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Comparative Effect of Saponin and Aqueous Saponin Extracts of *Lycopersiconesculentum* in Diabetic Blood

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Abstract: ***The aim of this study was to evaluate the effect of fresh aqueous extract, lyophilised extract and purified sample of Lycopersiconesculentum on normal and diabetic blood samples. Some of the physicochemical properties as well as antioxidant properties of human blood were studied. Firstly the fresh aqueous extracts of tomato plants were prepared and these extracts were lyophilized. 0.2 ml of blood was added to 0.2 ml of different concentrations of each extract in McIlvaine's buffer, and then incubated. The absorbance of the samples was determined by UV spectrophotometer. Among the studied extracts, normal blood showed the highest haemolytic effect when treated with fresh tomato extract. The values of emulsification index (E_{24}) and foam stability index (Fh) for each extract signify the surface activity. Diabetic blood showed more SOD activity than normal blood. Maximum catalase activity was found in diabetic blood containing fresh aqueous extract compared to normal blood. The results obtained showed that purified sample was more effective than fresh aqueous extracts and lyophilised extracts. It was found that saponin has more positive effect on diabetic blood and thus helps in maintaining hypoglycemic conditions.***

Keywords: Catalase, Emulsification index (E_{24}), Foam stability index (Fh), *Lycopersiconesculentum*, Lyophilised extract, Osmotic fragility, SOD.

1. Introduction

Saponins are glycosides containing one or more sugar chain on a triterpene or steroidaglyconebackbone also called sapogenin. They are categorized according to the number of sugar chains in their structure as mono, di or tridesmosidic. Monodesmosidicsaponins have a single sugarchain, normally attached at C-3. Bidesmosidicsaponins have two sugar chains, often with one attached through an ether linkage at C-3 and one attached through an ester linkage at C-28 (triterpenesaponins) or an ether linkage at C-26 (furastanolsaponins). The most common monosaccharides includeD-glucose (Glc), D-galactose (Gal), D-glucuronic acid (GlcA),D-galacturonicacid (GalA), L-rhamnose (Rha), L-arabinose(Ara), D-xylose (Xyl), and D-fucose (Fuc). Saponins are a large family of structurally-related compounds of steroid or triterpenoidaglycone (sapogenin) linked to one or more oligosaccharide moieties by glycosidic linkage. The carbohydrate moiety consists of pentoses, hexoses, or uronic acids. The presence of both polar (sugar) and nonpolar (steroid or triterpene) groups provide saponins with strong surface-active properties. Many saponins exhibit distinct foaming properties. They are also added to shampoos, liquid detergents, toothpastes and beverages as emulsifier and long-lasting foaming agent.

In addition, some pharmacological effects, such as molluscicidal,antinflammatory,antimicrobial,antihelmintic, antidermatophytic, antitussives and cytotoxic activities have been demonstrated in the plants.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 and fat- soluble components. They consist of a fat – soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water – soluble carbohydrates (sugar). 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. These natural soaps are toxic to bacteria and fungi and so form part of the plant's protection against disease.Scientists havedescribed about the bioactivesaponins with cancer related and immunomodulatory activity.Certainanti-inflammatory, antimicrobial,anthelmintic and antidermatophytic activity has been attributed to the saponins of plants.It is also found heavily in *Gynostemmapentaphyllum* (Genus *Gynostemma*, Family *Cucurbitaceae*) in a form called gypenosides, and in 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: leaves, stems, roots, bulbs, blossom and fruit. Commercial formulations of plant-derived saponinse.g. from the soap bark (or soapbark) tree, *Quillajasaponaria*, and from other sources are available via controlled manufacturing processes, which make them of use as chemical and biomedical reagents.

2. Materials and Methods

The tomato fruit sample was used in three forms namely lyophilised, fresh and purified forms. Lyophilised, fresh and purified forms of *Lycopersiconesculentum* were taken in 5 graduated test tubes with different concentrations i.e. 0.2,0.4,0.6 and 0.8ml, respectively and volume was adjusted to 5 ml by adding double distilled water,1 ml of blood (diabetic and normal blood) was added. The final concentration of saponin was 8µg100 ml^{-1}.Blood was

collected from Pathology Laboratory,Swaroop Rani Medical College, Allahabad (Uttar Pradesh).

Human blood was collected from healthy individuals and added to four tubes containing anticoagulant.After centrifuging 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 in 4°C for further experiments. The following parameters were assayed as follows:The emulsification index by the method of Carillo*et al.* (1996), foam stability index by the method ofNoudeh*et al.* (2008), sugar estimation by the method of Dubois *et al.* (1951), osmoticfragility by the method of Hitoshi and Hiroshi (2005).The superoxide dismutase activity was assayed by the method of Marklund and Marklund (1994) and catalase activity was assayed by the method of Beers and Sizer (1952).

3. Results and Discussion

In the present study the emulsification index of the tested extracts showed insignificant difference from each other. Diabetic blood when treated with purified sample was found to be higher (0.657±0.018µg ml^{-1}), whereas when normal blood treated with purified sample showed a lower range (0.655±0.032µg ml^{-1}).Similar convincing results have been obtained by Dehghan*et al.* (2008) who worked on the evaluation of aqueous extract of *Tribulusterrestris*L., *Trigonellafoenum-graecum*L. and *Echiumamoenum*. These plants contain saponins that can act on red blood cells (RBC) as a model of biological membranes.

The foam stability index of the diabetic bloodwhen treated with fresh sample was found to be higher (0.862±0.034µg ml^{-1}), whereas the range was less (0.802±0.046µg ml^{-1}) in the fresh sample when treated with normal blood. Foam production and stability depends on type and concentration of surfactants. The results of the present study also showed that fresh extract had more ability to produce foam than the lyophilised extract and purified form. Nowadays, a great deal of research is being carried out concerning the effect of surfactants on absorption. Some work on foam stability index was carried out by Noudeh*et al.* (2009).They reported values of Fh showed the extract of *T. terrestris*L. has the highest foam producing activity (14.42 mm).In addition it has been found that diabetic blood produced more foam than the normal blood.

In the present study the osmotic fragility increased with increase in concentration of saponin as well as PBS. The control sample has maximum osmotic fragility i.e %H (21.290±24.654). The normal sample when treated with lyophilised extract has least osmotic fragility (5.733±39.896) and the diabetic blood when treated with lyophilised extract has increased (6.941±6.204) in comparison to the normal blood. The normal sample showed the maximum osmotic fragility when treated with fresh extract (24.167±29.933), whereas the diabetic sample showed a significant decrease in the osmotic fragility when treated with the fresh extract(14.011±14.306). The normal blood has the maximum osmotic fragility (40.740±34.642) than the diabetic blood (6.220±5.115) and the control (4.883±4.904) when treated with the purified sample. Similar kind of work was done by Noudeh*et al.* (2009). According to him, the extract of *H. persicum*with lower haemolytic effect could be preferred in pharmaceutical preparation.

The fresh aqueous extract (0.983+0.068) when compared with the lyophilised sample shows a significant increase (1.086+0.061). The purified sample has the catalase activity (1.346+0.072). The purified sample has the maximum catalase activity when compared with the fresh aqueous extract and the lyophilised extract. ROS may alter superoxide dismutase and catalase in the presence of alloxan(Yadav*et al.*, 1997). The pyrogallol treated sample (2.284 +1.1815) showed highest SOD activity, the fresh aqueous extract (2.223+0.619) , the purified sample (1.448+0.789) and the lyophilised extract had least SOD activity (0.690+0.191). SOD is considered a primary enzyme since it is involved in the direct elimination of reactive oxygen species. SOD is an important defense enzyme which catalyses the dismutation of superoxide radicals. Free radical scavenging enzymes such as SOD protect the biological systems from oxidative stress. SOD and catalase provide the first defense against oxygen toxicity by catalysing the dismutation of superoxide anion to hydrogen peroxide and decomposition of hydrogen peroxide to water and molecular oxygen. According toMizobuchi*et al.* (1993) the serum SOD (s- SOD) activities in patients with *Diabetes mellitus* were assayed in order to evaluate its usefulness for monitoring of and also evaluate the relation between s-SOD activities and microangiopathies (nephropathy and retinopathy). s-SOD activities in DM patients were significantly higher than those in healthy controls (12.56+7.73 vs 10.51+ 1.69, $p< 0.01$),it has been suggested that the high s-SOD activity reflects the microangiopathic complications, particularly nephropathy.

The quantity of total soluble sugar was considerably high in normal blood when treated with the purified sample showed highest total sugar (1.346+0.072) than the lyophilised extract (1.086+0.061) and the fresh extract had the least total sugar (0.983+0.068). According to Mehrotra and Agarwal (2003),the quantity of total soluble sugar was higher in gall tissues as compared to the normal tissue. Sugar has large numbers of stereo-isomers, because it contains several asymmetric carbon atoms (Lindhrost and Thisbe, 2003).AlthoughLiu and Gutterman (2002)reported that glycemia increase the production of ROS and it may lead to increase in the lipid peroxidation in alloxan treated test animals.

Galls have often been described as physiological sinks. Increase in sugar content might be due to accumulation of these substances. This accumulation may involve the translocation of soluble sugars from the neighbouring healthy tissues to the physiological sinks. This view has been supported by the findings of Shaw and Samborski (1956). High sugar contents in young and mature galls may be due to increased metabolic activity under stress which may in turn be responsible for additional synthesis of sugar.

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Table 1: Comparison of specific activity of SOD and catalase in normal and diabetic blood using different sapon in extracts

Sample	*Specific activity of SOD (Umg^{-1})*	*Specific activity of catalase (Umg^{-1})*
Control	*1.21±0.32*	*1.12±0.18*
Fresh+ test extract	2.22±0.61	0.983±0.068
Lyophilised + test extract	0.69±0.19	1.086±0.061
Purified+ test extract	1.44±0.79	1.346±0.072
Pyrogallol+ test extract	2.28±1.81	—
Normal + fresh	2.31±0.629	0.99±0.07
Normal + Lyophilised	0.74±0.18	1.09±0.68
Normal + purified	1.51±0.72	1.35±0.08
Normal+ pyrogallol	2.71±1.92	—

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

Table 2: Comparison of total sugar in normal and diabetic blood using different saponin extracts

Parameter	*Amount (mg)*
Control	0.76+0.21
Fresh+ test extract	0.983±0.068
Lyophilised+ test extract	1.086±0.061
Purified+ test extract	1.346±0.072
Normal + fresh	0.82±0.07
Normal + Lyophilised	1.09±0.09
Normal + purified	1.41±0.08

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

Table 3: Comparison of emulsification index in normal and diabetic blood using different saponin extracts

Sample	*Emulsification index (µg/ml)*	*Foam stability index (µg/ml)*
Control	0.41±0.13	0.19+0.12
Test blood + fresh extract	0.532±0.019	0.862±0.034
Test + Lyophilised	0.535±0.019	0.44±0.087
Test + Purified	0.657±0.018	0.435±0.132
Normal blood + fresh extract	0.544±0.019	0.802±0.046
Normal+ Lyophilised	0.536±0.010	0.283±0.108
Normal + Purified	0.655±0.032	0.850±0.058

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

Table 4: Comparison of osmotic fragility in normal and diabetic blood using different saponin extracts

Sample	*%H (control)*	*%H (normal)*	*% H (test)*
Lyophilised	21.290±24.654	5.733±39.896	6.941±6.204
Fresh	4.883±4.904	24.167±29.933	14.011±14.306
Purified	4.883±4.904	40.740±34.642	6.220±5.115

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