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Effect of *Bauhinia variegata* L. and *Glycyrrhiza glabra* L. extracts on immunocompromised male wistar ratsShivani Chauhan^{*♦}, Kavita Rana^{**}, Arti Ghabru^{*}, Dinesh Sharma^{***}, Himani Sharma^{*} and Neerja Rana^{****}^{*}College of Horticulture and Forestry, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Neri-177001, Hamirpur, Himachal Pradesh, India^{**}Department of Microbiology, DAV University, Jalandhar-144012, Punjab, India^{***}Department of Veterinary Pharmacology and Toxicology, CSKHPKV, Palampur-176062, Himachal Pradesh, India^{****}Department of Basic Sciences, COF, Dr YSP UHF, Nauni-173230, Solan, Himachal Pradesh, India

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Abstract

Bauhinia variegata L. and *Glycyrrhiza glabra* L. were considered for the study to evaluate the effect on haematological parameters using their bark and roots, respectively. The plant materials were collected, shade dried and ground to obtain fine powder. Methanolic extraction (95%) was undertaken for preparing the extracts. For *in vivo* study, forty two rats were randomized into seven experimental groups (n=6) for twenty eight day. Group I received PBS (1 ml/100 g b.w, pH 7.4). Groups II, III, IV and V received cyclosporine (5 mg/kg b.w) for first 7 days. Group III received levamisol (3.5 mg/kg b.w). Group IV received *B. variegata* methanolic extract (BVME 250 mg/kg b.w.) for 21 days. Group V received *G. glabra* methanolic extract (GGME 250 mg/kg b.w.) for 21 days. Group VI received BVME (250 mg/kg b.w.) for 28 days. Group VII received GGME (250 mg/kg b.w.) for 28 days. *In vivo* studies results showed that the animals treated with *B. variegata* methanolic extract (BVME) alone had significantly ($p<0.05$) higher haemoglobin values when compared with control group and cyclosporine treated group. Lymphocyte count decreased significantly in immunocompromised rats fed on plant extracts of both *B. variegata* and *G. glabra*, but this decrease was more significant in BVME treated immunocompromised rats as compared to control as well as GGME treated immunocompromised rats. Initially (day 7) monocyte count was found to be decreased in all the Groups which on day 28 increased non-significantly in all the plant extracts treated groups as compared to control. RBC count remains highly significantly low in group II as compared to the control groups. In group treated with *B. variegata* and *G. glabra* alone number of RBC count was comparable to control animals, but *B. variegata* treated both immunocompromised as well as normal animals showed more RBC count as compared to the *G. glabra*. Similarly, Hb level was also significantly higher ($p<0.05$) in animals treated with *B. variegata* alone (Group VI) as compared to the control group. The immunostimulation in immunocompromised animals in terms of lymphocytic and monocytic percentage was seen only with *B. variegata* extract. BBME administration to both immunocompromised (Group IV) as well as to normal rats (Group VI) showed more PCV% as compared to *G. glabra*. Thus, it can be concluded that *B. variegata* is more effective as compared to *G. glabra* as positive affect on the haematology of normal as well as immunosuppressed animals was observed with BBME.

1. Introduction

All we require to stay in perfect health has been graciously provided by Mother Nature as they are excellent suppliers of nutrients and include a number of components that help fight off various diseases, plants meet the vast majority of our daily demands. Regular intake of plant-based foods (fruits, vegetables, seeds, flowers, and vegetables) has been linked with improved health and reduced likelihood of chronic diseases like cancer, diabetes, and rheumatoid arthritis (Swier *et al.*, 2023). Still, a huge variety of plants remain unknown for human consumption or local to a certain location. Since

the beginning of civilizations, plants have served as the foundation of traditional medicine. Different plant sections have different phytochemicals, which are crucial for both illness prevention and disease treatment.

In Indian Ayurvedic medical system, there are about 34 plants known as rasayans, which have a variety of pharmacological qualities, including antiageing, antibacterial, antirheumatic, anticancer, adaptogenic and antistress effects (Agarwal and Singh, 1999). Specifically, a rasayana “promotes cognitive ability and recall and increases immunity against disease” (presumably infections, suggesting the possibility of immunostimulant effects) and “produces longevity, regains youth, acquires a keen intellect and memory, attains freedom from diseases, acquires a lustrous complexion, and attains the stamina of a horse” (Soni and Sharma, 2018).

It also “antiages, lengthens life span.” adaptogens, immunomodulators, probiotics, and antimutagenics are all functions of rasayana plants. Numerous plants with possible immuno-

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modulatory properties have been identified; some of these have even undergone testing to determine how they affect animals and, to a lesser extent, humans (Bamne *et al.*, 2023; Tamoli *et al.*, 2022). The utility of phytochemicals in medicine has been established through extensive experimental trials. However, there are still a lot of therapeutic plants whose phytochemical profiles need to be investigated. Two of these trees include *G. glabra* (Licorice) and *B. variegata* (Kachnar), among many more.

An indigenous medicinal herb called *B. variegata* has rasayana like pharmacological effects (Agarwal and Singh, 1999). The open plantations and damp waste ground are frequent habitats for the *B. variegata* (Caesalpiniaceae) plant (Samant *et al.*, 2014). All of these can be found throughout India and go by different names. Among the biological and therapeutic qualities of the tree are its immunomodulating, antidiabetic, anti-inflammatory, antimicrobial, haematinic, haemagglutinating, cancer fighting, liver protective and antiulcer capabilities (Kumar *et al.*, 2016). In Ayurveda, *B. variegata* is used to cure leprosy, menorrhagia, blood impurities, tuberculous glands, wounds, ulcers, asthma, and other conditions (Kirtikar and Basu, 1993; Nadkarni, 2001). The powdered bark of the plant is an essential element in the herbal tonic Kanchanar guggul, an ayurvedic medication believed to enhance the development of white blood cells. Tannins, steroids, alkaloids, flavonoids, and saponin are found in the stem bark of *B. variegata* according to phytochemical analysis (Parekh *et al.*, 2006; Reddy, 2022). Vitamin C, quercetin, lupeol, flavonone, kaempferol and sitosterol have been identified in the stem bark of *B. variegata* which is extracted using ethanol (Raj Kapoor *et al.*, 2006).

G. glabra is part of the Fabaceae family, usually referred to as Leguminosae, which is one of the most well-known families of medicinal plants. The plant is suggested as a typical treatment for arthritis, bronchitis, coughing, and digestive issues. Specifically, it is still commonly used in conventional medicine to treat peptic ulcers, gastritis, respiratory infections, and tremors. Liquorice has good concentrations of amino acids, simple sugars and mineral salts like iron, selenium, sodium, copper, potassium, calcium, phosphorus, magnesium, silicon, zinc and manganese. According to Wang *et al.* (2015), there are additional starches, resins, pectins, gums and sterols. The primary active ingredient in roots is a compound called glycyrrhizin, a triterpenoid saponin that is almost fifty times sweeter than sucrose (Soulef *et al.*, 2021; Yu *et al.*, 2015). Liquorice's high flavonoid content gives it a yellow hue. Among the flavonoids discovered are flavonones, flavones, flavanonols, chalcones, isoflavans, isoflavones, and isoflavanones (Rizzato *et al.*, 2017). The strong antioxidant activity seen is likely caused by the phenolic content (Rackova *et al.*, 2007).

Despite having long standing usage and well established health advantages, the crops are nevertheless underutilized. However, *B. variegata* and *G. glabra* effect against cyclosporine induced immune suppression has not been investigated so far. Thus, this study intended to estimate the possible effect of the methanolic extract of these plants on haematology of immune suppressed in male wistar rats.

2. Materials and Methods

2.1 Plant material

The *B. variegata* bark was gathered from the vicinity of CSKHPKV in Palampur, Himachal Pradesh, India. The *G. glabra* roots were purchased from the Palampur local market. After being cleaned with

distilled water, these were shade dried for a period of 15 days, powdered and kept in an airtight container until needed again. The powder was utilized to make extracts. The plant material was identified at the Institute of Himalayan Bioresource and Technology, Palampur, Himachal Pradesh, India. Vocher No. PLP 22373 and PLP 22374.

2.2 Preparation of extract

100 g of *B. variegata* bark and *G. glabra* roots powder while constantly stirring were soaked separately in 800 ml of methanol. Filter paper was used to filter the mixture. The filtrates underwent vacuum drying at 40°C in a rotary vacuum evaporator. Following lyophilisation, the extracts were stored for later use at 4°C in airtight containers.

2.3 Experimental animals

Forty two male wistar rats (150-200 g) were employed in the investigation. The Central Animal House at Hisar Agricultural University provided the animals. The Institutional Animal Ethics Committee's approval was rigorously adhered to for all animal research. The animals were housed in polyacrylic cages with standard lighting and dark cycles, temperature ranges of 24-27°C and humidity ranges of 60-65%. They needed seven days to acclimate. The feed was acquired from Department of Animal Nutrition, College of Veterinary and Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. Approval No. (IAEC-Registration No. 259, CSK HPKV, Palampur, Letter No. QSD/VSR/COVAS/11/IAEC/-51-71 dated: 25-4-2011).

2.4 Preliminary phytochemical screening

Preliminary tests were carried out for the presence or absence of phytoconstituents like tannins, alkaloids, saponins, glycosides, flavonoids, sterols, reducing sugar and amino acids in the *G. glabra* and *B. variegata* methanolic extracts.

a. Test for tannins

About 200 mg of the plant extract was boiled with 10 ml of distilled water; and 0.1% ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

b. Test for alkaloids

The plant extract was dissolved in 100 ml of water, filtered, and cooked in steam with 2 ml of the filtrate and 3 drops of 1% hydrochloric acid. Then, 1 ml of the heated mixture was combined with 6 ml of the Mayer-Wagner reagent. The appearance of a cream or brown-red coloured precipitate indicated the presence of alkaloids.

c. Test for saponins

About 0.5 ml of the extract and 5 ml of distilled water were combined and agitated. Then, the formation of foam confirmed the presence of saponins.

d. Test for glycosides

To the extract, 5 ml Molisch's reagent and concentrated H₂SO₄ were added. Violet colour formation indicated glycosides presence.

e. Test for flavonoids

4 ml of extract solution, 1.5 ml of 50% methanol solution a small magnesium chunk were warmed. 5-6 drops of concentrated HCl were added, red colour was observed for flavonoids.

f. Test for steroids

About 1 ml of the crude extract was combined with 10 ml of chloroform and 10 ml of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

g. Test for reducing sugar

To 0.5 ml of plant extract, 1 ml of water, and 5-8 drops of Fehling's solution were added and heated. The presence of reducing sugar was indicated by the appearance of brick red precipitation.

h. Test for amino acids

Two drops of ninhydrin solution are added to 2 ml of the extract filtrate and purple colour proves the presence of amino acids.

i. Test for terpenoids

To 0.5 g of plant extract 10 ml of chloroform was added, shaken

and filtered. To this solution, 2 ml of acetic anhydride was added. Thereafter, the solution was dipped in ice and then 1 ml of concentrated sulfuric acid was carefully poured down on the side of the test tube to form a layer. Appearance of pinkish red colour indicated the presence of terpenoid nucleus.

2.5 Selection of doses

Following an overnight fast, a single bolus dose (2500 mg/kg) of root extract from *G. glabra* (n=5 per species) and bark extract from *B. variegata* (n = 5) was administered to ten male wistar rats. The rats were then observed for alterations in behaviour and mortality for a period of seven days. Since, the animals showed no signs of death upto a dose of 2500 mg/kg, and hence 1/10th of the dose, i.e., 250 mg/kg each was used as the therapeutic dose of *G. glabra* and *B. variegata*. Forty two animals were weighed, given numbers, and divided into seven groups of six animals each, as indicated in Table 1.

Table 1: Treatment protocol with dose quantity

Group	Treatment
I	Control {PBS (pH 7.4) 1 ml/100 g b.w} p.o.
II	Standard immunosuppressant: cyclosporine (i/p): 5 mg/kg b.w (for first 7 days).
III	Cyclosporine followed by standard immunostimulant (Levamisole; 3.5 mg/kg bw, p.o.).
IV	Immunosuppressant + 250 mg/kg b.w, p.o. of BVME (for 21 days)
V	Immunosuppressant + 250 mg/kg b.w, p.o. of GGME (for 21 days)
VI	250 mg/kg b.w, p.o. of BVME (for 21 days)
VII	250 mg/kg b.w, p.o. of GGME (for 21 days)

Immunocompromised rats of Groups IV and V received *B. variegata* bark extract (BVME) and *G. glabra* root extract (GGME) each 250 mg/kg/day, p.o., respectively, for 7th to 28th day of the study and rats in Groups VI and VII having normal immune status were also given same dose (250 mg/kg BW) of BVME and GGME, respectively, for 7th to 28th day of the study.

2.6 Immunosuppression studies

To develop immunosuppression, standard immune-suppressants cyclosporine was administered to the Groups II, III, IV and V. The immuno-suppression was checked by haematological investigations in blood samples withdrawn from retro orbital plexus.

2.6.1 Haematological investigations

On the 28th day of the experiment, blood samples were drawn from each group using a retro-orbital puncture into sterilized EDTA containing vials. A BC-2800vet, MIDRAY Auto haematology analyzer was used to analyze the haematological parameters, including red blood cells (RBC), hemoglobin (Hb), total white blood cell counts (WBC) and leucocyte counts.

2.6.2 Biochemical analysis

Each animal's retro-orbital plexus was used to harvest blood, which was then stored slantwise to facilitate serum separation. Following a centrifugation of the samples, the serum was utilized to estimate total protein and albumin using Erba Mannheim kits.

2.6.3 Growth response

The body weight of the animals was recorded on weekly intervals. The weight gain was calculated using weight on the first day and the last day of the experiment and expressed in terms of grams.

2.7 Statistical analysis

The data was analyzed by variance test using the Graph Pad Instat version 3.00 and the significance differences between mean values were determined using Turkey-Krammer multiple comparison test. The data is presented as mean \pm SE. The inter group comparison were made at 5% level of significance.

3. Results

3.1 Phytochemical constituents

The beneficial properties of medicinal plants are probably due to occurrence of various secondary metabolites, which helps in treatment of many ailments. Therefore, due to strong evidence obtained during screening of phytochemicals various secondary metabolites like flavonoids, phenols, tannins, saponins, alkaloids etc. were reported from the bark extract of *B. variegata* whereas, *G. glabra* methanolic

extract showed the presence of only steroids, saponins, flavonoides and amino acids (Figure1).

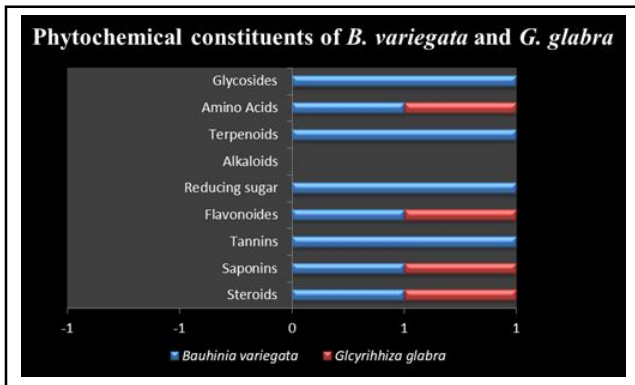


Figure 1: Phytochemicals present in the roots and stem of the plant extracts.

3.2 Haematological investigation

Blood samples were taken from Groups I through V on Day 7 of the

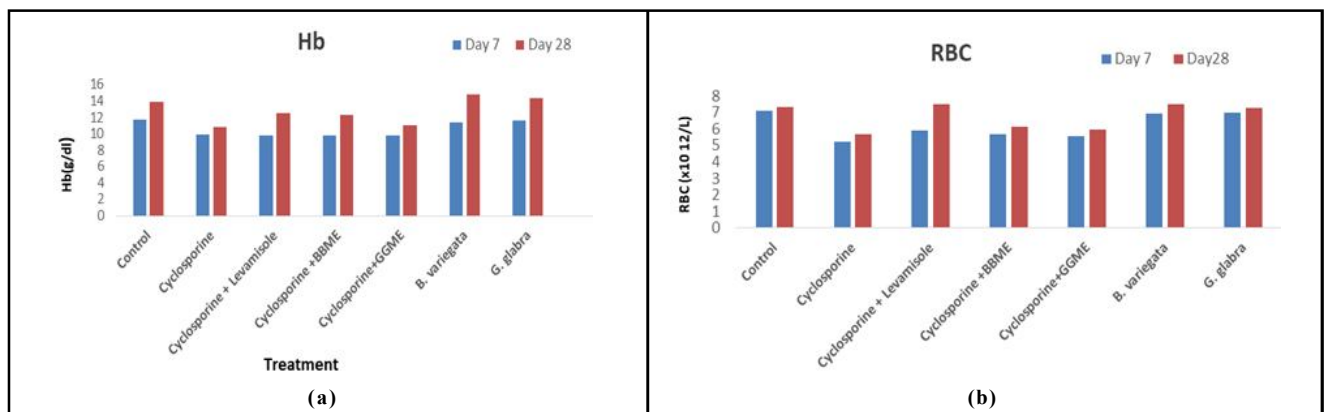


Figure 2: Effect of *B. variegata* and *G. glabra* extracts on, (a) haemoglobin (b) RBC count.

RBC in whole blood decreased significantly in all immunosuppressed groups as compared to the control group. After 28 days treatment with plant extracts, although the number of RBC increased as compared to Day 7, but significant increase was not observed in any group when compared with control. Also, the number of RBC remains highly significantly low in Group II as compared to the control

experiment following the last dose of the standard immuno suppressant. On Day 28, blood samples from all the groups of the experiment were taken *via* retro-orbital puncture of each animal into clean EDTA vials, and haematological parameters such as RBC, hemoglobin (Hb), lymphocyte percentage, monocytes, and packed cell volume (PCV) were analyzed.

There was significant decrease in Hb level on day 7 of experiment in all the immune-suppressed groups as compared to the control group (Figure 2a). It ranged between 9.74 to 9.95 g/dl in all the treated groups. But, on day 28th the experiment, there was an increase in Hb level of all the groups which was significant lower in Group II (10.86 g/dl) and Group V as compared to control group. Hb level of Group II animals (10.86 g/dl) remain significantly (11.1 g/dl, $p < 0.01$) low even after 28 days of experiment as compared to the control Group (13.85 g/dl). The non-immunocompromised animals treated with *B. variegata* extract (Group VI) had significantly higher haemoglobin values when compared with Group VII, *i.e.*, non-immuno compromised *G. glabra* extract treated group.

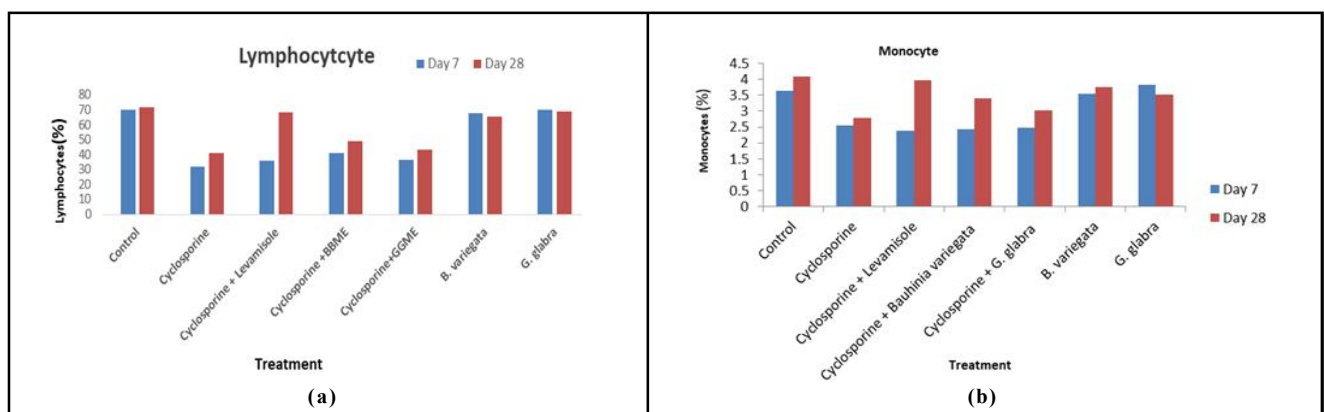


Figure 3: Effect of *B. variegata* and *G. glabra* extracts on, (a) Lymphocyte (b) Monocyte.

Similarly, on Day 7 of the experiment, lymphocyte count of all the immune suppressed groups was significantly low as compared to the control group. It ranged between 31.7 to 40.98% in all the treated groups whereas; on Day 28th of the study, an increase was observed in lymphocytes percentage in all the groups of the experiment (Figure

3a) except Groups VI and VII. However, in Groups II (40.78%), IV (48.92%) and V (43.075%), the level of lymphocytes increased, but was non-significant as compared to the control group (71.82%). As compared to Groups III on Day 28th, V and V. The trend continues with monocytes count in rats on day 7th and 28th of the experiment.

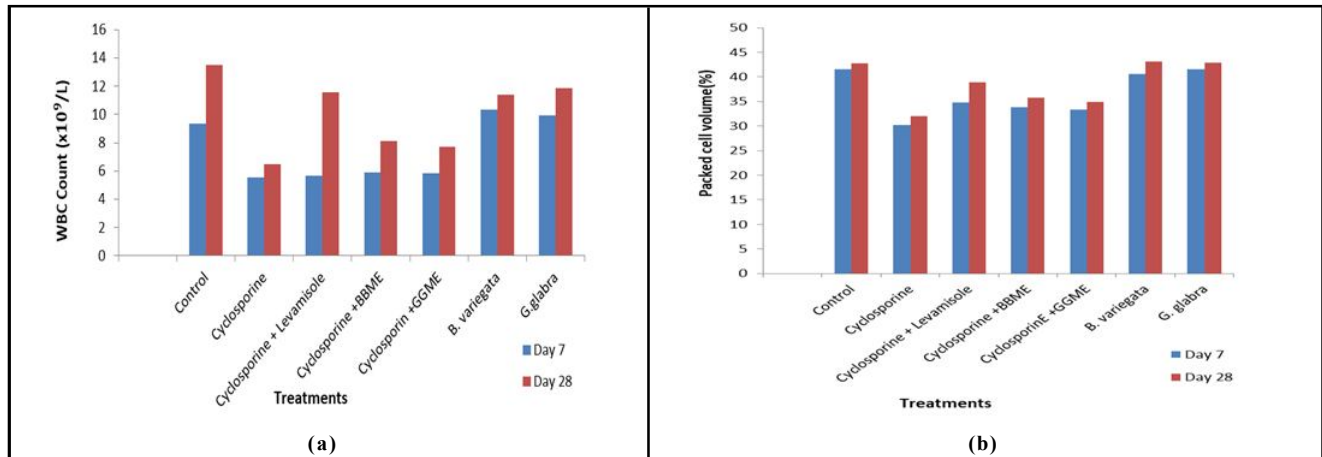


Figure 4: Effect of *B. variegata* and *G. glabra* extracts on (a) WBC count, (b) Packed cell volume.

It was found that on Day 7th after treatment with cyclosporine, the percentage of monocytes decreased significantly in all groups as compared to the control group. On Day 28, percentages of monocytes were significantly lower in Groups II, IV and V rats treated with immunosuppressant (cyclosporine) and with extracts, but in Group VI percentage of monocyte percentage increased non-significantly as compared to the control group (Figure 3b). There was reduction in WBC count after treatment with standard immunosuppressant in all the groups as compared to the control animals (Figure 4a). However, on Day 28 of the experiment, a slight increase in WBC

count was observed in all the immunosuppressed groups. There was a non-significant difference in an increase in the WBC count of *B. variegata* and *G. glabra* extract alone treated groups (VI and VII).

There was a decrease in PCV percentage in all the cyclosporine treated groups as compared to control on Day 7 of the experiment (Figure 4b). On the 28th Day, of the experiment, there was an increase in PCV values in all the extract treated groups. *B. variegata* extract alone treated animals (Group VI) showed non-significant increase in PCV percentage as compared to *G. glabra* extract alone treated group (Group VII).

Table 2: Effect of *B. variegata* and *G. glabra* extract on serum total protein and albumin

Group	Drug	Total protein (g/dl)	Albumin (g/dl)	Body weight (g)		
				Initial body weight	Final body weight	Weight gain
I	Control	8.02 ± 0.13	3.16 ± 0.18	185 ± 8.004	253.5 ± 8.53	68.5 ± 2.26
II	Cyclosporine	4.61 ± 0.20 ^{#####}	1.60 ± 0.13 ^{#####}	193.3 ± 7.22	212.8 ± 6.36 ^{***}	19.5 ± 2.89 ^{***}
III	Cyclosporine followed by levamisole	7.64 ± 0.18	2.59 ± 0.06	195.7 ± 5.04	230.3 ± 7.58	34.6 ± 9.6 ^{**}
IV	Cyclosporine followed by <i>B. variegata</i> bark extract	5.86 ± 0.20 ^{#####}	2.19 ± 0.05 ^{***}	196.7 ± 6.95	227.6 ± 9.21	30.9 ± 9.03 ^{**}
V	Cyclosporine followed by <i>G. glabra</i> root extract	6.28 ± 0.19 ^{#####}	2.24 ± 0.05 ^{***}	182.2 ± 5.37	206.5 ± 4.16 ^{**}	24.3 ± 6.98 ^{**}
VI	<i>B. variegata</i>	7.8 ± 0.19	2.65 ± 0.06 [*]	196.5 ± 4.28	253.8 ± 4.32	57.3 ± 0.91
VII	<i>G. glabra</i>	8.09 ± 0.17	2.85 ± 0.05	191.2 ± 4.84	243.8 ± 4.57	52.6 ± 1.43

*Compared with control, # compared with Group III, *#($p < 0.05$), ***#($p < 0.01$)#####($p < 0.001$).

3.3 Biochemical test

3.3.1 Total protein and albumin

Blood samples from the retro orbital plexus were taken on Day 28 of the trial, and they were placed into sterile, dry centrifugation tubes and heated to 37°C for 30 min. A mini centrifuge was used to separate the clear serum at 2500 rpm for 10 min, and total protein and albumin analyses were conducted as part of the biochemical examination. The impact of BVME and GGME on body weight, total protein and albumin is summarized in Table 2. In Group II, the amount of total protein was significantly low as compared to the control as well as Group III. Also, in Groups IV and V its level was significantly low as comparison to the control Group. But, in Groups VI and VII animals treated with *B. variegata* (7.8 g/dl) and *G. glabra* (8.09 g/dl) extracts only the total protein levels were quite close to control value (8.01 g/dl). On the last day of experiment, it was found that the amount of albumin was significantly low in Group II (1.60 g/dl) as compared to the control (3.16 g/dl). In Groups IV and V animals also the level of albumin was significantly low (2.19 g/dl, 2.24 g/dl, respectively) as compared to the control group.

3.3.2 Body weight

The body weight (g) was recorded on Day 1 and Day 28th of the study using digital weighing balance. On day one, there was non-significant difference in body weight of all the animals as compared to the control group. On Day 7, in immunocompromised groups, there was significant decrease in body weight of all the animals in Groups II-V and was non-significant. Later, on Day 15 of the study, there was slight increase in the body weight of all the animals except Group II as compared to 7th Day body weight. As compared to weight of control group animals, it was significantly low in Groups II, IV and V. On last day of treatment, *i.e.*, on Day 28th of the study, although the body weight increased in Groups II-VII as compared to Day 15th, but was still significantly low as compared to control group in animals of Groups II and V. In body weight measurement, although there was gain in body weight of all the animals as compared to Day 1. It was significantly low in all the immune suppressed rats as compared to control group. Among non-immune compromised rats *B. variegata* extract showed better effect on haematology of rats as compared to *G. glabra* extract.

4. Discussion

The presence of different secondary metabolites, which act as defensive mechanisms against several microbes, is likely the cause of the therapeutic qualities of medicinal plants (Britto and Sebastian, 2011). Thus, a number of secondary metabolites, including phenols, tannins, alkaloids, flavonoids, carotenoids and saponins were measured from different *B. variegata* flower extracts as a result of compelling evidence found during the screening of phytochemicals. Both *B. variegata* chloroform extract (BVC) and *B. variegata* methanolic extract (BVM) had a substantially larger quantity of total phenols (21.34 and 17.66 mg/g, respectively). Consequently, BVM and BVC have flavonoid contents of 18.16 and 12.18 mg/g,

respectively. In comparison to chloroform, methanol extracted a higher number of phytochemicals (Gul *et al.*, 2021). Methanol has been proven to be more effective in separating multiple phytoconstituents and has the potential to enhance the diversity and richness of phytochemical extraction. Finding the bioactive molecules with the help of the first approximation could pave the way for medication development and discovery (Maqsood *et al.* (2017).

There was significant increase in Hb and RBC contents of blood in broiler chickens treated with licorice extract against the aflatoxin toxicity for 35 days (Al-Daraji, 2012). The increase in erythrocyte count and hemoglobin concentration is due to the activation of the mechanism responsible for the migration of immature red blood cells and redistribution of peripheral blood cells from the bone marrow. It was supported by a significant increase in reticulocyte count. *G. glabra* activates proliferation and maturation in the bone marrow erythroid stem cells (Adamyan *et al.*, 2005). Ethanolic extract of *B. variegata* (250 mg/kg/day p.o) treatment in tumor bearing mice changed altered RBC and Hb values near to normal (Rajkapoor, 2003). Cyclosporine exerts its main pharmacological effect as an immunosuppressive by binding to a member of intracellular protein known as immunophilin, forming a complex that interferes with signalling pathway important for the clonal expansion of lymphocytes. There is no difference in the percentages of monocytes and lymphocytes in the quail's blood when dietary licorice at 0.5, 1, and 2 g/kg is consumed (Sedghi *et al.*, 2011) and broiler (Reda *et al.*, 2021). The study conducting by Gul *et al.* (2021) found the decrease in leucocyte in the blood profiling of albino mice fed on flower extract of *B. variegata*. In animals weakened immune system is responsible for rapid increase or decline in haematological parameters and these persistent conditions end in severe consequences (Carvalho *et al.*, 2011).

The results were similar with Rajkapoor *et al.* (2003) findings who reported that extract of *B. variegata* (250 mg/kg) increased the total protein content of the tumor induced mice. Decrease in albumin content has been observed in albino rats treated with *B. variegata* extract (Kohle *et al.*, 2015). Similarly, adding licorice root powder extract to broiler meals had no discernible effect on the serum albumin and globulin levels. 42 days old broiler hens fed on a diet having 1% licorice had higher body weights than the control group birds (Jagadeeswaran and Selvasubramanian, 2014).

5. Conclusion

Initial examinations were conducted to determine whether the methanolic extracts of *B. variegata* and *G. glabra* contained phytoconstituents such as sterols, alkaloids, flavonoids, glycosides, saponins, proteins, carbohydrates and tannins. In *B. variegata*, the results indicated the presence of steroids, saponins, amino acids, tannins, flavonoids, reducing sugars, terpenoides, and glycosides; in *G. glabra*, the methanolic extract revealed their existence. The haematological results revealed that both *G. glabra* and *B. variegata* are effective in maintaining haemoglobin level in immunosuppressed

rats as well as non-immunocompromised rats. The immune-stimulation by *B. variegata* in terms of lymphocytic and monocytic percentage was only seen in immunocompromised animals. *B. variegata* administration to both immune-compromised as well as to normal rats showed more PCV% as compared to *G. glabra*. Biochemical analysis showed that total protein and albumin was higher in *G. glabra* treated groups as compared to *B. variegata* treated groups. In male wistar rats, cyclosporine treatment was found to have negative effects on haematological parameters as well as on serum total proteins, and albumin, but the plant materials from *G. glabra* and *B. variegata* showed their effectiveness in reducing these negative effects to some extent but to precisely understand the mechanism underlying these plant's materials in improving haematological properties, more research is required. Therefore, this study provides scientific validation and support for the traditional usage of *G. glabra* roots and *B. variegata* bark to improve haematological parameters and overall health.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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