



ISSN (E): 2277- 7695  
 ISSN (P): 2349-8242  
 NAAS Rating: 5.03  
 TPI 2019; 8(4): 1146-1148  
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 www.thepharmajournal.com  
 Received: 18-02-2019  
 Accepted: 19-03-2019

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## ***In vitro* anticoagulant activity of *Datura stramonium* flower extracts on blood plasma of poultry bird**

**Sumit Sarkar, Anindya Bagchi, Anusree Raha, Prosenjit Mukherjee, Monit Paul, Sourav Roy and Rahaman Mehedi Mamud**

### **Abstract**

Haemostasis is the process of forming clots in the walls of damaged blood vessels to prevent abnormal bleeding and to maintain intravascular blood in a fluid state. There is an increasing need to source for pharmacological and medicinal materials from plant source. We aimed to study the possible coagulation effect of *Datura* flower extract and methanol extracts *in vitro* anticoagulant activity by using blood samples of poultry bird. *In vitro* coagulation effects of *Datura* extract in different concentrations 100, 125 and 250 microliter were examined in the blood samples of poultry bird by measuring prothrombin time (PT). The both extracts were found to inhibit coagulation process and significantly prolonged prothrombin time in a dose-dependent manner. The principle involved in this extracts in different concentrations inhibits clot formation and increases prothrombin time. This also subject to further studies on efficacy and safety, it can well be used, in the future, as a supplementary anticoagulant agent in cardio problems.

**Keywords:** Haemostasis, *in vitro* anticoagulant activity, prothrombin time, intravascular, blood

### **Introduction**

Haemostasis is an interaction process between coagulation and anticoagulants that retains the blood within the injured vascular system during periods of injury. Haemostasis comprises a complex mechanism that contains three major steps:

- 1) Vasoconstriction,
- 2) Temporary blockage of a break by a platelet plug, and
- 3) Blood coagulation, or formation of a fibrin clot.

The coagulation mechanism is a complex cascade mechanism involving the conversion of precursor enzymes (zymogens, procoagulants, and proenzymes) into the active enzymes. Mostly, substances that are necessary for coagulation are present in an inert form and converted to an activated state. Once, one active enzyme is formed it converts the next inactive zymogen to its active enzyme. This series process continues until a fibrin meshwork clot is formed. Protein cofactors, membrane phospholipids surfaces and calcium ions play an active role in the development of the fibrin clot <sup>[1]</sup>. Cardiovascular disorders include hypertension, cerebral haemorrhage, coronary thrombosis, arteriosclerosis, and congestive heart failure are caused by blood circulatory system as blood clotting disorders constitute a serious medical problem. The prothrombin time (PT) test also known as pro-test or PT test used to screen the extrinsic pathways and detects the deficiencies in Factors II, V, VII, and X. In the presence of calcium ions thromboplastin activates the extrinsic pathway in coagulation system and the subsequent clotting time depends on the concentration of Factors II, V, VII, and X. Thus, one or more of these clotting factors (VII and X) deficiency indicated by a prolonged PT and considered as abnormal <sup>[2-4]</sup>. The normal PT is 11-15 s. Except for nonsteroidal anti-inflammatory drugs (aspirin and indomethacin) some other important synthetic anticoagulant agents are heparin, ethylenediaminetetraacetic acid (EDTA), citrate, and warfarin have anti-inflammatory and anti-platelets activity <sup>[5]</sup>.

In India, the use of plants with widespread medicinal purposes for the prevention and/or treatment of various ailments is one of the most ancient traditional remedial forms of primary health care <sup>[6, 7]</sup>. Besides, the pharmaceutical properties anticoagulant drugs show serious side effects and also expensive. Hence, therefore, it is necessary to explore alternative anticoagulants. Since the plants are the safer source of medicine, this study is a preliminary attempt to investigate the *in vitro* anticoagulant activities of *Datura stramonium* flower extracts using standard experimental models in the blood samples of normal individuals.

**Materials and Method**

**Collection of plant material**

The flower of *Datura stramonium* were collected from Chakdaha, Nadia District, and West Bengal, India and were shade-dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with various solvents. Calcium Chloride was purchased from Merck India Pvt. Ltd.

**Preparation of plant extract**

*Datura stramonium* flowers were air dried at room temperature and crushed with a mortar vessel grinder. This plant material again dried at room temperature for two days. This plant material was soaked by suspending 5.85g of powder of *Datura stramonium* flower in 50ml chloroform and 70ml methanol by the process of percolation. After 24 hours the suspension was filtered through No.1 Whatman filter paper. The solvent was removed at low temperature (50-70 °C) under water bath by using moist heat process. They were preserved into sterile bottle kept in a refrigerator until used for further analysis.

**Phytochemicals**

Each extract (chloroform and methanol) of the flower of *Datura stramonium* was subjected to a preliminary phytochemical analysis for the detection of different phytochemical constituents present in extract using the different phytochemical test. Different crude extracts were

dissolved in respective solvent and used for qualitative phytochemical constituent’s confirmation such as alkaloids, flavonoids, tannins, phenols, saponins and glycosides.

**Determination of PT**

**Collection of blood and separation of plasma**

About 5 ml of blood was drawn from healthy poultry bird (having no medicine consumption history) by intravenous injection. To the 9 µl volume of blood, 1 µl volume of 3.8% trisodium citrate solution uses added to avoid natural coagulation process. Immediately centrifugation was carried out for 15 min at a rate of 3000 rpm to separate the blood cells from plasma for prothrombin time (PT) test.

Plasma sample was divided into five groups:

Group 1:-Control group- 0.2 ml plasma + 0.1 µl 0.9 % saline + 0.3 µl calcium chloride

Group 2: -1<sup>st</sup> Test group- 0.2 ml plasma + 0.062 µl plant extract + 0.3 µl calcium chloride

Group 3: -2<sup>nd</sup> Test group- 0.2ml plasma + 0.125 µl plant extract + 0.3 µl calcium chloride

Group 4: -3<sup>rd</sup> Test group - 0.2 ml plasma + 0.25 µl plant extract + 0.3 µl calcium chloride

Group 5: -Standard group- 0.2ml plasma + tranexamic acid solution + 0.125µl plant extract + 0.3 µl calcium chloride

All the tubes are tilted at an angle of 45° for every 30 seconds to measure the clotting time. Stop watch was used for measuring the clot formation. This time is called as PT.

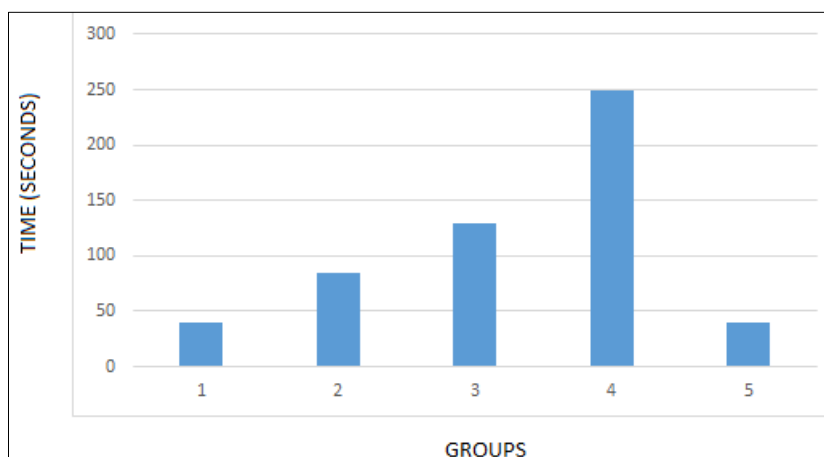
**Results and Discussion**

**Table 1:** Clotting time of different groups

Group	Test	Sample	Result
Group 1 : --0.2 ml plasma+0.1µl 0.9% saline+0.3µl calcium chloride	Control group		40sec
Group 2 : -- 0.2 ml plasma + 0.062µl plant extract + 0.3 µl calcium chloride	1 <sup>st</sup> Test group	Plant extract	85sec
Group 3: -- 0.2 ml plasma + 0.125 µl plant extract + 0.3 µl calcium chloride.	2 <sup>nd</sup> Test group	plant extract	129sec
Group 4 : -- 0.2ml plasma + 0.25 µl plant extract + 0.3 µl calcium chloride	3 <sup>rd</sup> Test group	plant extract	250sec
Group 5: -- 0.2 µl plasma +56% 0.125µl tranexamic acid solution + 0.125µl plant extract + 0.3 µl calcium chloride.	Standard group	plant extract	40 seconds

**Table 2:** Phytochemical screening of plant

Chemical Constituents (Process Name)	Result	Response
Alkaloid (Dragendroff Test)	Yellow Precipitate	Yes
Tannin (Ferric Chloride Test)	Greenish Colour	Yes
Phenol (Gelatin Test)	White Precipitate	Yes
Saponin	Foam	Yes
Flavonoid	Yellow Colour Change Into Colourless	Yes
Glycoside(Kellar Killani Test)	Reddish Brown/Greenish Blue	Yes



**Fig 1:** Clotting time of different groups

## Discussion

Coagulation is a process that occurs mainly due to the complex interaction of cellular and molecular components. Initially clotting involves common pathways both intrinsic and extrinsic pathway but lately it's found to be due to a balance between the procoagulants and anticoagulants here in group 4 crude concentration source most effective anticoagulant effect.

## Conclusion

Anticoagulant activity of *Datura stramonium* was not yet reported and this report was found to be the first investigation for PT test. Hence further identification and characterisation of active molecule responsible for activity to be found out in future. The study was carried out to evaluate the effect of *Datura stramonium* leaf extract for anticoagulant in blood sample of normal individuals by using principle of PT. the activity of future measurement for anticoagulation the experiment should go through coagulation and fibrinolysis assay, activated partial thromboplastin time, fibrinolytic activity, antioxidant activity, cytotoxic assay, haemolytic assay.

## Acknowledgement

The authors are thankful to the respected Principal Sir, Dr. Arnab Samanta, Netaji Subhas Chandra Bose Institute of Pharmacy, West Bengal for providing necessary facilities for the completion of research work.

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