolic extract of *Cordia sebestena* and ascorbic acid were found to be 300.25µg/ml and 261.52µg/ml respectively. The reported high haemolytic activity of the different solvent extracts of *Allium stracheyi* Baker towards human red blood cells (Mukherjee and Rajasekaran, 2010).

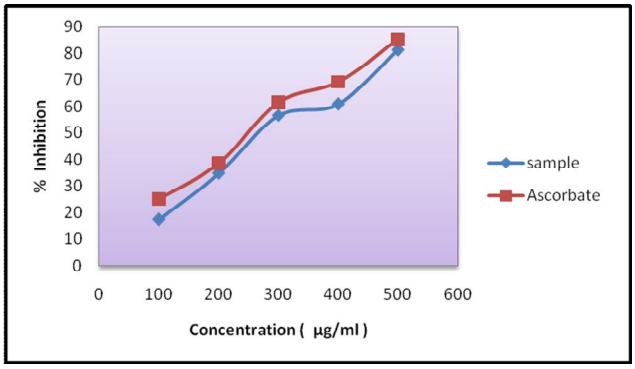

Figure 4: hypotonic solution induced haemolysis activity of *Cordia sebestena*

IV. CONCLUSION

In conclusion from the above study the hydroethanolic crude leaf extract of *Cordia sebestena* was found to be possess effective antidiabetic, antiinflammatory and antihaemolytic activity. Further studies are needed to investigate the *in vivo* antiinflammatory activity with animal model.

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