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Comparative Effect of Different Saponin Extracts of Tomato on Some Blood Parameters

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Abstract: ***The study was done to evaluate the effect of various extracts i.e. fresh, lyophilized and purified saponin of tomato (Lycopersicon esculentum) on emulsification and foaming index, osmotic fragility, , super oxide dismutase and catalase in cardiovascular patients' blood samples. Among the studied extracts, fresh tomato extract treatment showed the highest haemolytic effect in comparison to normal blood. Cardiovascular patients' blood showed more superoxide dismutase and catalase activities as compared to normal blood. Further purified saponin was found to be more effective than fresh aqueous and lyophilized extracts of saponin.***

Keywords: *Lycopersicon esculentum*, lyophilised extract, Fh, E_{24}, OF, catalase and SOD.

1. Introduction

Saponins, a class of natural surfactants, are sterol or triterpene glycosides that are present naturally in a wide variety of plants. They have detergent or surfactant properties because it contains both water - soluble (*e.g.* sugars) and fat- soluble (either a steroid or triterpenoid structure) components. Their ability to survive under adverse conditions makes them extremely valuable for human health.

Saponins containing plants often display a creamy, even foamy, texture that distinguishes them from other plant. They are added to shampoos, liquid detergent, toothpastes and beverages as emulsifier and long lasting foaming agent. Various medicinal properties such as anti-inflammatory, antimicrobial, anthelmintic, antidermatophytic, and cytotoxic activities have been attributed to the saponins of plants **(Francis *et al.*, 2002).**

The steroidal saponins are mainly found in monocotyledons whereas triterpene saponins are predominantly present in dicotyledons **(Sparg *et al.*, 2004).** Saponins are also found in the botanical family Sapindaceae, with its defining genus Sapindus (soapberry or soapnut), and in the closely related families Aceraceae (maples) and Hippocastanaceae (horse chestnuts). It is also found heavily in *Gynostemma pentaphyllum* (genus *Gynostemma*, family *Cucurbitaceae*) in a form called gypenosides, and ginseng (genus *Panax*, family *Araliaceae*) in a form called ginsenosides. Within these families, this class of chemical compounds are found in various parts of the plant *i.e.* leaves, stems, roots, bulbs, blossom and fruit. Commercial formulations of plant-derived saponins *e.g.,* from the soap bark tree, Quillaja saponaria, and from other sources are available *via* controlled manufacturing processes, which make them of use as chemical and biomedical reagents. The present study was taken up to evaluate the effect of different extracts of saponins on some physico– and biochemical parameters in the blood of cardiovascular patients'.

2. Materials & Methods

2.1 Preparation of fruit extracts

The three forms of tomato fruit samples *i.e.* lyophilised, fresh and purified forms (0.2 to 1.0 ml) were taken in test tubes and volume in each tube was adjusted to 5 ml by adding double distilled water followed by addition of 1 ml of respective blood sample. The final concentration of saponin was 8µg/100 ml.

2.2 Preparation of red blood cells suspension

Blood was collected from Pathology Laboratory**,** Swaroop Rani Medical College, Allahabad. The red cells suspension was prepared by the method of **Gould *et al.* (2008).** Human blood collected from healthy individuals (controls) was added to four tubes containing anticoagulant and centrifuged at 3000 rpm for 10 minutes. Plasma and buffy coat were removed and the erythrocytes were washed three times using Mcllvaine's buffer (pH 7.0) five times of their volume. Afterwards, by adding Mcllvaine's buffer, an erythrocyte suspension was prepared and kept at 4°C for further experiments.

2.3 Determination of Emulsification Index (E_{24})

The emulsification index was assayed by the method of **Carrillo *et al.* (1986)**. The emulsion stability was determined after 24 hours by using the formulae given:

E_{24} = Width of emulsified layer/Total height of the liquid or column

2.4 Estimation of foaming index (Fh)

The foam stability index was assayed by the method of **Noudeh (2008).** Fh was measured as foam height in graduated cylinder as given here.

Fh = Foam height at zero minute/Foam height after 5 minutes

2.5 Estimation of osmotic fragility (OF) in blood

The OF was assayed by the method of **Hitoshi & Hiroshi (2005).** The % haemolysis was calculated by using the following formula.

% Haemolysis = Change in OD x 100/maximum change in OD

2.6 Assay of superoxide dismutase

The superoxide dismutase activity was assayed by the pyrogallol auto oxidation method of **Marklund & Marklund (1994)**.The protein content in each sample was estimated by Lowry's method. The OD was read at 412 nm for 3 minutes at 30 seconds interval. The specific activity was calculated by the formulae:

$$\text{Specific activity} = \frac{(\Delta OD/\text{min.REF} - OD/\text{min.}) \times \text{reaction volume}}{\text{REF}/2 \times \text{sample volume} \times \text{protein volume (mg/ml)}}$$

2.7 Assay of catalase

The catalase activity was assayed by the method of **Beers & Sizer (1952).** The absorbance was measured at 240nm for time span of 3 mins every 30 sec interval against phosphate buffer (0.2 M,,pH 7.0) as blank. The catalase units were calculated as given below:

Specific activity = ΔOD/ml /minute × 1000 ÷ 43.6 mg/ml protein.

3. Results

3.1 Emulsification index (E24)

The observations made on emulsification index are shown in **Table 1.** In general there seems no effect of any treatments on emulsification index. The results obtained are at par normal blood.

3.2 Foam stability index (Fh)

The observations made on foam stability index in test and normal groups have been depicted in T**able 1**. The foam stability index of the cardiovascular patient's blood when treated with fresh sample was found to be higher (0.857±0.034µg/ml) whereas the range was less (0.802±0.046µg/ml) in the fresh sample when treated with normal blood. Cardiovascular patient's blood when treated with lyophilized sample was found to be significantly higher (0.402±0.087µg/ml) and in case when lyophilized sample was treated with normal blood showed a lower range (0.283±0.108µg/ml). Significant lower level of Fh was shown in the cardiovascular patient's blood when treated with purified sample (0.442±0.132µg/ml) whereas, a higher level of Fh was observed in the normal blood when treated with purified sample (0.850±0.058µg/ml).

Table 1: Emulsification and foam stability indices of normal and patients' blood (sample blood)

Parameters	*Emulsification index (µg/ml)*	*Foam stability index((µg/ml)*
Control	0.41±0.13	0.19+0.12
Sample blood + fresh extract	0.540±0.019	0.857±0.034
Sample blood + lyophilized extract	0.535±0.019	0.402±0.087
Sample blood + purified extract	0.658±0.018	0.442±0.132
Normal blood + fresh extract	0.544±0.019	0.802±0.046
Normal blood+ lyophilized extract	0.536±0.010	0.283±0.108
Normal blood + purified extract	0.655±0.032	0.850±0.058

Note: Statistical differences were assayed by student's t-test. Values were expressed as Mean ± SD.

3.2 Osmotic fragility

The results of osmotic fragility in the fresh, lyophilized and purified extracts are shown in T**able 2.** The control showed a maximum osmotic fragility (21.290±24.654) in lyophilized extract. The normal sample when treated with lyophilized extract had least osmotic fragility (5.733±39.896) and the cardiovascular patients' blood when treated with lyophilized extract increased (6.968±6.204) in comparison to the normal blood.

Table 2: Osmotic fragility of normal and patients' blood

Parameter	*Osmotic fragility*		
	Fresh Extract	*Lyophilized extract*	*Purified extract*
%H (control)	4.883±4.904	21.290±24.65	4.883±4.904
%H (normal)	24.167±29.933	5.733±39.896	40.740±34.642
% H (test)	18.011±20.306	6.968±6.204	8.120±5.115

Note: Statistical differences were assayed by student's t-test. Values were expressed as Mean ± SD.

In case of fresh extract, the normal blood sample showed the maximum osmotic fragility (24.167±29.933) whereas, the cardiovascular patients' blood sample showed a decrease in the osmotic fragility (18.011±14.306). In case of purified extract, the normal blood had the maximum osmotic fragility (40.740±34.642) than the cardiovascular patient's blood (8.120±5.115) and the control (4.883±4.904).

3.3 Superoxide dismutase (SOD) activity

The results of SOD specific activity in fresh, lyophilized, purified samples in normal and cardiovascular patients' blood samples are depicted in **Table 3**. The pyrogallol treated samples showed highest SOD specific activity (2.88±1.81) followed by the fresh aqueous extract (2.40±0.62), purified sample (1.67±0.79) and the lyophilized extract (0.89±0.20 U/mg).

3.4 Catalase activity

The results of catalase specific activity in all groups with various extracts of saponin are shown in T**able 3.** The fresh aqueous extract showed lesser activity (0.102±0.068U/mg) than the lyophilized sample (1.21±0.071U/mg). The purified sample had the maximum catalase activity (1.46±0.82 U/mg) in comparison with the fresh aqueous and the lyophilized

extracts. In general, all the samples of patients' blood showed more specific activity than the normal blood.

4. Discussion

Emulsification index is the extent to which the viscosity of serum in blood emulsion is determined. Saponins have a high capacity for solubilising monoglycerides. Saponins help inducing and stabilizing emulsion.

Table 3: Specific activities of super oxide dismutase and catalase in normal and patients' blood

Parameter	*Superoxide dismutase (Units/mg protein)*	*Catalase (Units/mg protein)*
Control	1.21±0.32	1.12±0.18
Patient' blood+ fresh extract	2.40±0.62	0.102±0.07
Patient' blood + lyophilised extract	0.89±0.20	1.21±0.07
Patient' blood + purified extract	1.67±0.79	1.46±0.82
Patient' blood + pyrogallol	2.88±1.81	---------
Normal blood + fresh extract	2.31±0.63	0.99±0.07
Normal blood + lyophilised extract	0.74±0.18	1.09±0.68
Normal blood + purified extract	1.51±0.72	1.35±0.08
Normal blood + pyrogallol	2.71±1.92	---------

Note: Statistical differences were assayed by student's t-test. Values were expressed as Mean ± SD.

Emulsifying index is also directly related to surface tension and ability in micelle production. In this study the emulsifying index of the test extract were insignificant from each other. Regarding the surfactant activity of saponins, this activity of the extract could be attributed to their similar structure of saponins which have detergent or surfactant properties because they contain both water- soluble and fat-soluble components. These insignificant differences may be due to the same type of saponin and similar quantity. Present study implied that maximum emulsification index was in fresh extract. Similar findings were obtained by **Oakenfull & Sidhu (1989).**

Foam stability index is the ratio of foam formed before foam formation settling down. Foaming ability of surfactants is a property which will help improve the existence of surfactants in a solution. Furthermore, this ability can be used in order to compare the detergency properties of detergents with high ability of foaming production. Foam production and stability depends on type and concentration of surfactants. The results of the present study showed that fresh extract had on ability to produce foam more than the lyophilized and purified extracts. Work on foam stability index carried out by **Noudeh *et al.* (2009)** also in addition it was observed that blood of heart patients produced more foam than the normal blood.

Osmotic fragility is the ease with which RBCs undergo lysis, when kept in hypotonic solutions. When the red cells are placed in hypotonic saline medium, water is drawn in by the higher intracellular osmotic pressure. Consequently the cells become spherical and ultimately the red cell membrane gives way with liberation of haemoglobin into the surrounding fluid. The importance of osmotic fragility estimation lies in the fact that it provides information about the total status of red cell metabolism and membrane stability. This haemolytic activity may be due to effect on cell membrane, altering the sodium- potassium and calcium magnesium, ATPase activity or insertion of the hydrophobic saponin nucleus into the lipid bilayer. In the present study the haemolytic activity increased with increase in concentration of saponin as well as PBS. Similar kind of work was done by **Noudeh *et al.* (2009).** In addition normal blood showed higher haemolytic activity than those from cardiovascular blood.

Superoxide dismutase and catalase provide the first defence mechanism against oxygen toxicity. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of cardiovascular disease. The present study confirmed that saponin shows more catalase activity on diseased blood in comparison to normal blood. Lyophilized extract of *Lycopersicon esculentum* has pronounced effect on catalase activity than the fresh and purified aqueous extract.. In comparison to control, saponin has no pronounced effect on catalase activity. The work of **Moussa (1996)** supports the above result. SOD is an important defence enzyme which catalyzes the dismutation of superoxide radicals making the system free of oxidative stress. The current study also showed that SOD activity was higher in cardiovascular patients' blood samples than normal blood. **Fukal *et al.* (2002)** reported similar results**.**

5. Conclusion

Cardiovascular patients' blood showed more catalase and superoxide dismutase activities as compared to normal blood. Lyophilised, fresh extracts and purified samples did not show any prominent effect on patient's blood. Maximum catalase activity was observed in cardiovascular patients' blood containing fresh aqueous extract as compared to normal blood. Among all, the purified sample when treated with cardiovascular patients' blood had much higher effect on catalase activity. The above results indicate that saponin had positive effect on cardiovascular patients' blood and is therefore good for cardiovascular patients'.

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