

Indian Journal of Experimental Biology Vol. 59, August 2021, pp. 547-555



A preliminary study on perioperative hemostatic effect of spray dried powder of *Chromolaena odorata* leaf extract

Tania S. Paul¹*, Biswadeep B. Das¹, Mangesh P. Gurav¹, Yogesh P. Talekar² & Kishori G. Apte²

¹APT Research Foundation (a DSIR, SIRO Recognised Research Laboratory), Pune, Maharashtra, India ²APT Testing and Research Pvt. Ltd. (Formerly, National Toxicology Centre), Pune, Maharashtra, India

Received 31 October 2019; revised 19 August 2020

Accidents or surgery often cause internal haemorrhage in liver and arteries which may lead to patient morbidity and mortality. The current hemostatic agents used for treatment like collagen, oxidized cellulose, and chitosan suffer from side effects which include infection, inflammation and even sepsis. In the present study, we studied the spray dried powder (SDP) of the aqueous extract of the leaves of *Chromolaena odorata* (L.) R.M.King & H.Rob., commonly known as Siam weed or Common floss flower and also Christmas bush, for its hemostatic efficacy in two experimental models of surgery. Firstly, the SDP was screened through standard pharmacognostical parameters, and a part of the liver was lacerated in rats and femoral artery was transected in rabbits to assess the blood loss in pre-weighed gauze with and without treatment. In the liver laceration model, an effective blood loss reduction of 54.30 % was observed with oral administration of SDP 7 days prior to surgery. Similarly, application of SDP at the site of artery transection caused 70.36% reduction in blood loss as compared to the control rabbit artery. The results suggest that oral delivery and/or application of SDP of *C. odorata* by formulating it in a suitable drug delivery tool could minimize perioperative bleeding in hepatic and arterial tissue and improve recovery.

Keywords: Blood loss, Common floss flower, Christmas bush, Hemostatis, Internal haemorrhage, Liver laceration, Siam weed, Wound healing

Haemorrhage can be categorized as internal or external depending on the site of injury. Non compressible traumatic injury is one of the leading cause of death globally¹. Hepatic trauma or surgery leads to excessive blood loss and haemorrhage due to it is diffuse in nature. Massive trauma or acute coagulopathy can lead to large blood vessel damage where natural blood clotting ability becomes insufficient. Currently deployed hemostatic agent to manage the excessive blood loss includes fibrinogen concentrate, tranexamic acid, synthetic platelets² and topical hemostatic agents containing collagen, oxidized cellulose, and chitosan. However, use of these products have been reported to cause allergies, embolism, increase the risk of infectious diseases and low pH-induced inflammation. Studies also suggests that cellulose haemostatic agents are used in internal wounds of liver and spleen but some complication like sepsis may occur in few patients³. Therefore, a search for a rapid hemostatic agent that is nonantigenic, efficacious, absorbable and having ability to staunch bleeding at internal sites is the need of the hour.

Plants belonging to Asteraceae (Compositae) family are known for their wound healing and hemostatic activity. The most important genus include Achillea, Ageatum, Arctium, Artemisia, Caesulia, Centaurea, Chromolaena, Cichorium, Dendranthema, Eclipta, Gundelia, Helichrysum, Saussurea, Scorzonera, Sonchus, Tagetes, Tanacetum, Tridax and Vernonia⁴. The Siam weed or Common floss flower, Chromolaena odorata (L.) R. M. King & H. Rob., formerly known as Eupatorium odoratum Linn., has been studied extensively for its pharmacological and therapeutic potential including anti-inflammatory activity⁵, membrane stabilizing activity⁶; inhibition of hydrated collagen lattice contraction by normal human dermal fibroblasts⁷; decreasing the bleeding time and blood volume of wounds⁸; and diuretic⁹ wound healing^{10,11} and erythropoietic activity¹²; healing of skin infection¹³; and also antimicrobial propertv¹⁴.

To ensure the quality of herbal raw material, pharmacognostical, physicochemical and phytochemical

^{*}Correspondence:

Phone: +91 9767997581 (Mob.)

E-Mail: taniapaul6@gmail.com

evaluations play a pivotal role¹⁵. Moreover, pharmacognostical tests also help in distinction from other allied species and adulterants and guarantee the safe use of quality products and combat seasonal variations, if any¹⁶. Microscopy and macroscopy of leaves of C. odorata is well documented and reported¹⁷. Hence, emphasis has been given on other pharmacognostical parameters in the present study. In addition, our previous study revealed the hemostatic efficacy of the spray dried powder (SDP) in other bleeding models of thrombocytopenia, heavy use of anticoagulant and gastric ulcer when administered orally¹⁸. However, its efficacy in preventing blood loss in internal hemorrhagic situation has not been studied until now. Thus, in the present work, we have made an attempt to lay down the hemostatic potential of spray dried powder of C. odorata in surgery models of liver laceration and femoral artery injury when administered by oral and topical route, respectively.

Material and Methods

Collection and Authentication of plant material

The leaves of *Chromolaena odorata* (L.) R. M. King & H. Rob. was collected from Vadgaon khurd, Pune district of Maharashtra and was authenticated by Dr. J. Jayanthi from Botanical Survey of India (BSI), Pune under the number BSI/WRC/Cert./2014. A voucher specimen of the herbarium was submitted in APT Research Foundation, Pune for further reference.

Preparation of plant extracts

The leaves were cleaned and shade dried in a shade dryer and the dried leaves obtained were powdered. It was then extracted in soxhlet apparatus with Millipore RO water. The aqueous extract obtained was concentrated in rotary evaporator under vacuum and percent yield was determined by using the formula: Weight of the Extract/Weight of the plant material $\times 100^{19}$.

Preparation of Spray dried powder (SDP)

The liquid extract obtained was first atomized and allowed to contact with hot air which results in evaporation to yield dried particles. These particles are subsequently separated from the air stream by pressure of cyclone mixer inbuilt in spray drying machine. Briefly, the blower was started and desired inlet temperature of 150°C and outlet temperature of 80°C was set in the panel²⁰. The heater was started till the desired temperature is achieved followed by starting the compressor at 0.70 kg/cm². Later, the feed

pump was set at 14 RPM to achieve feed rate of about 750 mL/h. It was ensured not to have particles which might block nozzle of the atomizer. The powder obtained after spray drying was tightly packed in air tight containers for further experiments.

Pharmacognostical screening of SDP

The SDP of C. odorata was subjected to various pharmacognostical screening procedures such as phytochemical screening²¹. Further, fluorescent characteristics of SDP was done by treating it with chemical reagents and were observed in day light as well as under UV radiation. Fluorescent analyses of all the plant powders were carried out according to the standard protocols²². Physicochemical evaluation of the fine powder was performed following an established protocol 23 . The parameters evaluated were moisture content, extractive yield, spray dried yield, crude fibre content, total ash, water soluble ash, acid soluble ash, sulphated ash. Determination of heavy metals, such as Arsenic (As), Chromium (Cr), Lead (Pb), Mercury (Hg), Cadmium(Cd) and Iron (Fe) in the fine powder was performed using atomic absorption spectroscopy (AAS) (model no.: AA-6300 Shimadzu) according to standard protocols²⁴. Calibration of the instrument was performed using a blank solution to zero. The standard samples were loaded followed by the test sample and the absorbance was recorded and data expressed as mg/kg. To monitor the microbial contamination in the SDP of C. odorata, microbial analysis was carried out using 1.0 g of spray dried powder in 20 mL of sterile distilled water as stock solution. One mL from the above stock solution was mixed by vortexing into 9 mL sterile normal saline for one hour. One mL from different dilutions (Stock, 10^1 , 10^2 , 10^4 & 10^6) was poured on Nutrient Agar (NA) plates and Chloramphenicol Yeast Glucose Agar (CYGA) plates by pour plate method. The NA plates were kept at 37°C and CYGA plates were kept at 28-30°C for incubation for 24 h and 48-72 h, respectively25. Similarly, 1.0 mL each of the same samples was poured on Mac Conkey's Agar plate, Brilliant Green Agar plate for detection of E. coli, and Salmonella-Shigella spp., respectively. At the end of the incubation period, the number of colony-forming units per gram (CFU/g) was calculated.

Accelerated shelf life storage stability study on SDP was conducted by keeping the samples at room temperature of 37°C on Day '0' and at 54°C for 14 days²⁶. Briefly, on the day of analysis, 20 mg of powdered sample was weighed into a 100 mL

volumetric flask and 30 mL of water was added and sonicated for about 5 min to dissolve. Finally, volume was made up to 100 mL with water. A portion of this solution was filtered through Whatman 41 filter paper and analyzed by HPLC-DAD method in Agilent 1260 HPLC with DAD Detector using C18 (250 mm length \times 4.6 mm i.d. \times 5 μ m particle size. The mobile phase used was acetonitrile: water (35:65) with flow rate of 1.0 mL /min and run time was 10 min. Similarly, 18 months' shelf life storage stability study on spray dried powder was conducted by keeping the samples in stability chamber at temperature $40\pm2^{\circ}$ C and relative humidity of $75\pm5\%$ for 18 months. Samples were made into aliquots and analyzed on 0, 3rd, 6th, 9th, 12th, 15th and 18th month time points by HPLC-DAD method as mentioned above.

Spectral fingerprinting of SDP of C. odorata

The spray dried powder obtained was taken and 2 mg/2 mL solution was prepared. The sample was run in a UV visible spectrophotometer to obtain the fingerprinting from 200-900 nm²⁷. For the HPLC of SDP, a 20 µL from 5% aqueous solution is made up to 10 mL by distilled water. The solution was filtered through 0.22 µ filter and 4 mL each was distributed in two vials and sent for analysis to Venture Centre, Pune. HPLC peak were observed using the solvent system methanol:water at the ratio 60:40, run time 1.0mL/minute and detected at 268 nm. Subsequently, HPTLC of spray dried powder was conducted by macerating 2 g of powdered drug with 10 mL methanol (5-7 h). The crude extract was filtered and concentrated to dryness and from that, 5 mg of the residue was dissolved in 10 mL of methanol. About 10 µL of the sample was injected to CAMAG Linomat 5 "Linomat5-210175" S/N 210175 (1.00.13) and sample allowed to run in the Solvent system of n-Hexane: Ethyl acetate (6:4). The stationary phase used was 5×10 cm TLC silica gel 60 F₂₅₄ (Merck). For derivatization, the plate was immersed into anisaldehyde sulphuric acid reagent for 1 second then heated at 120°C for 5 min²⁸. The plates were scanned in CAMAG TLC scanner 4 at 254 nm, 366 nm and 540 nm wavelength.

Ultimately, particle size analysis of SDP was done by preparing a solution with concentration 1.0 mg/mL of SDP. From that 400 μ L was made upto 12 mL with methanol. About 3 mL sample was analyzed with Brookhaven Instrument Corp. and analyzed with 90+ particle sizing software Ver 4.12.

Pharmacological hemostatic efficacy of *C.odorata Animal experimentation for liver laceration model*

All animal study was done as per the approval Institutional Animal Ethics Committee of conducted by following the principles of CPCSEA, GoI. The study was approved by IAEC with the protocol no. RP. No. 06/1617 of APT Testing and Research Pvt. Ltd. Pune, and conducted by following the principles of CPCSEA. Twelve male Wistar rats weighing 200-250 g were housed under standard conditions of temperature (24±2°C) and relative humidity (40-60%) with 12 h light/dark cycle. Animals were fed on standard commercial pellet diet and water ad libitum. Rats were divided into two groups of Control and Test (n=6). Animals were anesthetized 75 mg/kg ketamine hydrochloride +10 mg/kg xylazine i.p.²⁹. The rats were positioned with the tail toward the surgeon. A midline laparotomy was performed to expose the abdominal cavity, and the left lobe of the liver was exposed. A 3.0×1.5 cm section of the edge of the left liver lobe was transected to cause liver injury³⁰. Preweighed gauze was placed inferior to the liver before injury. The gauze was replaced and weighed at intervals. The process was repeated for 3 min or until blood stops flowing from the lacerated area.

Animal experimentation for Femoral Artery Injury model

One of the objectives of the present study was to assess the surgical hemostatic effects of topical application of SDP on major arterial vessel injury. The study was approved by IAEC with the protocol no. RP. No. 06/1617 of APT Testing and Research Pvt. Ltd. Pune, and conducted by following the principles of CPCSEA. Six New Zealand white rabbits were used for the study and housed under standard conditions of temperature and relative humidity with 12 h light/dark cycle. Animals were fed on standard commercial pellet diet and water *ad libitum*. SDP coated gauze was topically applied to the femoral artery puncture in 1 extremity in each animal (femoral artery 1), but not to the other extremity, which served as the control (femoral artery 2).

All of the rabbits underwent surgery under proper general anaesthesia without intubation. Anaesthesia was provided via intramuscular injection of ketamine hydrochloride 35 mg/kg and xylazine hydrochloride 5 mg/kg³¹. After administration of anaesthesia, both femoral arteries were explored using a sterile surgical technique, and separated from the femoral vein and femoral nerve. The femoral arteries were rounded with tapes and a 21G injector needle was inserted to induce major bleeding. When bleeding began, compression with control gauzes was immediately applied in the right leg and test gauzes were immediately applied in the left leg and the bleeding time was calculated. The blood loss was monitored by measuring the weight of the gauzes before and after decompression³².

Statistical analysis

Results are expressed as percentages and Mean \pm SD. For animal Studies, data are analyzed by Student's T-Test and compared with non-treated control animals.

Results

Pharmacognostical screening of SDP

Data in Table-1 represent the various phytochemicals present in the crude powder, aqueous extract and SDP of C. odorata. Followed by this, the fluorescence of SDP of C. odorata was monitored in visible and UV light as depicted in Table 2. The physicochemical parameters were also analysed to detect the presence of inorganic and organic material present in the SDP of C. odorata which is tabulated in Table 3. Weeds such as C. odorata generally uptake heavy metals from the soils which in turn may attribute as a contaminant in the process of developing a herbal medicine. Therefore, the presence or absence of these heavy metals were analyzed in the present study and found to be in the permissible limits

| Table 1 — Preli | minary phytochemical | screenin | g data of | | | | | | | | |
|-----------------------|-----------------------|--------------------------|------------------|-----------------|--|--|--|--|--|--|--|
| Chromolaena odorata | | | | | | | | | | | |
| Secondary metabolites | Name of test | Crude Powder of CO | Aq. Ext of CO | SDP of CO | | | | | | | |
| Flavonoids | Alkaline reagent test | ++ | ++ | $^{++}$ | | | | | | | |
| Alkaloids | Dragendorff's Test | + | + | + | | | | | | | |
| Carbohydrate | Benedicts Test | + | + | + | | | | | | | |
| Glycosides | Keller Killiani Test | - | - | - | | | | | | | |
| Saponins | Foam Test | +++ | +++ | +++ | | | | | | | |
| Proteins | Biuret Test | - | - | - | | | | | | | |
| Phenols and Tannins | Ferric Chloride test | + | + | + | | | | | | | |
| Phytosterols | Salkowski Test | - | - | - | | | | | | | |

except Cadmium as given in Table 4. In the same way, the SDP of C. odorata was assessed for its microbial load with special reference to E.coli, Salmonella and fungi and was found to have negligible count when grown in controlled environment as illustrated in Fig. 1. Accelerated shelf life testing of SDP was undertaken to do comparative analysis of SDP after storage at 37°C on

| Table 2 — Fluoroscence analysis of SDP of C. odorata | | | | | | | | | | |
|---|---|--------------------|-------------|-------------------|-----------------|--------------|---------------|-----------------------|---|--|
| Tests | | | Visible | | U١ | UV (354 nm) | | | | |
| Powder+distilled water | | | | brown | | | black | | | |
| Powder+acetone | | | C | dark green | | | pink | | | |
| Powder+ethanol | | C | dark green | | pi | pinkish red | | | | |
| Powder+benzene | | C | olive green | | | pinkish red | | | | |
| Powder+cl | Powder+chloroform | | (| dark green | | | brown | | | |
| Powder+m | nethanol | | | (| dark green | | | green | | |
| Powder+petroleum ether | | | | yellow | | | orange | | | |
| Powder+glacial acetic acid | | | C | olive green | | | greenish blue | | | |
| Powder+su | ulphuric ad | cid | | rec | reddish brown | | | green | | |
| Powder+nitric acid | | | orange | | | brown | | | | |
| Powder+HCl | | | green | | | green | | | | |
| Powder+5 | Powder+5% FeCl ₃ yellowish gre | | n greer | n d | ark green | | | | | |
| Powder+5% I ₂ | | | brown | | | gray | | | | |
| Powder+picric acid | | gre | enish | yellow | gre | enish blacl | k | | | |
| Powder+1 | N NaOH | | | bro | brownish yellow | | v | orange | | |
| Powder+1 | N NaOH- | ⊦meth | nano | l yel | yellowish green | | ı | brown | | |
| Table 3 — Physicochemical analysis of SDP of <i>C</i> odorata | | | | | | | | | | |
| Parame | tors | 1 st Bo | atch | 2 nd B | atch | 3rd B | atch | 1 th Batch | | |
| I arano | ntont | 75.0 | 1.0/ | 2 D | 004 | 72.0 | 20/ | 74 0004 | | |
| Moisture Content 75.01 | | 1 70 | 705 | 77.49% 73.92 | | ∠ 70 0.0/ | 74.00% | | | |
| Extractive yield 16.00 | | J%0 04 | 20.3 | 28.30% 29 | | U%0 | 12 00% | | | |
| Spray Drieu | Tielu | 1.14 | · 70 | 0.79 | 1 70 20/ | 0.70 | 0 70 NO/ | 0.820/ | | |
| Total ash | | 12.00 | 50/ | 14.5 | 5%0 20/ | 12 2 | 70/ 70/ | 0.85% | | |
| 10tai asii Watar Saluh | la Ash | 12.0. |)%))/ | 14.3 | 0%0 10/ | 2.00 | / %0 | 15.15% | | |
| water Solub | A 1 | 4.43 | % | 3.4. | 3.41% 3.80% | | 1%0 10/ | 4.08% | | |
| Acid Soluble | e Asn | 1.02 | .% 4.07 | 4./4 | 2% | 3.8/ | % 50/ | 3.3/% | | |
| Sulphated A | sn | 16.74 | 4% | 1/.1 | 2% | 17.3 | 5% | 16.63% | | |
| Table 4 — Heavy Metal analysis of SDP of Chromolaena odorata | | | | | | | | | а | |
| | Permissi | ble | Cr | ude | CD | νD. | | | | |
| Parameters | limits | | pov | vder | SL | n n | In | ference | | |
| | ppm/(mg | /kg) | (mg | g/kg) | (mg/ | kg) | | | | |
| Arsenic | 5 | | < | 0.2 | <0 | .2 | Less | than limit | | |
| Chromium | 2 | | < | 0.5 | <0 | .5 | Less | than limit | | |
| Lead | 10 | | 2 | .93 | 1.3 | 35 | Less | than limit | | |
| Mercury | 0.2 | | < | 0.2 | <0 | .2 | Less | than limit | | |
| Cadmium | 0.3 | | 0 | .98 | 0.5 | 59 | More | e than limit | t | |
| Iron | - | | 104 | 48.4 | 298 | 3.6 | | | | |
| | | | | | | | | | _ | |



Mac Conkey Agar

Brilliant Green Agar

CYGA Agar

day 0 and at 54°C for 14 days. No significant deviations in peak shape were observed. Similarly, no significant deviations in peak shape were observed on 0, 3^{rd} , 6^{th} , 9^{th} , 12^{th} , 15^{th} and 18^{th} month time points by HPLC indicating it can be safely stored and used for the stipulated period without change in colour, odour and texture of SDP of *C. odorata*.

Spectral fingerprinting of SDP of C.odorata

The Fig. 2 showed the UV Visible spectra of SDP at 200 nm to 900 nm which was similar in every batch of collection. In Fig. 3A, the HPLC spectral image showed presence of small peaks and an unidentified peak at 3.4 retention time at 268 nm. Unlike the HPLC data, the HPTLC data showed multiple number of bands at 254, 366, after derivatization and at 560 nm, respectively as seen in Fig. 3B.

Figure 4 shows that the spray dried sample obtained from laboratory spray drier has uniformity in size distribution of particles which may suggest that the sample is not adulterated with mixed dimension particles.

Pharmacological hemostatic efficacy of C. odorata

Animal experimentation for liver laceration model was conducted by pretreatment of rats for 7 days at 500 mg/kg dose orally. This dose was selected based on the 90 days sub-acute oral toxicity study done as per OECD guidelines 408. The treatment has showed statistically significant reduction in the blood loss in dissected liver tissue by 54.30% (P < 0.05). Rats were humanely sacrificed after 72 hours as there was no mortality in test as well as the control groups. The amount of blood loss and number of gauges required to achieve bleeding hemostasis is illustrated in Fig. 5.



Fig. 2 — The UV visible spectra of the SDP of *Chromolaena* odorata at 200-900 nm



Fig. 3 — (A) HPLC spectral fingerprinting of SDP of *C. odorata*; and (B) HPTLC spectral fingerprinting of SDP of *C. odorata* with RF values







Fig. 5 — The graphs showing reduction of bleeding diathesis in rats treated with SDP of *Chromolaena odorata*

Animal experimentation for femoral artery injury model was performed in rabbits to monitor the duration of the blood flow from transacted artery after topical administration of SDP of *C. odorata* at the site of injury. The right limb of 3 rabbits served as control and the left limb of those 3 rabbits served as test. To avoid blood loss due to ambiguity between right and left limb, in the second set of experiment, the left limb of 3 rabbits served as control and right limb of those 3 rabbits served as test. The mean blood flow time in minutes was found to be 27 ± 5 min in control whereas it was reduced to 8 ± 3 min in test treated group indicating statistical significant (*P* <0.05) hemostatic effect of *C. odorata*.

Discussion

In a recent review published it is reported that 90% of the combat casualties are due to haemorrhage in mostly truncal, junctional and extremities of the body³³. In both civilian and military trauma, deaths due to haemorrhage occur in the first several hours after injury. Therefore, fast acting efficacious hemostatic agent is of utmost need to save lives³⁴. The latest trend is to provide handy and portable hemostatic products such as Combat Gauze in emergency medical services³⁵. It is always desirable that hemostatic agent should have high stability at extreme conditions and most importantly have the properties of being quick and effective, biodegradable, easily administrable, sterilizable and biocompatible³⁶.

Scientific reports suggest that for externally accessible injuries, robust research is done and biomaterials including glues, bandages, tamponades, tourniquets, dressings, and procoagulant powders have come up. In contrast, challenge lies with the treatment of internal non-compressible haemorrhage that depends on transfusion of whole blood or blood's hemostatic components like platelets, fibrinogen, and coagulation factors with many shortcomings³⁷.

In the present study, focus was to develop a hemostatic agent that will meet the above criteria and can be formulated for two routes of dosing (oral and at site of injury). Ample evidence suggests that patch products enable delivery of pro-coagulants to defined areas and chances of dilution and/or displacement is lesser³⁸. But before working on the aforementioned principles for developing biopharmaceutical hemostatic agent, some basic pharmocognostical aspects have to be studied. Due to the lack of

reproducible quality of herbal products, major concerns have developed on the value of herbal drugs which leads to impediments on their effective commercialization³⁹.

In the present study, phytochemical analysis was done for detecting the presence of bioactive components followed by fluorescence test which is known to exhibit various chemical constituents of the plants that either shows fluorescence in visible or ultraviolet light⁴⁰.

For determining the physicochemical parameters, extractive values are important parameter as it determines adulterated materials and solubility of the chemical constituents. Ash value also gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug⁴¹. Similarly, presence of increased heavy metal content in herbal materials leads to decreasing quality of the herbal product, which directly or indirectly becomes responsible for causing several adverse effects⁴². *C. odorata* was found to have more iron in leaves and all other harmful heavy metals were within the permissible limits established by WHO.

Another important parameter in maintaining the quality of herbal drug is microbial analysis of the extract which indicates that the powdered drug is free from specific pathogens such as Escherichia coli, Salmonella species, Pseudomonas aeruginosa and Staphylococcus aureus including Fungi⁴³. In the present study, the total microbial load was within the permissible limits. Nowadays, spectral analysis is gaining importance as a tool for fingerprinting and identification of wide range of substances in less time⁴⁴. Therefore, C. odorata was collected in four seasons and were analyzed by UV Visible spectrum, HPLC and HPTLC fingerprinting methods to identify the seasonal variations if any. It is important that spray dried powder (SDP) of C. odorata remain physically stable throughout their shelf life, and for that no or a minimal change in the particle size distribution is necessary. The mean droplet size was found to be around 951 nm with a polydispersity index of 0.225 indicating good uniformity amongst the samples. The Baseline index (B. I) was seen to be 98.66% for this trial which is relative to the accuracy of the data.

The future perspective of the SDP is to formulate it into drug loaded/coated bandages that will help as hemostatic agent in perioperative bleeding. Therefore, the pharmacognostic and physicochemical characters is vital source of information and provided suitable standards to determine the quality of the SDP of the leaf extract of *C. odorata* for future investigations.

After standardization of the quality of C. odorata, emphasis was given on its role in hemostatc surgery model. Uncontrollable haemorrhage is a significant concern in veterinary and human surgery which occurs as a result of blunt or penetrating trauma and leads to increased morbidity and mortality⁴⁵. The liver is the most vessel-rich organ and does not have smooth muscle and has few collagen tissues for which vasoconstriction cannot occur and parenchymal stitches do not get benefited by the resistance created by collagen fibers⁴⁶. Presently, hemostatic strategies for traumatic and surgical bleeding includes: biologically derived materials like albumin, collagen, gelatin, polypeptides, keratin, chitosan, cellulose, and dextran; Synthetically derived materials, such as biologically mimetic adhesives, in situ forming sealants, direct activators and aggregators, and aluminosilicates, and intravenously administered hemostatic agents like coagulation factors, antifibrinolytic agents, and lyophilized or frozen platelets⁴⁷.

Internal or external wounds can be incision, laceration, puncture, abrasion, avulsions or amputation in nature. In most types of wounds, the blood vessels may rupture which include arteries, veins and capillaries. The venous and the capillary rupture does not cause much blood loss as compared to arterial rupture because oxygenated dark red blood oozes as spurts from broken arteries rather than steady flow in veins. This fact has motivated us to study a model of blood loss in femoral artery damage by placing pre-coated gauze with the proposed hemostatic agent SDP. In the present study, the SDP effectively controlled blood loss in the liver laceration model when SDP was administered orally and the reduction percent of the amount the blood loss in dissected liver tissue by 54.30%. The practical implementation of this oral therapy can be used for patients with acute coagulopathies and the ones who use blood thinning medications prior to pancreatobiliary or pancreaticoduodenectomy or any major or minor surgeries. Similarly, in the femoral artery damage models SDP was applied at the site of injury and it caused 70.36% reduction in blood loss as compared to control artery. Another important aspect due to which there is an inclination towards herbal

source of hemostatic agent is because the fibrinogen, thrombin and synthetic hemostatic material are costly and scarcely available⁴⁸. Therefore, it can be hypothesized that the present intervention can be used for sports injury as well as traumatic injuries to overcome blood loss to some extent.

Conclusion

Results of this study demonstrated the hemostatic efficacy of the spray dried powder (SDP) of the aqueous extract of Chromolaena odorata leaves by improved hemostasis in both, the surgical model of hepatic haemorrhage and the arterial damage. As evident from the results, a statistical significant reduction in blood loss proves its candidature for possible use as hemostatic agent that is non allergic, fast acting and with strong adherence to the site of injury. From this preliminary study, we can only hypothesize that oral delivery and/or application of SDP of C. odorata to bleeding hepatic and arterial tissue would result in decreased haemorrhage and improved post operational recovery/survival. The expected outcome from this preliminary study gives us motivation to work extensively on its active molecules isolation and warrant more elaborate study in future for developing biopharmaceuticals from C. odorata.

Acknowledgement

This work was supported by Biotechnology Ignition Grant-04, BIRAC, Government of India under Grant number: BIRAC/VENTURE0012/BIG-04/14, for the project title: "To develop a novel, cost-effective, nonallergic herbal formulation for management of spontaneous bleeding and allied complications in Multiple Coagulation Disorders." The authors are thankful to Dr. Mrs. VS. Keskar, Maarc Labs Pvt. Ltd. Pune, India for performing Ash Values, Crude Fibre and Heavy Metal Analysis of samples. We appreciate the support received from Miss. Edna Joseph, Assistant Lab Manager, at the DBT-BIRAC supported Venture Center BioIncubator at CSIR-NCL, Pune, India in performing the UV, HPLC and Particle size Analysis of the samples. Also, we thank Mr. Sampat Shinde, Method Development Analyst and Mrs. Anjali Katariya, Manager QC & QA at Reve Pharma Nashik for HPTLC analysis of samples. Further, we acknowledge Mr. Nitin Ghatpande, Ross Life Science Pvt. Ltd, Pune for Shelf Life Testing and team of APT Research Foundation for the help and support extended during animal studies.

Conflict of Interest

Authors declare no competing interests.

References

- 1 Norton R & Kobusingye O, Injuries. *N Engl J Med*, 368 (2013) 1723.
- 2 Peng HT, Hemostatic agents for prehospital haemorrhage control: a narrative review. *Military Med Res*, 7 (2020) 1.
- 3 Khoshmohabat H, Paydar S, Makarem A, Karami MY, Dastgheib N, Zahraei SAH, Rezaei R & Nezhad GSM, A review of the application of cellulose hemostatic agent on trauma injuries. *Open Access Emerg Med*, 11 (2019) 171.
- 4 Suntar I, The medicinal value of Asteraceae family plants in terms of wound healing activity. *FABAD J Pharm Sci*, 39 (2014) 21.
- 5 Owoyele VB, Adediji JO & Soladoye AO, Anti-inflammatory activity of aqueous leaf extract of *Chromolaena odorata*. *Inflammopharmacol*, 13 (2005) 479.
- 6 Umukoro S & Ashrobi RB, Evaluation of Anti-inflammatory and membrane stabilizing effects of *Eupatorium odoratum*. *Int J Pharmacol*, 2 (2006) 509.
- 7 Phan TT, Hughes MA, Cherry GW, Le TT & Pham HM, An aqueous extract of the leaves of *Chromolaena odorata* (formerly *Eupatorium odoratum*) (Eupolin) inhibits hydrated collagen lattice contraction by normal human fibroblasts. *J Altern Complement Med*, 2 (1996) 335.
- 8 Pandith H, Thongpraditchote S, Wongkrajang Y & Gritsanapan W, *In vivo* and *in vitro* hemostatic activity of *Chromolaena odorata* leaf extract. *Pharmaceut Biol*, 50 (2012) 1073.
- 9 Vijayaraghavan K, Rajkumar J, Bukhari SNA, Al-Sayed B & Sayed MA, *Chromolaena odorata*: A neglected weed with a wide spectrum of pharmacological activities (Review). *Mol Med Rep*, 15 (2017) 1007.
- 10 Omokhua AG, McGaw LJ, Finnie JF & Staden JV, *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. *J Ethnopharmacol*, 183 (2016) 112.
- 11 Vijayaraghavan K, Rajkumar J & Seyed MA, Efficacy of *Chromolaena odorata* leaf extracts for the healing of rat excision wounds. *Vet Med*, 62 (2017) 565.
- 12 Henshaw UO, Ifeyinwa MO, Emmanuel KU & Item JA, Some hematological parameters of Wistar rats treated with *Chromolaena odorata* leaf extracts. *J Biol Res*, 90 (2017) 51.
- 13 Panyaphu K, On TV, Sirisa-Ard P, Srisa-Nga P, Chansakaow S & Nathakarnkitkul S, Medicinal plants of the Mien (Yao) in Northern Thailand and their potential value in the primary healthcare of postpartum women. *J Ethnopharmacol*, 135 (2011) 226.
- 14 Stanley A, Imeobong JI, Asemota EA, Obioma OO, Okpokam DC & Agu VO, Effect of ethanolic extract of *Chromolaena odorata* on the kidneys and intestines of healthy albino rats. *Int Med Res*, 6 (2017) 292.
- 15 Nandyala VNASR & Chandrasekhar KB, Pharmacognostical standardization & phytochemical evaluation of *Alphonsea* sclerocarpa Thwaites Bark & Leaves. *Pharmacogn J*, 9 (2017) 196.
- 16 Ankad GM, Konakeri RT, Hegde HV & Roy S, Variation in pharmacognostic characters and polyphenolic contents

among four species of medicinal plants from the Genus Spermacoce (Rubiaceae). *Indian J Pharm Sci*, 77 (2015) 446.

- 17 Pitakpawasuthi Y, Palanuvej C & Ruangrungsi N, Microscopic leaf constant numbers of *Chromolaena odorata* in Thailand. *Pharmacog J*, 10 (2018) 95.
- 18 Paul T, Das B, Ingale SP, Killedar N & Apte KG, Oral intake of polyphenols of *Chromolaena odorata*: A perspective in peptic ulcer, thrombocytopenia, and heparin-induced bleeding diathesis in rodent model. *Phcog Res*, 10 (2018) 426.
- 19 Satish P & Sunita K, Antimalarial efficacy of *Pongamia pinnata* (L) Pierre against *Plasmodium falciparum* (3D7 strain) and *Plasmodium berghei* (ANKA). *BMC Complement Altern Med*, 17 (2017) 1.
- 20 Tan SP, Kha TC, Parks SE, Stathopoulos CE & Roach PD, Effects of the spray-drying temperatures on the physiochemical properties of an encapsulated bitter melon aqueous extract powder. *Powder Technol*, 281 (2015) 65.
- 21 Subba, Rai AR & Kumar S, Phytochemical screening, physico-chemical analysis and antioxidant activity of some ethnomedicinal plants from Sikkim Himalaya. *Indian J Nat Prod Resour*, 9 (2018) 235.
- 22 Gupta PC, Sharma N & Rao CV, Pharmacognostic studies of the leaves and stem of *Careya arborea* Roxb. *Asian Pac J Trop Biomed*, 2 (2012) 404.
- 23 Mushtaq A, Akbar S, Zargar MA, Wali AF, Malik AH, Dar MY, Hamid R & Ganai BA, Phytochemical screening, physicochemical properties, acute toxicity testing and screening of hypoglycaemic activity of extracts of *Eremurus himalaicus* Baker. in normoglycaemic wistar strain albino rats. *Biomed Res Int*, 2014 (2014) 1.
- 24 Maranhao TA, Silva JSA, Andrade RM, Bascunan VLAF, Oliveira FJS & Curtius AJ, Determination of As and Hg in acetic acid extract by vapor generation coupled to atomic spectrometry for solid waste classification. *Microchem J*, 106 (2013) 139.
- 25 Noor R, Huda N, Rahman F, Bashar T & Munshi SK, Microbial contamination in herbal medicines available in Bangladesh. *Bangladesh Med Res Counc Bull*, 39 (2013) 124.
- 26 Aksoy A, Yavuz O, Guvenc D & Das YK, Effects of the high temperature on the physical and chemical properties of some public health insecticide formulations. *Asian J Chem*, 19 (2007) 5681.
- 27 Muthukumar H, Palanirajan SK, Shanmugam MK & Gummadi SN, Plant extract mediated synthesis enhanced the functional properties of silver ferrite nanoparticles over chemical mediated synthesis. *Biotechnol Rep*, 26 (2020) e00469.
- 28 Senguttuvan J & Subramaniam P, HPTLC Fingerprints of various secondary metabolites in the traditional medicinal herb *Hypochaeris radicata* L. J Bot, 2016 (2016) 1.
- 29 Davis JA, Mouse and rat anesthesia and analgesia. *Curr Protocols in Neuro Sci*, 42 (2008) A4B21.
- 30 Morgan CE, Prakash VS, Vercammen JM, Pritts T & Kibbe MR, Development and validation of 4 different rat model of uncontrolled hemorrhage. *JAMA Surg*, 150 (2015) 316.
- 31 Koura Y, Fukushima A, Nishino K, Ishida W, Nakakuki T, Sento M, Yamazoe K, Yamaguchi T, Misyoshi T & Ueno H, Inflammatory reaction following cataract surgery and

implantation of acrylic intraocular lens in rabbits with endotoxin-induced uveitis. *Eye*, 20 (2006) 606.

- 32 Ulus AT, Turan NN, Ozyalçın S, Aydog G, Ulus F, Goker H & Haznedaroglu IC, Surgical and histopathological effects of topical Ankaferd® hemostat on major arterial vessel injury related to elevated intra-arterial blood pressure. *Turk J Hematol*, 28 (2011) 206.
- 33 Peng HT, Hemostatic agents for prehospital hemorrhage control: a narrative review. *Military Med Res*, 7 (2020) 1.
- 34 Stephens CT, Gumbert S & Holcomb JB, Trauma-associated bleeding: management of massive transfusion. *Curr Opin Anesthesiol*, 29 (2016) 250.
- 35 Sigal A, Martin A & Ong A, Availability and use of hemostatic agents in prehospital trauma patients in Pennsylvania translation from the military to the civilian setting. *Open Access Emerg Med*, 9 (2017) 47.
- 36 Khosmohabat H, Paydar S, Kazemi HM & Dalfardi B, Overview of agents used for emergency hemostasis. *Trauma Mon*, 21 (2016) 1.
- 37 Hickman DA, Pawlowski CL, Sekhon UDS, Marks J & Sengupta A, Biomaterials and advanced technologies for hemostatic management of bleeding. *Adv Mater*, 30 (2018) 1700859.
- 38 Navarro A & Brooks A, Use of local pro-coagulant haemostatic agents for intra-cavity control of haemorrhage after trauma. *Eur J Trauma Emerg Surg*, 41 (2015) 493.
- 39 Subha TS, Gnanamani A & Mandal AB, Pharmacognostic evaluation of *Acorus calamus* L. *Pharmacogn J*, 3 (2011) 24.
- 40 Bora NS, Kakoti BB, Yadav P, Gogoi B & Borah S, Phytophysicochemical, acute and subacute toxicity studies of

Garcinia lanceifolia Roxb.-A rare ethnomedicinal plant of Assam, India. *Indian J Nat Prod Res*, 8 (2017) 360.

- 41 Kishor kumar V & Lalitha KG, Pharmacognostical studies on the root of *Anacyclus pyrethrum* DC. *Indian J Nat Prod Res*, 3 (2012) 518.
- 42 Singh R, Gautam N, Mishra A & Gupta R, Heavy metals and living systems: an overview. *Ind J Pharmacol*, 43 (2011) 253.
- 43 Ghosh D, Mondal S & Ramakrishna K, Pharmacobotanical, physicochemical and phytochemical characterisation of a rare salt-secreting mangrove *Aegialitis rotundifolia* Roxb., (Plumbaginaceae) leaves: A comprehensive pharmacognostical study. *S Afr J Bot*, 113 (2017) 212.
- 44 Mukhi S, Bose A, Panda P & Rao MM, Pharmacognostic, physicochemical and chromatographic characterization of Samasharkara Churna. J Ayur Int Med, 7 (2016) 88.
- 45 Rosselli DD, Brainard BM & Schmiedt CW, Efficacy of a topical bovine-derived thrombin solution as a hemostatic agent in a rodent model of hepatic injury. *Can J Vet Res*, 79 (2015) 303.
- 46 Aysan E, Bektas H, Ersoz F, Sari S, Kaygusuz A & Huq GE, Ability of the Ankaferd blood stopper® to prevent parenchymal bleeding in an experimental hepatic trauma model. *Int J Clin Exp Med*, 3 (2010) 186.
- 47 Behrens AM, Sikorski MJ & Kofinas P, Hemostatic strategies for traumatic and surgical bleeding. J Biomed Mater Res A, 102 (2014) 4182.
- 48 Bhatnagar M, Parwani L, Sharma V, Ganguli J & Bhatnagar A, Hemostatic, antibacterial biopolymers from *Acacia arabica* (Lam.) Willd. And *Moringa oleifera* (Lam.) as potential wound dressing materials. *Ind J Exp Biol*, 51 (2013) 804.