Comparative hemostatic activity of sweet potato (*Ipomea batatas Linn.*) leaf aqueous and ethanolic crude extracts in New Zealand white rabbits

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Abstract - Coagulation disorders are among the most commonly presented clinical symptoms in most healthcare scenarios. These can be due to platelet dysfunction commonly acquired due to medication, prescribed or over-the-counter and may underlie an increased risk of bleeding. To alleviate such anomaly, a common countermeasure is the correction of the patient's platelet count and the incessant monitoring of various clotting factors. Recent studies, anecdotal accounts, and folkloric practices suggest the activities of sweet potato (Ipomea batatas L.) decoction on platelet generation and hemostasis. The present study was therefore designed to scientifically test and compare the hemostatic activity of sweet potato leaf aqueous and ethanolic crude extracts primarily on platelet count, clotting time and prothrombrin time using New Zealand White rabbits as models after 10 days of treatment. Both extracts at 150 mg/kg have significantly increased ($p < 0.05$) the platelet count and significantly decreased ($p < 0.05$) the clotting time and prothrombin time. It was revealed that both crude leaf extracts of Ipomea batatas L. have a good hemostatic activity with aqueous extract the most promising one based on the total mean value and possibly interact with both intrinsic and extrinsic pathways. The results obtained could be of value in the prevention of bleeding tendencies and also provide a rationale for ethnotherapeutic indication of

the plant. Moreover, absence of adverse effects and death following oral administration of both extracts at doses of 300, 600, and 2000 mg/kg in rabbits suggests that the plant preparations are acutely non-toxic.

Keywords- Ipomea batatas, coagulation disorders, hemostasis, platelet, clotting time, prothrombin time

INTRODUCTION

Platelets play a key role in the initiation and propagation of the hemostatic response (Elchoufani et al., 2008). Decrease in platelet count is known as thrombocytopenia (Zhou and Schmaier, 2005). When platelets and clotting factors circulate in an inactive form, blood flows freely through the vascular system (Kamal et al., 2007). Once damage is detected, platelets respond rapidly through a cascade of events or steps which sequentially involves their adherence to sites of vessel injury, activation of internal signaling pathways, aggregation to form plugs, and acceleration and localization of the coagulation reactions leading to the formation of a hemostatic plug stabilized with a fibrin meshwork, thus preventing hemorrhage (Elchoufani et al., 2008; Zhou and

Schmaier, 2005). However, if any of the pathways is defective, platelet function may be impaired. This platelet dysfunction may be acquired which is the most common due to medication, prescribed or over-the-counter or may be congenital which is about 10% of cases in which patients have prolonged bleeding (Zhou and Schmaier, 2005).

Such disorders and many other diseases have been known to be treated with herbal remedies. It is considered to be the oldest remedies known to mankind (Kumar et al., 2010a; Kumar et al., 2010c; Parekh and Chanda, 2007). Adedapo et al., (2009) as cited by Abubakar in 2009a, revealed that these practices have been passed on from generation to generation. According to Tripathi and Tripathi (2003) as cited by Odesanmi et al. in 2009, medicinal plants are vital sources of life saving drugs for the majority of the world's population. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Rajeh et al., 2010).

The plant Ipomoea batatas is a crop plant commonly called sweet potato (Udoh et al., 2010). It is a specie of the morning glory family Convolvulaceae which is widely grown in tropical, subtropical, and warm temperate regions (Gonzales et al., 2008; Srisuwan et al., 2006) and is tolerant of many diseases and pests (Islam et al., 2002). It has smooth, lightly moderate green leaves sometimes with a considerable amount of purple pigmentation especially along its veins (Antia et al., 2006) and the edible tuberous root is long and tapered with colour ranges from red, purple, brown to white (Udoh et al., 2010). This plant has also different aromatic smell that varies with the variety (Kwach et al., 2010). According to Scott et al. (2000) as cited by Wireko-Manu et al. in 2010, more than two billion people in Asia, Africa, and Latin America will depend on this crop for food, feed and income by 2020.

Sweet potato is a major world crop and considered to be one of the most versatile yet under-exploited crop specie in the world (Gonzales et al., 2008). It is a good vegetable source for consumption of man (Tairo et al., 2008; Antia et al., 2006). The leaves can contribute significantly to the nutrient requirements of humans (Oduro et al., 2008) because they contain an appreciable amount of nutrients, and are rich in vitamins and minerals such as vitamin A, B2, B6, C, and E, magnesium, phosphorus, calcium, iron, sodium, potassium, manganese, copper, and zinc. It contains low levels of toxicants whose value can be reduced by cooking (Saritha et al., 2010; Udoh et al., 2010; Adewolu et al., 2008; Antia et al., 2006). Further study revealed that the leaves also contain anthocyanin and polyphenolic compound (Udoh et al., 2010), protein and crude fibre which are important for addressing deficiency diseases and colon diseases (Abubakar et al., 2010b). The leaves are also used in the treatment of hookworm, hemorrhagic and abscesses, while the tuber is used for the treatment of asthma (Udoh et al., 2010). The leaves also possess anti-hyperglycemic activity (Li et al., 2009) and used in the treatment of diabetes (Islam, 2006), platelet-generating potential (Osime et al., 2008), and the edible plant parts have a high amount of dietary fiber which contains abundant soluble components, pectin or hemicellulose, suggesting that physiological functions may be higher in sweetpotato fibers than in other crops (Yoshimoto et al., 2005). The leaf meal has

good potential for use as one of the protein sources in Tilapia zilli diet (Adewolu et al., 2008) and even the concoction of non-alcoholic beverage (Wireko-Manu et al., 2010). It is also a suitable substrate for the efficient production of biofuel ethanol using S. cerevisiae (Saritha et al., 2010) and has potential for other uses such as fuel and animal feed in which the leaves and vines are used for feeding rabbits, sheep, goats and cattle (Okungbowa and Osagie, 2009). Hong et al. (2003) as cited by Adewolu et al. in 2008, reported that the leaves of sweet potato can be harvested many times throughout the year.

In summary, sweet potato shows much promise as therapeutic agent that raises platelet population on the grounds of recent studies (Osime et al., 2008) which are undoubtedly beneficial to man. It also contains tannins that arrest bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs (Bamidele et al., 2010).

Taking these into account, the researchers decided to perform a comparative study to assess which plant's extracts will generate a stronger effect on hemostasis while utilizing rabbits that are well established as laboratory animal (Carter, 2007) and have been used in a large number of studies since the expansion of basic human knowledge in the fields of physiology, biochemistry and molecular biology and the consequent therapeutic material, can be combined with the use of laboratory animals in almost all of man's research efforts. Among all strains of rabbits, the New Zealand is the most used in the laboratory (Yanni, 2004).

MATERIALS AND METHODS

Plant Materials

The fresh, fully grown, intact leaves of I. batatas (Li et al., 2009) were used in the study. The plant was grown last May in Alangilan, Batangas and a voucher specimen was submitted to the Herbarium of University of Santo Tomas, España, Manila for authentication. The leaves were collected in the month of June-August, 2011 and were washed thoroughly in running tap water to remove debris (Udoh et al., 2010; Okungbowa and Osagie, 2009). Then, the washed materials were air-dried under shade for about one week (Basma et al., 2011; Ogbulie et al., 2007) and later oven-dried at 180°C for 20 minutes. The dried materials were grinded into fine powder using a clean and dry electrical grinder. The powder was stored in a clean labelled airtight bottle until needed for use (Udoh et al., 2010; Antia et al., 2006).

Preparation of Crude Extracts

The powdered plant materials were extracted by the researchers using two solvents namely distilled water and 95% ethyl alcohol at the experimental laboratory of Lyceum of the Philippines University–Batangas. However, due to small amount of crude ethanolic extract obtained, the researcher decided to have the said extract prepared at Department of Science and Technology.

Aqueous Crude Extraction

A weighed quantity of the leaf-powder sample (10 g) was dissolved in 100 ml of distilled water in a conical flask. The solution of the plant sample was covered and was subjected to shaking motion for about 24 hours at room temperature. The solution was filtered using muslin cloth and Whatman filter paper (Singh et al., 2011; Rafat et al., 2010; Ogbulie et al., 2007). The aqueous extract solution was evaporated using petri dish in ambient atmosphere at 45°C until the sample produced a semisolid mass and was stored in refrigerator below 10°C until its use (Kumar et al., 2010b; Odesanmi et al., 2009). The same procedure was employed for ethanolic extraction.

Ethanolic Crude Extraction

The powdered sweet potato leaves about 605 grams were soaked in 2.5 L of 95% ethyl alcohol for 48 hours. The filtrate obtained was concentrated using rotary evaporator at 60°C for 1 hour. The concentrated extract was further evaporated using water bath at 60°C to obtain a semi-solid extract. The extract was stored in airtight amber bottle in refrigerator below 10°C until its use. The extraction proper was performed at the Department of Industrial Technology in the Department of Science and Technology in Parañaque City, Manila.

The percent yield was calculated using the expression: Yield (%) = (Weight of Dried Extract/Weight of Sample Used) x 100 (Odesanmi et al., 2009).

For administration, a weighed quantity of the extract was reconstituted in 3 ml of NSS.

Test Animals

Fourty healthy New Zealand white rabbits of both sexes weighing 1.4 ±.4 kg (3-month old) were used in the study. The test animals (1-month old) were obtained from Lipa City, Batangas. They were housed in mesh wire cages at a temperature of 25 ± 2°C and was observed under a 12-h light/dark periodicity in a well ventilated room. They were fed with standard rabbit pellets every morning and afternoon and with clean water ad libitum. The rabbits were divided into 2 groups; the first group for determination of selected hemostatic parameters and the second group for acute toxicity. They were placed in different cages (2 rabbits of same sex in each cage) for proper identification. The rabbits were allowed to acclimatize for 1 month and were fed until the desired weight was obtained prior to the experiment (Ghasi et al., 2011; Mikail, 2010; Tijani et al., 2010; Odesanmi et al., 2009). Ethical approval for the use of laboratory animals was secured from the Bureau of Animal Industry (BAI).

Design of Experiment

Platelet count was obtained upon gaining the desired weight to have a baseline value before the administration of the extracts of Ipomea batatas.

A total of 30 rabbits were able to be utilized in the study due to mortality during acclimatization. Body weight change was obtained before and after the study.

First Group (for hemostatic parameters determination)

i.) Group 1 (2 rabbits)– 3 ml normal saline(Bamidele et al., 2010)
ii.) Group 2 (4 rabbits)– 150 mg/kg *Ipomea batatas* aqueous crude extract
iii.) Group 3 (4 rabbits)– 150 mg/kg *Ipomea batatas* ethanolic crude extract

(Ghasi et al., 2011)

The extracts were orally administered with the aid of different plastic syringe and received the above treatment for 10 days, respectively. After 24 hours of the last treatment, the rabbits were subjected to analysis (Udoh et al., 2010; Odesanmi et al., 2009).

Second Group (acute toxicity)

i.) Group 1 (2 rabbits) – 3 ml distilled water
ii.) Group 2 (3 rabbits) – 300 mg/kg *I. batatas* aqueous crude extract
iii.) Group 3 (3 rabbits) – 300 mg/kg *I. batatas* ethanolic crude extract
iv.) Group 4 (4 rabbits) – 600 mg/kg *I. batatas* aqueous crude extract
v.) Group 5 (4 rabbits) – 600 mg/kg *I. batatas* ethanolic crude extract
vi.) Group 6 (2 rabbits) – 2000 mg/kg *I. batatas* aqueous crude extract
vii.) Group 7 (2 rabbits) – 2000 mg/kg *I. batatas* ethanolic crude extract

The rabbits were also treated orally and were observed for signs of toxicity like behavioural changes (which include but not limited to paw-licking, salivation, stretching, lacrimation, diarrhea, lethargy, sleep, convulsion, and coma) and mortality for the first 4 critical hours and thereafter for 72 hours (Mikail, 2010; Tijani et al., 2010).

Sample Analysis

Method of Blood Collection

The hair/fur around the site of collection was shaved and cleaned with 70% isopropyl alcohol (Ghasi et al., 2011). Blood was collected via the marginal ear vein for platelet count and clotting time test and a cardiac puncture was done using 3cc TERUMO® syringe with 23Gx1" for Prothrombin time test (Rafat et al., 2010).

Platelet Count

Platelet count was determined using Unopette® System ("BD Unopette™ System Test", 2009) and was promptly counted using a modified Neubauer hemocytometer.

Determination of Clotting Time

The slide method of clotting time was used in the study. Blood was collected via the marginal ear vein through skin puncture. The first drop of blood was wiped due to tissue fluid contamination. Then, the second drop of blood was placed on a clean slide and the timer was started upon contact of the blood to the slide. A clean needle was then passed through the blood every 30 seconds until a fibrin strand was observed. The time taken for fibrin strand to form was noted (Brown et al., 1993).

Determination of Prothrombin Time

Blood was collected into sample vials containing sodium citrate in the ratio 1:9 with the blood sample. The blood was centrifuged at 1000 g for 15 min to obtain platelet poor plasma. Neoplastine© reagent was placed in a water bath at 37°C; and 100 ul of test plasma was also placed in a test tube and prewarmed to 37°C in an incubator. A 200 ul of warmed Neoplastine© was then forcibly added to the test plasma and the stopwatch was started. The solution was continuously mixed with an applicator stick until a clot was observed and the time taken for clot to form was noted. Precaution was taken to perform test within 3 hours of blood collection since the labile factor deteriorates quickly at room temperature (Bamidele et al., 2010).

Statistical Analysis

All numerical data was evaluated statistically using Mann–Whitney U test (Mann–Whitney–Wilcoxon (MWW) or Wilcoxon rank-sum test). A value of $p < 0.05$ was considered significant.

RESULTS

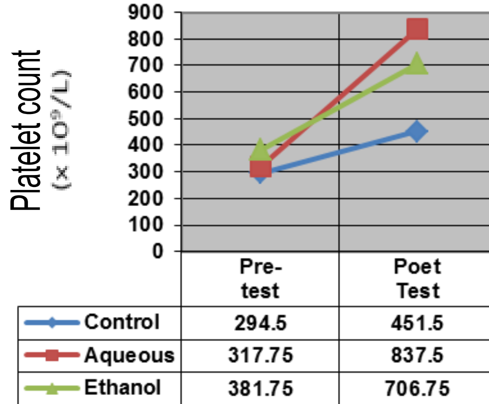
The aqueous crude extract of Ipomea batatas yielded 11.82% from 150 g of sample used and 7.56% for ethanolic crude extract from 78 g of sample used. The ethanolic extraction done at DOST yielded 2.187% from 605 g of sample used.

Platelet Count

The mean pre-test platelet count in the control group was 294.5 x 109/L, while those of Group II and Group III were 317.75 x 109/L and 381.75 x 109/L respectively. On the other hand, the mean post test platelet count in the control group was 451.5 x 109/L, whereas those of Group II and Group III were 837.5 x 109/L and 706.75 x 109/L respectively as shown in Figure 1. A demonstrable rise

of 519.75 x 109/L of platelets was exhibited by the aqueous extract and only 325 x 109/L for ethanolic extract.

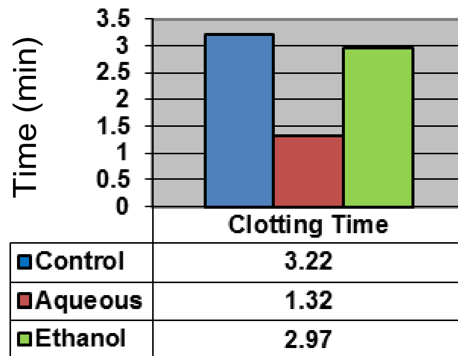
Figure 1
Effect of different groups on PlateletPopulation in New Zealand White Rabbits



Clotting Time

The mean post test clotting time in the control group was 3.22 minutes, while those of Group II and Group III were 1.32 and 2.97 minutes respectively as illustrated in Figure 2. Aqueous extract had a significant decrease of 1.9 minutes and only 0.25 minute for ethanolic extract.

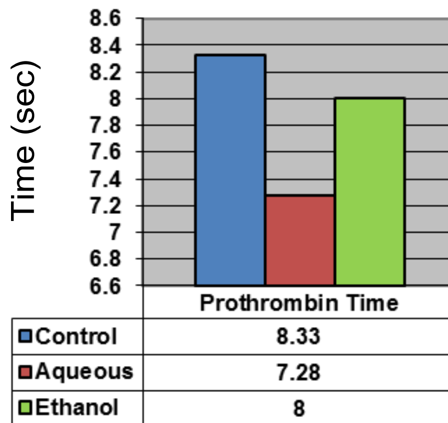
Figure 2
Effect of different groups on ClottingTime in New Zealand White Rabbits



Prothrombin Time

The mean post test prothrombin time in the control group was 8.33 seconds, whereas those of Group II and Group III were 7.28 and 8.00 seconds respectively as demonstrated in Figure 3. A significant decrease of 1.05 second was demonstrated by aqueous extract and only 0.33 second for ethanolic extract.

Figure 3
Effect of different groups on Prothrombin Time in New Zealand White Rabbits



Acute Toxicity

Acute toxicity studies revealed no obvious signs of toxicity or any significant changes in general behavior of the test animals exposed to both extracts. There was no mortality or any toxic reactions observed at the doses used in the study. All the test animals were alive, healthy, and active throughout the observation period.

DISCUSSION

The present study was carried out to assess the activities of Ipomea batatas aqueous and ethanolic crude extracts on the haemostatic mechanism primarily on platelet count, clotting time, and prothrombin time in New Zealand White rabbits to compare and contrast the actions of the said extracts. These indices are measure of blood coagulation; clotting time measures the intrinsic pathway while the prothrombin time measures the extrinsic pathway (Bamidele et al., 2010).

The results of the present study have proven that both of the extracts, particularly aqueous crude extract, have considerable effect on the selected hemostatic parameters by significantly increasing the platelet population and significantly decreasing the clotting time and prothrombin time. This result concerning the platelet generation agree with the reported findings of Osime et al. (2008) with regards to the use of aqueous leaf extract of the plant.

Clotting time test is a qualitative measurement of factors involved in the intrinsic pathway. Thus, deficiency in the factors of the intrinsic pathway (VIII, IX, XI, XII) will affect the result. Conversely, prothrombin time is a screening test for the extrinsic clotting system, that is, factor VII. Nonetheless, both tests can also detect deficiencies of factors involved in the common pathway (V, X, prothrombin and fibrinogen) as cited by Bamidele et al. (2010). Therefore, from the results obtained, there was significant decrease in clotting time and prothrombin time by the extracts particularly aqueous extract, reflecting that there was an increase in one or more of the clotting factors involved in the coagulation pathway. The leaf of Ipomea batatas L. also contains tannins that arrest bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs (Bamidele et al., 2010).

Table 1
Statistical analysis between the two extracts and the control group

Var 1	Var 2	z-value	Probability Value	Significance	Decision
Platelet Count					
Control	Aqueous	-1.019	0.308	Not Significant	Fail to Rejct Ho
Control	Ethanolic	-1.698	0.089	Not Significant	Fail to Rejct Ho
Clotting Time					
Control	Aqueous	-1.892	0.064	Not Significant	Fail to Rejct Ho
Control	Ethanolic	0.00	1.00	Not Significant	Fail to Rejct Ho
Prothrombin Time					
Control	Aqueous	-1.879	-0.935	Not Significant	Fail to Rejct Ho
Control	Ethanolic	0.6	0.355	Not Significant	Fail to Rejct Ho

Table 2

Statistical analysis between aqueous & ethanolic crude extract of *Ipomea batatas* L.

Var 1	**Var 2**	**z-value**	**Probability Value**	**Significance**
Aqueous extract		-2.323	0.029	Significant
	Ethanolic extract	-2.309	0.021	Significant

Statistically, it has been noted that there is no significant difference between the effect of aqueous and ethanolic crude extracts of Ipomea batatas at $p < 0.05$ level of significance (Table 1). Nonetheless, both of the extracts' hemostatic activities were significant at the said value (Table 2).

On the other hand, the normal control group (0.95% NSS) has shown a demonstrable raise in the platelet count, nevertheless, it represents a similar deviation of value, as seen in humans.

Moreover, absence of adverse effects and death following oral administration of aqueous and ethanolic crude extracts at doses of 300 to 2000 mg/kg in rabbits suggests that the plant preparations are acutely non-toxic. It has been observed as well that there was slight increase in body weight of rabbits after thestudy indicating that the treatment did not adversely affect the health status of the rabbits.

The researchers pursued on this study due to the scarcity of published research regarding the potentials of Ipomea batatas on the hemostatic mechanism.

CONCLUSION

Both extracts of Ipomea batatas have significant hemostatic activities with aqueous extract, the most promising one based on the total average mean. It could be of value in the prevention of bleeding tendencies. The results obtained from the study also provide a rationale for ethnotherapeutic indication of the plant.

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