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Potential effect of Asparagus racemosus root extract on experimental anemic and thrombocytopenic conditions in rats

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ABSTRACT

Purpose: To study the potential effect of Asparagus racemosus extract on experimental anemic and thrombocytopenic condition in rats.

Approach: The anti-anemic and anti-thrombocytopenic activities of chloroform:methanol (2:1) extract of Asparagus racemosus was studied using phenylhydrazine and heparin-induced models, respectively. Three doses of Asparagus racemosus viz., 250, 500 and 750 mg/kg (p.o, daily for 10 days) were tested in rats, and observations such as levels of RBC count, haemoglobin, mean corpuscular haemoglobin (MCH), WBC, bleeding time and clotting time were recorded. Ferrous sulphate and prednisolone were used as standard agents for anti-anemic and anti-thrombocytopenic activities, respectively.

Finding: The anti-anemic study indicated that Asparagus racemosusextract at 750 mg/kg ($p<0.01$) increase significantly the number of RBC, Hb and significantly decrease ($p<0.01$) MCH and WBC when compared with anaemic group. In anti-thrombocytopenic study, extract at 750 mg/kg decreased significantly ($p<0.01$) bleeding time and clotting time compared to heparin group. Our observations indicated that root extract of Asparagus racemosuspossess exhibited anti-anemic and anti-thrombocytopenic properties and these could be related to the phytoconstituents such as alkaloids, saponins, phytosterols, triterpenoids, polyphenols, carbohydrate, flavonoids, glycosides, phenolic compounds and tannins present in the extract.

Originality: The present study is the first time evaluation of the anti-anemic and anti-thrombocytopenic effect of Asparagus racemosus.

Conclusion: from our study Asparagus racemosus possess exhibited anti-anemic and anti-thrombocytopenic properties.

Keywords: *Asparagus racemosus; Anemia; Thrombocytopenia; Phenylhydrazine; Heparin.*

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INTRODUCTION:

Some of the important disorders of blood include anemia and thrombocytopenia.Anemia is the condition in which there is a deficiency of red blood cell or of hemoglobin in the body which results in the disturbance of the oxygen transport and,thrombocytopenia refers to disorder in which there is relatively decrease in thrombocyte commonly known as platelet[1]. Anemia and thrombocytopenia both are one of the public health problems most widespread, especially in developing countries. There are many disorders which is associated with anemia and thrombocytopenia like Thrombotic microangiopathies (TMAs) which is present with thrombocytopenia, microangiopathic hemolytic anemia, and symptoms of microvascular occlusion[2].

The experimental anemic condition in rats can be induced by the administration of phenylhydrazine (PHZ).PHZ induced anaemia is associated with activation of immune activation in the effected biological system[3]. PHZ activates of immune cellsand cause oxidative damage tohaemoglobin and membrane

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phospholipids, resulting in the lysis of erythrocytes[4].

Heparin can be used to induce thrombocytopenia. It reacts with platelet factor 4 (PF4) forming "Heparin-PF4" immune complex. Against these, antibodies are produced resulting in destruction of platelets[5].

Asparagus racemosus (family Asparagaceae) also known by the name Shatavari is one of the well known drug in ayurveda, effective in treating madhur rasam, madhur vipakam, seet-veeryam, somrogam, chronic fever and internal heat[6,7] .In modern Ayurvedic practices the roots of plant are considered to be effective as antispasmodic, appetizer, stomach tonic, aphrodisiac, astringent, antidysentiric, laxative, anticancer, antiinflammatory, blood purifier, antitubercular, antiepileptic and also in night blindness, kidney problems and in throat complaints[8] .

In India and China, this plant is given as a powerful diuretic in cholera and rheumatism[9]. Reports indicate that the pharmacological activities of A. racemosus root extract include antiulcer, antioxidant, and antidiarrhoeal, antidiabetic and immunomodulatory activities. Although, the ancient ayurvedic literature reported that Shatavari Rasayana have several beneficial effects on blood disorders[10], however, the same is not well documented. Hence, in the present study the chloroform:methanol root extract of Asparagus racemosus was tested against phenylhydrazine and heparin-induced anemic and thrombocytopenic conditions, respectively in rats.

MATERIALS AND METHODS

Experimental Animals

Sprague Dawley ratsof either sex weighing between 200 and 250 gm were used and maintained at room temperature, 40-60% humidity and 12±1 hour light-dark cycle. The animals were maintained under standard conditions in an animal house approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). All the rats were provided with normal pellet diet and water ad libitum, prior to the dietary manipulation. Before starting the study, approval from Institutional Animal Ethics Committee (CPCSEA No. 2015/PCOL/02) was obtained for the procurement of animals.

Plant Extract

A gift sample of chloroform: methanol (2:1) extract of Asparagus racemosus root was obtained from Green Chem Herbal extracts and formulation, Bangalore. Three doses of root extract of Asparagus racemosusviz., 250, 500 and 750 mg/kg was tested in this study[11]. The extracts were administered by oral route daily for 10 days and the samplings were done one hour after the last dose ie., on 10th day.

Phytochemical screening:

The phytochemical constituents such as alkaloids, saponins, phytosterols, triterpenoids, polyphenols, carbohydrate, flavonoids, glycosides, phenolic compounds and tannis, protein andsteriodswere tested in the extracts of Asparagus racemosus according to the standard procedures[12].

Animal grouping:

Sprague Dawley rats were divided into six groups and each consisted of six animals as follows: group-1 served as normal (negative) control, group-2 as positive (anemic or thrombocytopenic) control, groups 3 to 5 as treatment groups for testing low (250 mg/kg), medium (500 mg/kg) and highest (750 mg/kg) dose of Asparagus racemosus and group-6 as standard (ferrous sulphate - 100 mg/kg / prednisolone 1 mg/kg).

Induction of Anemia by Phenyl hydrazine

Anemia was induced by intraperitoneal administration of Phenylhydrazine 60 mg/kg, body weight for 2 days[13]. The parameters tested for anemia include haemoglobin content, RBC count, mean corpuscular haemoglobin (MCH) and WBC count[14].

Induction of thrombocytopenia by Heparin

Subcutaneous injection of low molecular weight Heparin, at the dose of 2000 IU/kg was given to rats daily for 10 days[15].Blood samples were tested to confirm thrombocytopenia and include bleeding time (Duke's method) and clotting time (Lee and White method).

Additionally, in both the studies influence of disease and treatment on body weight was recorded.

Statistical analysis

The results of this study are expressed as mean ±Std error of mean (Mean± SE). Statistical analysis was done byusing One-way ANOVA followed by Dunnet multiple comparision test. All values of $p<0.05$ were consideredstatistically significant.

RESULTS

A. Phytochemical analysis

The phytochemical analysis of chloroform: methanolicextract of Asparagus racemosus bark revealed the presence of active ingredients such as alkaloids, saponins, phytosterols, triterpenoids, polyphenols, carbohydrate, flavonoids, glycosides, phenolic compounds and tannis, protein butsteroids are absent [Table 1].

Table 1 : Various chemical compounds identified in Asparagus racemosus root

S.NO	Chemical Groups	Result
1	Alkaloids	+
2	Protein	+
3	Phenolic compounds and Tannins	+
4	Steroids	-
5	Flavanoids	+
6	Glycosides	+
7	Carbohydrate	+
8	Phytosterols	+
9	Triterpenoids	+
10	Saponins	+

Signs: + = Present; - = Absent

B. Effect of Asparagus racemosus extract on experimental anemia[Table 2]

After injection of phenylhydrazine to rats of the six groups except the normal group, there was a significantly ($p<0.01$) decrease in red blood cells and haemoglobin when compare with normal group and there was a decrease ($p<0.01$) in the mean cell haemoglobin and WBC count when compare with normal group. An increased number of red blood cells and haemoglobin was observed after treatment in the following days. Group 3 (250 mg/kg) and group 4 (500 mg/kg) showed decrease ($p<0.01$) in number of RBC count, haemoglobin and increase in the number of mean cell haemoglobin and WBC count when compared with the normal control group, but when group 3 compared with the anemic control group there was an increase ($p<0.05$) in the number of RBC, haemoglobin and decrease ($p<0.05$) in the number of mean cell haemoglobin and WBC count ($p<0.01$). When group 4 compared with the anemic control group there was an increase ($p<0.01$) in the number of RBC, haemoglobin and decrease ($p<0.01$) in the mean cell haemoglobin and WBC. Group 5 showed no significant change in RBC, haemoglobin, mean cell haemoglobin and the WBC when were compared with the normal control group, but when compared with anemic group there was an increase ($p<0.01$) in the RBC, haemoglobin and decrease ($p<0.01$) in the mean cell haemoglobin and WBC. When rats treated with standard drug there was a decrease in RBC, haemoglobin and increase in the mean cell haemoglobin and WBC when compared with the normal control group but when compared with the anemic group there was an increase ($p<0.01$) in the number of RBC, haemoglobin, mean cell haemoglobin and WBC.

Table 2 : Evaluation parameters for PHZ Induced Anemia in rats

S.no	GROUPS	RBCX1012/L	Hemoglobin g %	MCH (pg)	WBC x109 /L
1	Group I -Control	7.26±0.05	12.4±0.2	15±0.30	9.28±0.31
2	Group II- Anemic control	3.56±0.16**	7.9±0.15**	22.39±0.88**	18.34±0.17**
3	Group III- Low dose (250mg/kg)	4.26±0.02**c	8.57±0.08**c	20.4±0.24**c	12.7±0.12**b
4	Group IV- Medium dose (500mg/kg)	5.75±0.2**b	9.57±0.13**b	17.98±0.33**b	10.16±0.19*b
5	Group V- High dose (750mg/kg)	7.42±0.2b	12.63±0.13b	16.36±0.38b	9.35±0.14b
6	Group VI - Standard ferrous sulphate (100mg/kg)	6.66 ±0.27**b	11.63±0.23*b	16.11±0.52b	10.26±0.27*b

Results are presented as Mean ± SD. (n=6)
*Values are given as mean ± SEM, (n=6/group). ***p <0.001, **p<0.01, *p<0.05 when compared with control. [a]p<0.001,[b]p<0.01, [c]p<0.05 When compare with anemic control.*

There was a weight loss in rats after induction of anemia with PHZ ($p< 0.01$) when compared with normal control group. After treatment with drug, the weight was significant higher in group 5 ($p<0.01$) than the other group when compared with the normal control group. There was no significant change in weight when group 3 and 6 were compared with anemic group [Table 3].

Table 3 : Effect of A. racemosus root extract on the weight of PHZ induced anemia in rats

S.No	GROUPS	Initial weight before Inducing anaemia	Weight after inducing anaemia (g)	Weight after treatment with A. racemosus extract (g)
1	Group I -Control	245±0.88	246.16±1.83	250.16±0.98
2	Group II- Anemic control	251.5±0.56**	238.83±0.4**	241.83±0.6**
3	Group III- Low dose (250mg/kg)	245.6±0.88b	234.83±0.40**c	243.8±1.1**
4	Group IV- Medium dose (500mg/kg)	252.16±0.79**	239.3±0.71**	262.6±1.02b
5	Group V- High dose (750mg/kg)	254.83±0.65**c	240.83±0.47**	274.5±0.85**b
6	Group VI - Standard ferrous sulphate (100mg/kg)	245.5±0.62b	235±0.73**c	245.8±1.01**

C. Effect of Asparagus racemosus extract on experimental thrombocytopenia [Table 4]

Table 4 : Evaluation parameters for heparin Induced thrombocytopenia in rats

S.No	GROUPS	BLEEDING TIME (sec)	CLOTTING TIME (sec)
1	Group I -Control	88.38±1.15	140.03±0.96
2	Group II- thrombocytopenia control	132.06±0.76**	195.03±1.92**
3	Group III- Low dose (250mg/kg)	119.1±0.81**b	181.85±1.5**b
4	Group IV- Medium dose (500mg/kg)	106.48±1.92**b	168.05±1.49**b
5	Group V- High dose (750mg/kg)	94.8±1.61**b	145.73±0.96*b
6	Group VI – Standard prednisone (1mg/kg)	113.7±0.49**b	155.48±0.63**b

Results are presented as Mean ± SD. (n=6)
*Values are given as mean ± SEM, (n=6/group). ***p <0.001, **p<0.01, *p<0.05 when compared with control. [a]p<0.001,[b]p<0.01, [c]p<0.05 When compare with thrombocytopenia control.*

After injection of heparin to rat, there was a significant increase ($p<0.01$) in the bleeding and clotting time when were compared with the normal control group. Group 3, group 4 and standard group (group 6) showed increase ($p<0.01$) in the bleeding and clotting time when compared with the normal control group and showed decrease ($p<0.01$) in the bleeding and clotting time when compared with thrombocytopenic group. Group 5 showed increase in the bleeding ($p<0.001$) and clotting time ($p<0.01$) when compared with the normal control group and decrease ($p<0.01$) in the bleeding and clotting time when were compare with the thrombocytopenic group.

There was no significant decrease in weight after induction of thrombocytopenia with heparin when were compared with the normal control group. After treatment with drug only group 4 and 5 showed significant increase in weight when were compare with the normal control group [Table 5].

DISCUSSION

Thrombotic microangiopathies (TMAs), Thrombotic Thrombocytopenic Purpura—Hemolytic Uremic Syndrome (TTP-HUS), HELLP (Hemolysis,Elevated liver enzyme and low platelet count) syndrome, hypersplenism etc. all these disorders are associated

Table 5 : Effect of A. racemosus root extract on the weight of heparin induced thrombocytopenia in rats

S.No	GROUPS	Initial weight before inducing of Thrombocytopenia	Weight after inducing of thrombocytopenia	Weight after treatment with A. racemosus extract
1	Group I -Control	250.5?0.34	256.83?0.7	261?0.93
2	Group II- thrombocy-topenia control	248.5?0.9	251.3?0.84**	254.33?0.49**
3	Group III- Low dose (250mg/kg)	249.16?0.87	250.66?0.76**	259.5?1.20**b
4	Group IV- Medium dose (500mg/kg)	251.6?0.67	253.5?0.99	269.83?1.30**b
5	Group V- High dose (750mg/kg)	252.5?0.76	254.66?0.5	275.83?0.30**b
6	Group VI – Standard prednisone (1mg/kg)	248.8?0.47	250.5?1.38**	258.16?0.70**c

Results are presented as Mean ± SD. (n=6)
*Values are given as mean ± SEM, (n=6/group). ***p <0.001, **p<0.01, *p<0.05 when compared with control. [a]p<0.001, [b]p<0.01, [c]p<0.05 When compare with thrombocytopenia control.*

with anemia and thrombocytopenia and affecting large number of population. 40% of cases are seen with Thrombotic Thrombocytopenic Purpura—Hemolytic Uremic Syndrome (TTP-HUS)[16]and 4-12% of the patients are affected with HELLP syndrome.[17,18]

Anemia and thrombocytopenia generally occur in pregnant women, about 7 – 10 % pregnant women are suffering from anemia and thrombocytopenia and 1- 4 % thrombocytopenic and anemic neonates are born every year[19,20].

Phytochemical analysis revealed the presence of large chemical groups that are: alkaloids, saponins, phytosterols, triterpenoids, polyphenols, carbohydrate, flavonoids, glycosides, phenolic compounds and tannis and protein. Alkaloids have been proven effective against anti-platelet macrophages in patients suffering from Idiopathic Thrombocytopenic Purpura (ITP) [21]. Saponins are known to inhibit platelet adhesion and aggregation, prevent thrombosis and also improve microcirculation[22].Saponin containing in herbs have been successfully used in the management of liver inflammation, as tonic sedative formulas, to promote and vitalize blood circulation[22-23]. Flavonoids have anti-anaemic potential and veinotonic properties, which protects the blood capillaries[24].

With regard to the weight of the rats, there was a reduction in body weight after induction of anemia by phenylhydrazine [Table 3] but there was not significant change in body weight after induction of thrombocytopenia by heparin [Table 5]. During the treatment weight was increased in both anemic and thrombocytopenic groups when compare with normal group, but no weight again was observed after treatment with standard drug in both cases.

There was a significant decrease in RBC and haemoglobin after admistration of phenylhydrazine in anemic group when were compared with normal group which show induction of anemia. After treatment with the different doses of Asparagus racemosus extract, there was significantly increase in the RBC count and haemoglobin level as well as it maintain MCH value and decrease WBC to normal when were compared with anemic group.

In heparin induced thrombocytopenia group 2 showed a significant increase in the bleeding time and clotting time when were compared with a control group which show induction of thrombocytopenia. After treatment with drug there is significant reduction in the bleeding time and clotting time when were compared with thrombo-cytopenic group.

CONCLUSION:

The injection of phenylhydrazine to rats caused a haemolytic anemia characterized byreducing hematological parameters. The oral administration of extract Asparagus racemosus root in the dose of 750 mg/kg/day significantly (P<0.01) increased RBC count and haemoglobin level and decrease WBC count. It also increased the weight significantly (p<0.01) when compare with control group and anemic group. Injection of heparin cause thrombocytopenia and increase the bleeding and clotting time. After treatment with Asparagus racemosus root extract at a dose of 750 mg/kg/day there was a significant (p<0.01) decrease in bleeding time and clotting time when compare with thrombocytopenic group.

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