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COMPARATIVE STUDIES OF SAFI AND FERROUS GLUCONATE ON HUMAN VOLUNTEERS

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ABSTRACT:

Majority of Pakistani population (about 70 percent) lives in far-flung rural areas where modern medicines are not available. Much of this population must therefore rely on traditional herbal medicine for relief from diseases. This signifies the importance of Eastern System of medicine in our society and stresses the need of research for these herbal medicines. Safi is one such herbal drug and is said to be used as a blood purifier. Besides this it shows a wide range of pharmacological actions. In the present study effects of Safi and an allopathic hematinic Sangobion were observed on hematological and biochemical parameters of human volunteers. Significant changes were noted in some of these parameters after 21 days long oral administration of Safi and Sangobion.

INTRODUCTION

Hematological parameters have an extremely high degree of importance in respect of the functions performed by blood to meet the complex requirements of the various physiological systems in the body and in maintaining the life itself Any fault in these parameters may lead to various pathological conditions like anemias, prolonged bleeding and clotting time (Mitsutani 1985).

Literature reveals that Safi is composed of aqueous extract of organic and inorganic compounds from *Bauhinia variegata* Linn., *Curcuma caesia, Chrozophora plicata, Canscora decussata* Schult, *Cassia angustifolia* Vahl, *Cuscuta reflexa* (Roxb.), *Fumaria officinalis, Lavandula stoechas* Linn., *Melia azadirachta* Linn., *Nymphaea lotus* Linn., *Ocimum canum* Sims, *Pterocarpus santalinus* Linn., Rosa *damascena* Mill., *Swertia chirata, Sphaeranthus indicus* Linn., *Smilax china* Linn., *Terminalia chebula* Retz., *Tinospora cardifolia* and *Tephrosia purpurea.* These plants are used as tonic, carminative, blood purifier, alterative, deobstruent, antipyretic, analgesic, alexipharmic, resolvent of inflammation, antihelmintic, antibiotic, antibilious, repurcussive and used in rheumatism, in enlarged liver and spleen (Chopra *et al.,* 1956).

These plants, used in the preparation of the drug i.e., "Safi" have tannic acid, glucose, gum, essential oils, sugar, resin, albuminoids, anthraquinone derivatives, glucosides, chrysophanic acid, emodin, sennoside A and B, fumaric acid, protopine, choline, alkaloids, nonacosane (hydrocarbon), sterols, 7-methoxy coumarin, gallic acid, fatty acids (oleic, linoleic, palmitic, stearic and arachidonic), salts containing Fe, K, Ca, nitrates, carbonates, magnesium, vitamins especially vitamin C and carotene (Chopra *et al.,* 1982). The drug (Sangobion), according to the information leaflet published by Merck, contains per 5 ml of syrup:

Ferrous gluconate	129.5 mg
Vitamin B_1	1 mg
Vitamin B_2	1 mg
Vitamin B_6	1.5 mg
Nicotinamide	15 mg
Biotin	300 mcg

The present study is designed to evaluate the effects of Safi and Sangobion on some hematological and biochemical parameters in human volunteers.

MATERIAL AND METHOD

Two groups of 10 human volunteers were selected regardless of their age and sex, from low-income society and were tested to determine pretreatment values of hematological and biochemical parameters. The drugs were obtained from medical stores and distributed to the volunteers. They were advised to take the medicines for 3 weeks orally. The recommended daily doses of Safi and Sangobion were:

Safi	10 ml
Sangobion	15 ml

After the period of 21 days volunteers were tested to determine the effects on following parameters:

1. Bleeding time
2. Clotting time
3. Erythrocyte count
4. Hemoglobin percentage
5. Leucocyte count
6. Platelet count
7. Erythrocyte sedimentation rate
8. Osmotic fragility
9. Fibrinogen level
10. Glucose concentration
11. Protein concentration
12. Lipid concentration

1. Bleeding Time:

Bleeding time was measured by using Duke's method which is based on measuring the time interval from inflicting a standard wound until the bleeding stops (Duke, 1912; Adelson and Crosby, 1957; Heinrich. 1962).

2. Clotting Time:

Fingertip was punctured with a blood lancet and blood was taken up into a capillary tube, after every 30 seconds appearance of clot was checked.

3. Erythrocyte Count:

For counting red blood corpuscles, blood is diluted with Hayem's reagent and red blood cells were counted in 80 small squares of Neubauer's chamber (Haug, 1959).

4. Hemoglobin Percentage:

Sahli' s method is used to estimate the concentration of hemoglobin in blood.

5. Leucocyte Count:

Turk's solution is used for diluting white blood cells. The counts were taken in 4 large squares of the Neubauer chamber and counting was carried out under low magnification (about x 100).

6. Platelet Count:

Ammonium oxalate solution was used and thrombocytes were counted in 4 large squares (Brecher et al., 1953).

7. Erythrocyte Sedimentation Rate (ESR):

ESR is determined by Westergren method (Westergren, 1922).

8. Osmotic Fragility:

The resistance of erythrocytes against a hypotonic sodium chloride solution was tested by preparing a dilution series.

9. Fibrinogen Level:

Citrated plasma was prepared by mixing the sodium citrate solution and whole blood in 1:9 ratio. This citrated plasma was centrifuged at 3000 rpm, placed in water bath and supernatant was mixed with fibrinogen reagent. Either complete clot or thread like appearance is noted. Time consumed in this clot formation relates to fibrinogen level (Koepke *et al.*, 1975).

10. Glucose Concentration:

Glucose concentration was estimated on the basis of Trinder reaction (Trinder, 1969).

11. Total Protein Concentration:

Biuret method was used for the determination of total proteins in serum (Henry *et al.*, 1974).

12. Total Lipid Concentration:

Sulphophosphovanifline method was used for the determination of total lipids in serum (Zoeliner, 1962).

CALCULATIONS

The level of significance (probability) is calculated by applying paired t-test.

RESULTS

Results are summarized in Table 1 and 2.

1. Effect of Treatment on Bleeding Time: In Sangobion group normal bleeding time was noted as 227.8 seconds. After treatment bleeding time was found to be decreased to 221.8 seconds. Second group which received Safi also showed decrease in bleeding time. The normal bleeding time was 240.1 seconds and after treatment with Safi for 21 days it was decreased to 235.4 seconds. The results were statistically significant (Fig. 1).

2. Effect of Treatment on Clotting Time:

In both cases clotting time was reduced after treatment. Pretreatment value in Sangobion group was 347.6 seconds and in Safi group 296.1 seconds. After treatment clotting time was recorded as 343.3 and 289.6 seconds respectively in Sangobion and Safi. Results were insignificant in Sangobion (Fig. 2)

3. Effect of Treatment on Erythrocyte Count:

The erythrocyte count was increased after treatment with drugs. The count of red blood cell in pretreatment examination was found to be as 3.7 M/cmm in Sangobion group and 3.69 M/cmm in Safi group. Mean erythrocyte count was increased to 3.81 M/cmm in Sangobion and 4.38 M4mm in Safi group (Fig. 3). Results were significant.

4. Effect of Treatment on Hemoglobin Concentration:

The study so far shows increase in hemoglobin concentration. Safi is more effective in increasing hemoglobin as compared to Sangobion. Normal value of hemoglobin concentration in Sangobion was 11.2 g and 11.47 g in Safi group. Mean Hb concentration was increased to 11.45 g in Sangobion and 13.5 g in Safi group after treatment of 3 weeks (Fig.4). Increase in hemoglobin concentration was statistically significant.

5. Effect of Treatment on Leucocyte Count:

Decrease in leucocytes was observed after 3 weeks treatment by both drugs. This decrease was more prominent in Safi group. Normal and treated values of Sangobion group are 7050/cmm and 6660/cmm respectively. Normal and treated values of Safi group are 8763/cmm and 6305/cmm. (Fig. 5). Decrease in leucocytes by Safi was statistically significant.

6. Effect of Treatment on Platelet Count:

Platelets were significantly increased by both drugs. Normal platelet count of Safi and Sangobion group is 224,500/cmm and 233,500/cmm respectively. Whereas after treatment this count was increased to 247,000/cmm and 253,500/cmm by Safi and Sangobion respectively (Fig. 6).

7. Effect of Treatment on E.S.R.:

E.S.R. is decreased by both drugs. Normal E.S.R. values were 25.9 mm in Sangobion and 18.6 mm in Safi. Treated E.S.R. values were 16.4 mm in Sangobion and 10.2 mm in Safi (Fig. 7). Both results were statistically significant.

8. Effect of Treatment on Osmotic Fragility:

No change was observed in osmotic fragility of erythrocytes by both drugs Mean osmotic fragility was 0.40 in Sangobion group and 0.41 in Safi group (Fig. 8).

9. Effect of Treatment on Fibrinogen:

Insignificant increase in fibrinogen level was observed by both treatments. Normal fibrinogen value was 260 mg in Sangobion and 247.3 mg in Safi group. Treated values for Safi and Sangobion group were 255.6 mg and 272.3 mg respectively (Fig. 9).

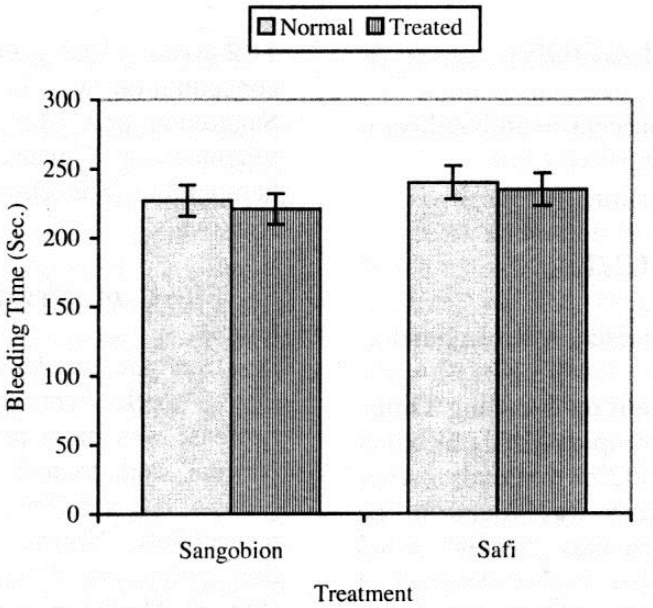

Fig. 1: Effect of treatment on bleeding time.

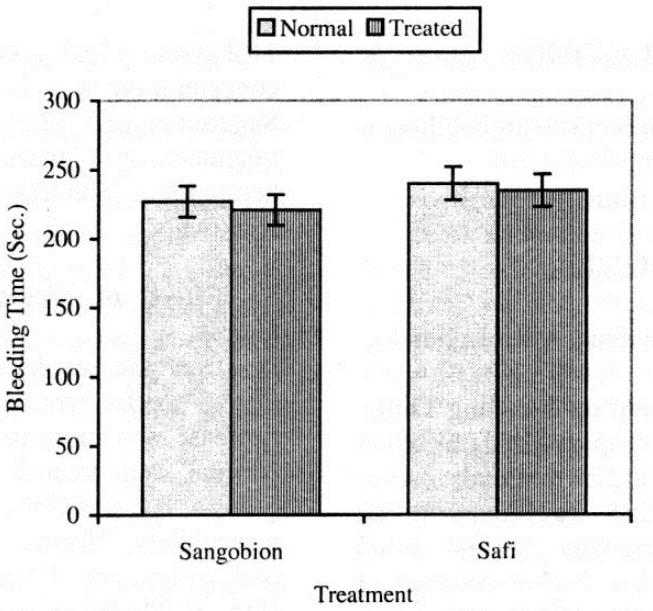

Fig. 2: Effect of treatment on clotting time.

10. Effect of Treatment on Glucose Concentration:

Glucose concentration was significantly increased by both drugs. Normal glucose level in Sangobion group was 105 mg and 104.1 mg in Safi group. After treatment of 3 weeks this level was increased to 114.6 mg in Sangobion group and 128.2 mg in Safi group (Fig. 10).

11. Effect of Treatment on Total Protein Concentration:

Level of protein was increased significantly by both drugs. Normal values were 7.48 g and 7.54 g for Sangobion and Safi. Treated protein levels were 7.65 g and 7.72 g for Sangobion and Safi (Fig. 11).

12. Effect of Treatment on Total Lipid Concentration:

Total lipid concentration was increased by Sangobion and Safi. Normal mean lipid

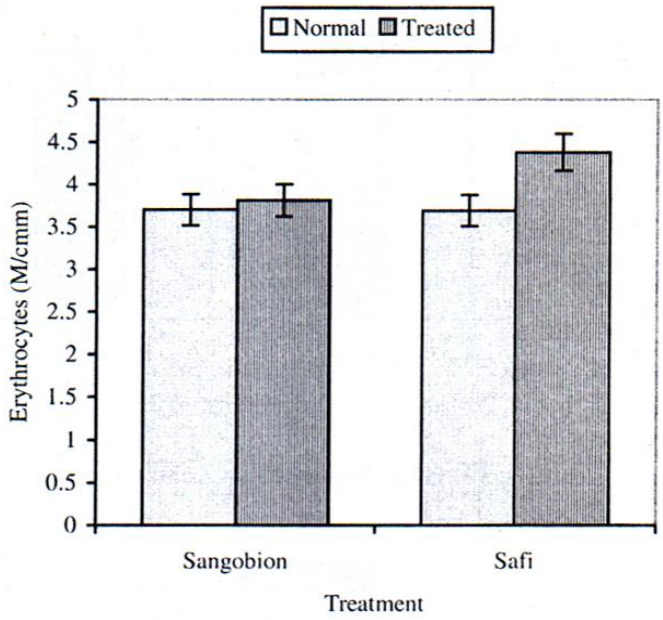

Fig. 3: Effect of treatment on Erythrocytes.

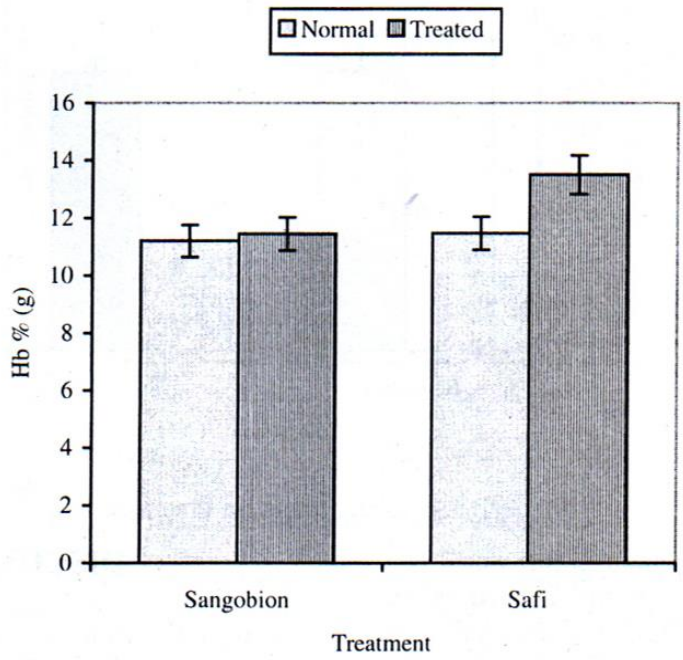

Fig. 4: Effect of treatment on Hemoglobin.

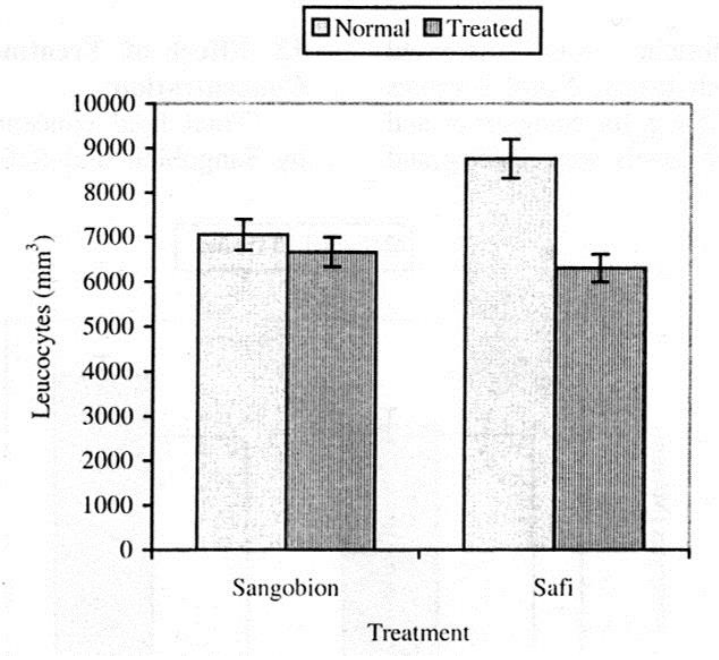

Fig. 5: Effect of treatment on Leucocytes.

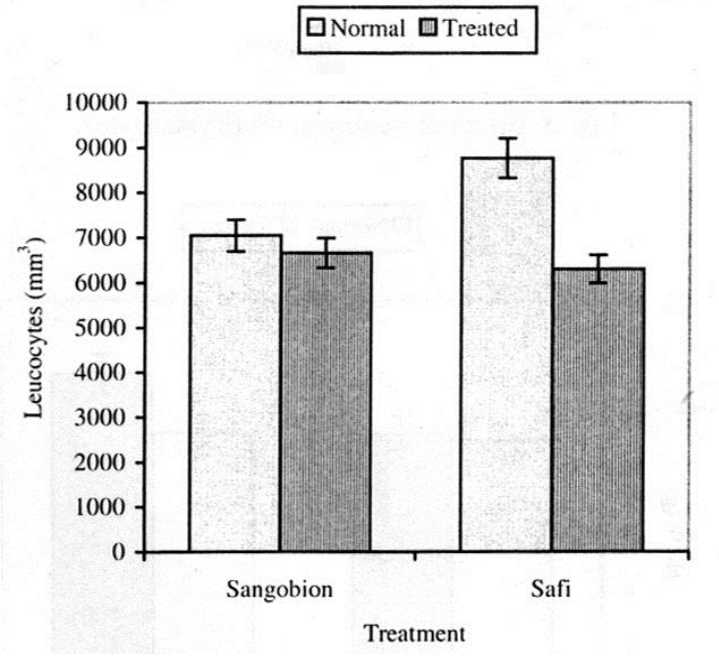

Fig. 6: Effect of treatment on Platelets.

concentration in Sangobion group was 704.3 mg and 736 mg in Safi group. Treated values were 744 mg and 732.5 mg for Safi and Sangobion respectively (Fig.12). Increase shown by drugs was statistically insignificant.

DISCUSSION

It has been revealed that Safi and Sangobion show an increase in erythrocyte count and hemoglobin concentration after 3

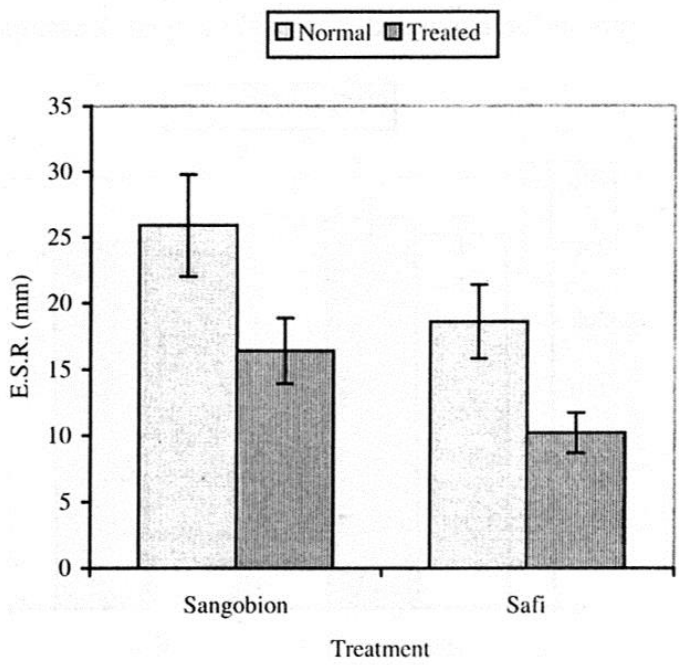

Fig. 7: Effect of treatment on E.S.R.

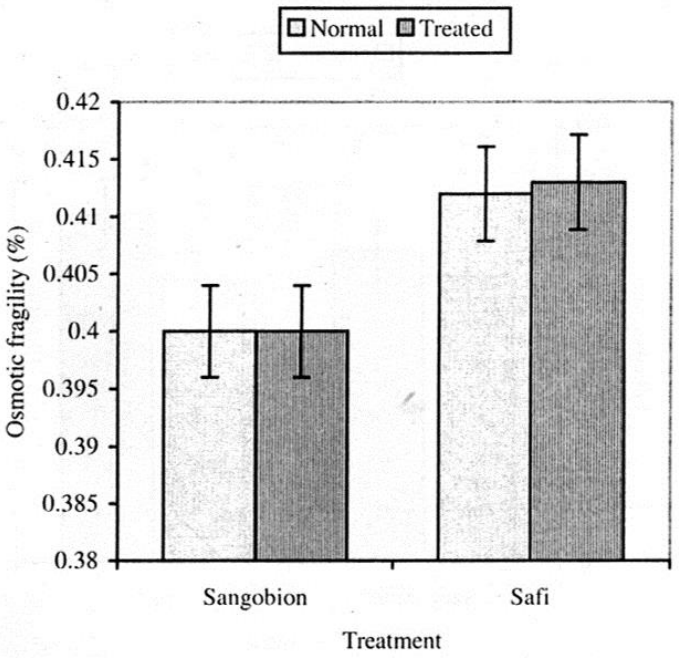

Fig. 8: Effect of treatment on Osmotic fragility.

weeks treatment. The increase in hemoglobin and erythrocytes was more prominent by Safi. Increase in Hb concentration may be probably due to the iron content, because the Iron has the capability to increase erythropoietic production and 80% of the body iron is used for this purpose (Sjaastad *et al.*, 1996; Ahluwalia, 1998). Minerals and vitamins present in the herbal drug Safi may be responsible for increase in RBC count.

Vitamin C is an important antioxidant which is necessary for the synthesis of red blood were noted which are correlated with increase in fibrinogen. Unsaturated fatty acids of Safi cells (RBC)

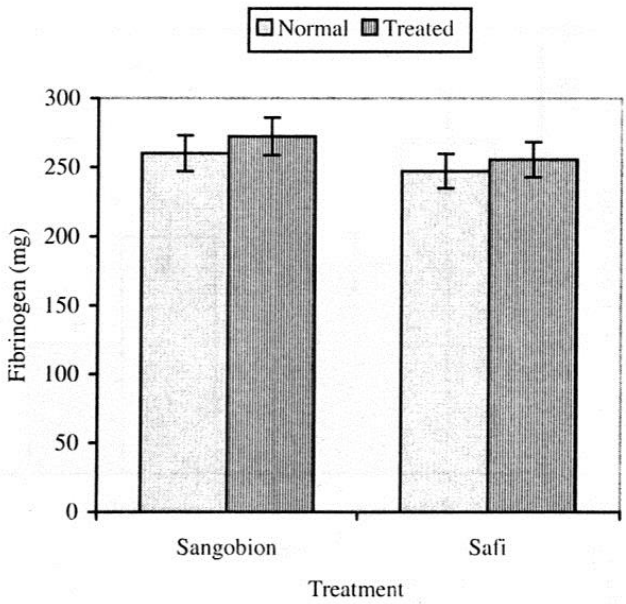

Fig. 9: Effect of treatment on Fibrinogen.

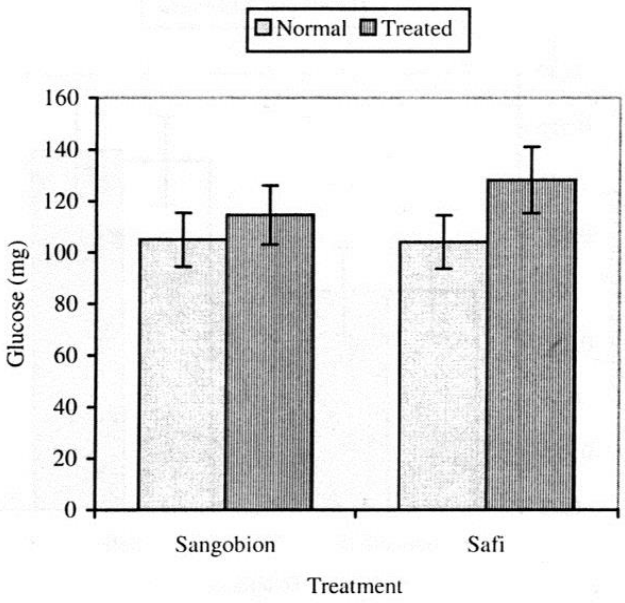

Fig. 10: Effect of treatment on Glucose.

indirectly (Behrman et al., 1987). Vitamin A can also increase hemoglobtn level (Kolsteren *et ca.*, 1999).

Decreased bleeding and clotting times probably contributed to TxA_2 formation. TxA_2 involvement is a possibility in the decrease of bleeding and clotting time. Increase in platelets is also responsible for decreasing clotting and bleeding times.

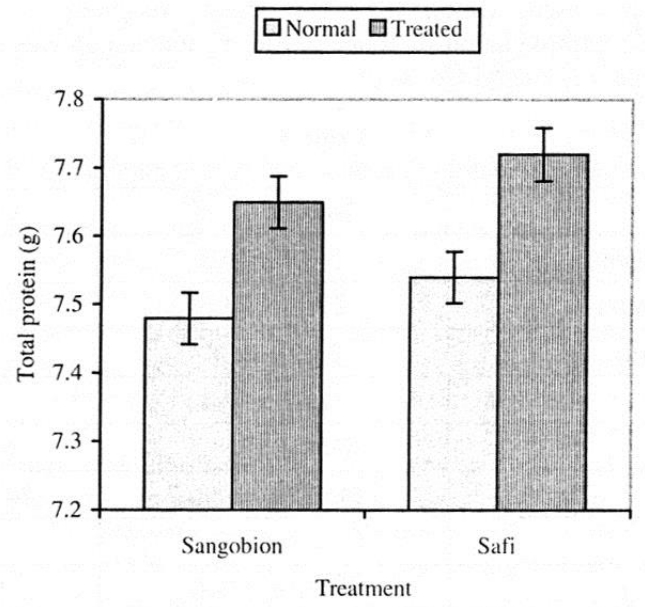

Fig. 11: Effect of treatment on Total protein.

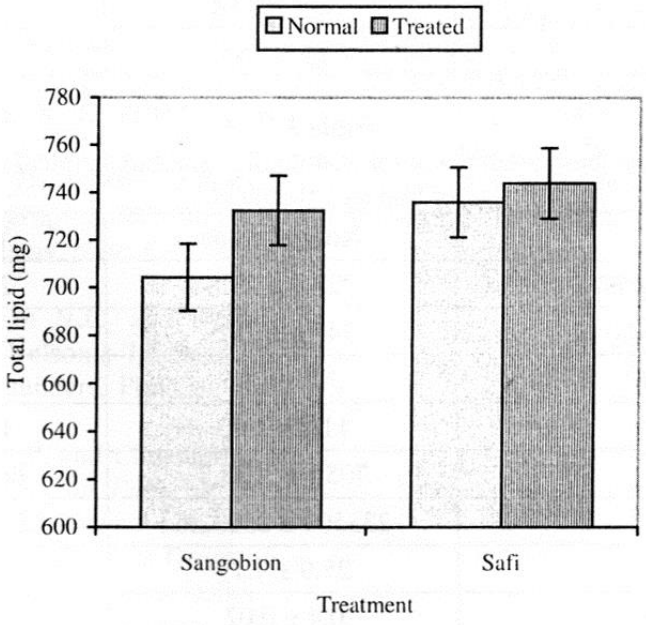

Fig. 12: Effect of treatment on Total lipid.

By the oral administration of the drugs used in this study, concentration of protein increases. Amino acids present in Safi may be responsible for increase in protein. Sangobion contains ferrous gluconate which is responsible for increased synthesis of hemoglobin and indirectly affects red blood cells count. Vitamin niacin of Sangobion acts as coenzyme for wide variety of proteins. This may be responsible for increasing proteins and for catalyzing oxidative reduction reactions essential for tissue

respiration. In present study protein fibrinogen is also increased. Biotin is a cofactor for enzymatic carboxylation of four substrates: (1) Pyruvate, (2) Acetyl Co-A, (3) Propionyl Co-A, (4)13-methyl crotonyl Co-A. These reactions contribute to glucose formation and gluconeogenesis.

Table 1
Effect of Safi treatment on hematological and biochemical parameters of human volunteers

	Normal value	Treated value
Bleeding time (seconds)	240.1 ± 56.5	235.4 ± 55.6*
Clotting time (seconds)	296.1 ± 61.5	289.6 ± 63.1*
Erythrocyte (M/cmm)	3.69 ± 0.38	4.38 ± 0.41*
Hemoglobin (g)	11.47 ± 0.91	13.5 ± 0.88*
Leucocytes (/mm^3)	8763 ± 1714.5	6305 ± 1403.6*
Platelets (/mm^3)	224500 ± 14990.7	247000 ± 23828**
E.S.R. (mm)	18.6 ± 8.7	10.2 ± 5.4**
Osmotic fragility (%)	0.412 ± 0.006	0.413 ± 0.004
Fibrinogen (mg)	247.3 ± 36.02	255.6 ± 38.78
Glucose (mg)	104.1 ± 23	128.2 ± 17.2*
Total protein (g)	7.54 ± 0.12	7.72 ± 0.1*
Total lipid (mg)	736 ± 95.1	744 ± 59.4

Table 2
Effect of Sangobion treatment on hematological and biochemical parameters of human volunteers

	Normal value	Treated value
Bleeding time (seconds)	227.8 ± 23.8	221.8 ± 23.9*
Clotting time (seconds)	347.6 ± 29.5	343.3 ± 28.4
Erythrocyte (M/cmm)	3.7 ± 0.46	3.81 ± 0.46*
Hemoglobin (g)	11.2 ± 1.43	11.45 ± 1.47*
Leucocytes (/mm^3)	7050 ± 2058.7	6660 ± 1995.6*
Platelets (/mm^3)	233500 ± 25932.6	253500 ± 16840.7*
E.S.R. (mm)	25.9 ± 9.19	16.4 ± 7.16
Osmotic fragility (%)	0.4 ± 0.02	0.4 ± 0.02
Fibrinogen (mg)	260 ± 40.7	272.3 ± 35.5
Glucose (mg)	105 ± 32	114.6 ± 26.0*
Total protein (g)	7.8 ± 0.29	7.65 ± 0.26*
Total lipid (mg)	704.3 ± 116.6	732.5 ± 57.8

Data are expressed as mean ± S.D. each figure is the mean of ten readings. Significant differences by student's t-test. *P < 0.05, **P<0.01 as compared to normal.

In present study osmotic fragility was not changed. Antioxidant vitamin may probably be responsible for decreased hemolysis. E.S.R. reduction is probably related to high erythrocyte count and prevention of hemolysis by drugs. Present work also shows increased glucogenesis. Safi contains precursors of carbohydrate and components of Shikimate and TCA cycle which probably contribute to increased glucose concentration. Increase in lipid can be related to contribution of sterol and sitosterols in cholesterol formation by esterification with fatty acids.

Safi cotnains quinones and coumarins. Coumarins inhibit platelet aggregation induced by adenosine. In present study platelet count was increased. This increase may be related to increased erythropoiesis. Administration of drugs in diabetic and hypercholesterolemic patients should be carefully monitored.

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