

# Hematological and immunomodulatory evaluation of methanolic extract of *Sorghum bicolor* leaves

Maheswara Reddy Mallu, Srinivasa Reddy Ronda, Srinivasulu Kamma, Siva Reddy Golamari, Sandeep Vemula

Department of Biotechnology, Centre for Bioprocess Technology, KLEF, Vaddeswaram, Guntur, Andhra Pradesh, India

## Abstract

**Introduction:** *Sorghum bicolor* has widely reported ethnomedicinal uses which include its use for stimulation and purification of blood as well as body defense. **Aim:** The study aims to determine the hematological and immunological activity of *S. bicolor* leaf base extracts. **Materials and Methods:** The immunological effect of the leaf base extract was evaluated using tests on hematological indices and activated charcoal clearance assay for macrophage phagocytosis. **Results:** The results revealed that the extract of 100–300 mg/kg formed no significant change in the packed cell volume, hemoglobin, red blood cell count, total leukocyte count, monocyte, basophil, eosinophil, bleeding time, and clotting time. The dose of 100 mg/kg produced a significant increase in neutrophil and decrease in lymphocyte. Platelet count was significantly increased at the dose of 300 mg/kg. The extract showed strong stimulation of phagocytic rate at the dose of 300 mg/kg. **Conclusion:** The results did not validate the ethnomedicinal use of the plant leaf base for stimulation of blood production. It, however, showed improvement of non-specific immune responses involving phagocytosis and inflammation.

**Key words:** Ethnomedicinal, hematology, immunomodulatory, inflammation, phagocytosis, *Sorghum bicolor*

## INTRODUCTION

*S. bicolor* belonging to Gramineae family has been reported widely for its ethnomedicinal uses in different parts of the world.<sup>[1]</sup> Sorghum phytochemicals include the use of the plant in the purification and stimulation of blood production.<sup>[2]</sup> It is used to improve the body resistance, blood formation, and as well as fertility.<sup>[3]</sup> Activation of the host's immunological system by any foreign stimulus leads to a spectrum of cellular and humoral events comprising many effector mechanisms involving several cell types, cell products, and soluble serum factors.<sup>[4]</sup> The cellular constituents include mononuclear phagocytes (e.g., monocyte of the circulatory blood and macrophages found in body tissues), granulocytes (neutrophils, eosinophils, and basophils), platelets, and lymphocytes. Some of these cells such as basophils, platelets, neutrophils, and others such as the mast cells, enterochromaffins participate in immunological reactions through the release of chemical substances (mediators)<sup>[5]</sup> that have a variety of biological activities such as increased

vascular permeability,<sup>[6]</sup> contraction of smooth muscle,<sup>[7]</sup> and enhancement of the inflammatory response.<sup>[8]</sup> However, increasing understanding of the relationship between the immune system and cancer points<sup>[9]</sup> out that there is more than one side to this tale, this process, therefore, shows a positive correlation between the hematological activities of the body and the immunological processes. It is an indication that boosting the body's hematological level will possibly boost the immune state of a host.<sup>[10]</sup> Hence, the present work was to add to the directory of agents that can modulate the immunological responses. Immunotherapy could provide an alternative to conventional chemotherapy against variety of diseased conditions; achieve desirable effects of preventing an infection rather than treating it at an advanced stage.<sup>[11]</sup>

### Address for correspondence:

Maheswara Reddy Mallu,  
Department of Biotechnology, Centre for Bioprocess  
Technology, KLEF, Vaddeswaram, Guntur,  
Andhra Pradesh, India.  
E-mail: mahesh\_bt@kluniversity.in

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## MATERIALS AND METHODS

### Extraction of Plant Leaves

Leaves of *S. bicolor* were collected from Guntur District, Andhra Pradesh, India. Leaves were pulverized in a mortar and made into smoothie.<sup>[12]</sup> 100 g of the smoothie sample was cold macerated in 1 L of 70% methanol over 48 h period on an orbital shaker. The extract was then filtered using centrifugal filters (Millipore) which was then placed on water bath to allow evaporation of the solvents and consequent concentration of the extract for subsequent studies. A yield of 18% extract was obtained.

### Hematological Studies

All the animals were anesthetized with chloroform, sacrificed, and their blood collected in EDTA anticoagulant bottles by trained technician from Sri Raghavendra Biotechnologies, Bangalore, Karnataka. The standard method of Baker<sup>[13]</sup> was used to measure the hematological indices which included hemoglobin, packed cell volume, red blood cell count, total leukocyte count differential leukocyte count, platelet count, bleeding time, and clotting time.

### Activated Charcoal Clearance Assay

The blood samples were each lysed in 3 mL of distilled water to perform activated charcoal clearance assay.<sup>[14]</sup> The optical density was then measured with ultraviolet-visible recording spectrophotometer (GENESYS™ 10S) at 650 nm using pre-injection blood sample as blank. The graph of the absorbance was then plotted against time in minutes. The rate of charcoal clearance termed phagocytic index was calculated as the ratio of slope of regression line of treated groups to the slope of regression line of the control.<sup>[15]</sup> Values <1.0 showed no effect, values 1.0–1.5 showed slight stimulation of phagocytic rate while values >1.5 showed strong stimulation of phagocytic rate.<sup>[16]</sup>

### Statistical Analysis

All data are presented as means  $\pm$  S.E.M. Statistical significance was determined by analysis of variance (one-way ANOVA and Bonferroni *post hoc* test), independent sample *t*-test, and where appropriate, Kruskal–Wallis and Mann–Whitney *U*-test. Relationships between variables were assessed using Pearson's correlation. Statistical significance was assumed at  $P < 0.05$ . Statistical analyses were performed using the SPSS 13.0.

## RESULTS

### Hematological Studies

Both increase and decrease were observed at all the tested doses for such parameters as hemoglobin, packed cell volume,

total red blood cells, total leukocyte count, and bleeding time. However, none of these changes was significantly different from the control. Increases and decreases were also observed in neutrophil and lymphocyte indices. However, the increase observed for neutrophil was only statistically significant ( $P < 0.05$ ) at the dose of 100 mg/kg while the decrease observed in lymphocyte was only significantly ( $P < 0.05$ ) different from control at dose of 100 mg/kg also. There was a general but non-significant decrease in the monocyte count. The basophil and eosinophil counts were just as the control count. A general but non-significant decrease was observed in the clotting time while there was increased platelet count in all the doses. The platelet increase was significant at the dose of 300 mg/kg [Tables 1 and 2].

### Activated Charcoal Clearance Assay for Macrophage Phagocytosis

The present study revealed that the ratio of the slope of the regression line for extract-treated groups to the slope of the regression line of the control has phagocytic indices of  $-20.0$ ,  $-1.8$ , and  $2.8$  for 100 mg/kg [Figure 1], 200 mg/kg [Figure 2], and 300 mg/kg [Figure 3] doses of *S. bicolor* leaf base extract, respectively.

## DISCUSSION

The study revealed that the extract had no significant effect on the hemoglobin, packed cell volume, red blood cell count, total leukocyte count, monocytes, basophil, eosinophil, bleeding time, and clotting time of Wistar rats treated for 14 days.<sup>[17]</sup> The neutrophils and platelets of the 14-day-treated rats were, however, significantly ( $P < 0.05$ ) increased at 100 mg/kg and 300 mg/kg doses, respectively, while their lymphocytes were significantly ( $P < 0.05$ ) decreased at 100 mg/kg. The non-significant effect of the extract on most of the hematological indices could, therefore, mean that these blood cell production sites were not made hematopoietically active by the extract. Significant increase ( $P < 0.05$ ) in neutrophil at the dose of 100 mg/kg might be because neutrophils are usually increased in acute conditions and are usually short lived.<sup>[18]</sup> Neutrophils are rapidly mobilized during periods of physical stress and can, therefore, give rise to increased neutrophil count. The result also showed that platelet was increased at all the tested doses of 100–300 mg/kg with statistically significant ( $P < 0.05$ ) increase at 300 mg/kg. In this study, activated charcoal was used as the inert particulate matter and the result showed no activity by *S. bicolor* leaf base extract on macrophage phagocytosis at doses of 100 and 200 mg/kg while the dose of 300 mg/kg showed strong stimulation of phagocytic rate. It is, therefore, possible that the extract at the high dose of 300 mg/kg primarily activated macrophages which, in turn, secrete cytokines to stimulate other immunocytes like neutrophils. This has been suggested for similar plant activities by Makare *et al.*<sup>[19]</sup> This could, therefore, account

**Table 1:** Effect of 70% methanolic extract of *S. bicolor* leaves on some hematological indices of blood sample

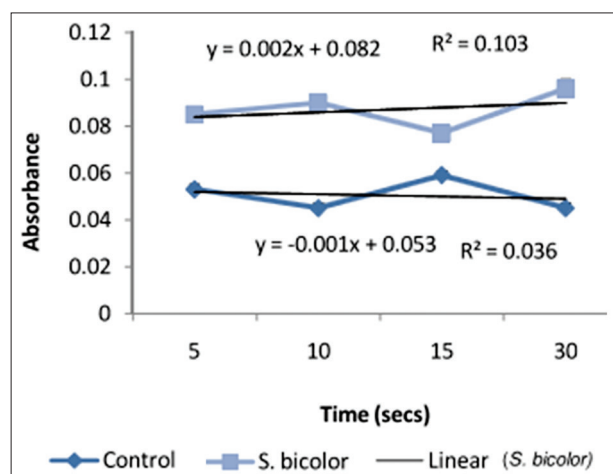
Treatment (14 days)	Hb (g/dL)	PCV (%)	RBC ( $\times 10^{12/L}$ )	WBC ( $\times 10^9/L$ )	Platelet ( $\times 10^9/L$ )	Bleeding time (sec)	Clotting time (sec)
Control	12.72 $\pm$ 0.38	38.0 $\pm$ 0.9	4.84 $\pm$ 0.16	7.78 $\pm$ 0.59	468.0 $\pm$ 14.5	31.4 $\pm$ 4.1	43.2 $\pm$ 2.7
<i>S. bicolor</i> 100 mg/kg	12.16 $\pm$ 0.55	36.4 $\pm$ 1.5	4.78 $\pm$ 0.24	8.22 $\pm$ 1.00	476.0 $\pm$ 17.7	38.0 $\pm$ 6.4	38.0 $\pm$ 2.5
200 mg/kg	12.86 $\pm$ 0.60	38.4 $\pm$ 1.7	5.10 $\pm$ 0.13	8.28 $\pm$ 1.20	491.0 $\pm$ 18.7	41.4 $\pm$ 6.7	36.4 $\pm$ 2.8
300 mg/kg	12.26 $\pm$ 0.30	36.6 $\pm$ 1.9	4.86 $\pm$ 0.29	7.50 $\pm$ 1.10	514.8 $\pm$ 18.9*	23.2 $\pm$ 3.1	38.4 $\pm$ 3.7

WBC: White blood cell, RBC: Red blood cell, *S. bicolor*: *Sorghum bicolor*

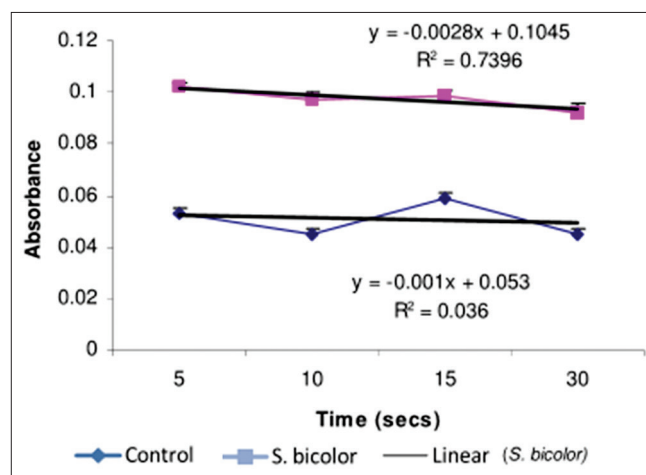
**Table 2:** Effect of 70% methanolic extract of *S. bicolor* leaves on differential leukocyte count of blood sample

Treatment (14 days)	DLC (%)				
	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Control	11.8 $\pm$ 1.2	87.8 $\pm$ 0.9	0.4 $\pm$ 0.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>S. bicolor</i> 100 mg/kg	20.0 $\pm$ 4.2*	79.8 $\pm$ 2.2*	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
200 mg/kg	19.0 $\pm$ 4.3	80.8 $\pm$ 2.0	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
300 mg/kg	11.6 $\pm$ 1.6	88.2 $\pm$ 1.7	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

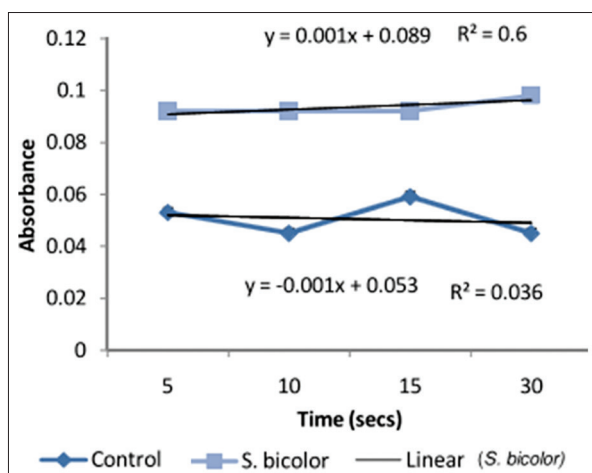
*S. bicolor*: *Sorghum bicolor*



**Figure 1:** Activated charcoal clearance assay for macrophage phagocytosis, blood sample treated with 70% methanolic extract of *Sorghum bicolor* leaves (100 mg/kg)



**Figure 3:** Activated charcoal clearance assay for macrophage phagocytosis, blood sample treated with 70% methanolic extract of *Sorghum bicolor* leaves (300 mg/kg)



**Figure 2:** Activated charcoal clearance assay for macrophage phagocytosis, blood sample treated with 70% methanolic extract of *Sorghum bicolor* leaves (200 mg/kg)

for the increase in the neutrophils observed in the hematology study. The statistically significant ( $P < 0.05$ ) rise in activated charcoal clearance by extract at 300 mg/kg dose could also be indicative of stimulation of reticuloendothelial system.<sup>[20]</sup>

## CONCLUSION

The present study evaluated the extract for hematological and immunomodulatory properties part, of which it is ethnomedicinally used. The immunological models adopted in the evaluation took into consideration both specific and non-specific types of immunity. It is worth noting, however, that the results of the hematological investigation did not support the ethnomedicinal use of the plant leaves for stimulation of blood production. However, the determination of the effect of hematological indices using the entire leaf of

*S. bicolor* plant is recommended instead of just the dark red base of the leaf attached to the sucker.

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