

Protection of Cyclophosphamide Induced Myelosuppression by Extracts of Asparagus setaceus Kunth and Caesalpinia volkensii Harm in Albino Rats

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Abstract

*Cancer is a leading cause of disease worldwide. In 2012, there were an estimated 14.1 million new cases of cancer in the world. Chemotherapy has been one of the ways to manage cancers. Unfortunately, chemotherapeutic agents used have been observed to have toxic side effects limiting their use. Accordingly, several new strategies are being developed to control and treat cancer. One such approach could be a combination of an effective phytochemical with chemotherapeutic agents, which when combined would enhance efficacy while reducing toxicity to normal tissues. There is a continued interest and need for the identification and development of nontoxic and effective chemopreventive compound that can reduce the side effects of cyclophosphamide. This study was conducted to establish the protective effects of extracts of *Asparagus setaceus* Kunth and *Caesalpinia volkensii* Harm against cyclophosphamide induced myelosuppression in albino rats. In one experimental setup, WBC count was first conducted in all groups of animals. Myelosuppression was then induced by treating animals with single doses of cyclophosphamide and another WBC count done on day 4. The animals were then orally administered with the various extracts and WBC count done on day 9. In another experimental setup, the protective effect of the extracts against cyclophosphamide induced myelosuppression in the experimental rats was assessed. WBC count was first conducted in the groups of animals followed by extract treatments and another WBC count. The animals were then treated with single dose of cyclophosphamide and WBC counts done at various days. The results were analyzed using student *t*-test. WBC count was found to be significantly reduced following cyclophosphamide treatment. Administration of dichloromethane leave and all ethanolic, methanolic and aqueous extracts were found to reverse leukopenia in albino rats. Hexane extracts did not reverse leukopenia.*

The extracts were also found to protect the animals against cyclophosphamide induced myelosuppression when animals were first treated with the extracts. These results suggest that the extracts of the two plants are able to reverse myelosuppression induced by cyclophosphamide treatment opening up the possibilities for the use of plant products in the prevention of adverse bone marrow effects associated with cyclophosphamide treatment.

Keywords: Extracts, WBC counts, Cyclophosphamide, Chemopreventive, Myelosuppression, Immunomodulants

Introduction

Cancer is the third leading cause of death worldwide, preceded by cardiovascular and infectious diseases. It is a generic term for a group of more than 100 diseases that can affect any part of the body [1]. Although there are many therapeutic strategies including chemotherapy to treat cancer, high systemic toxicity and drug resistance limit the successful outcomes in most cases. Most of the synthetic chemotherapeutic agents available today are immunosuppressant, cytotoxic and exert several side effects. Cyclophosphamide is probably the most common antineoplastic used in cancer chemotherapy and is an essential component of several effective chemotherapeutic formulae. It has a broad spectrum of activity against a variety of cancers. However, cyclophosphamide shows potent immunosuppressing properties [2,3] having suppressive and cytotoxic activity. It has a number of side effects in long term treatment. The major side effect of this drug is on immune system and haematological suppression [4,5]. Cyclophosphamide administration induces acute and transient myelosuppression, primarily through damage to rapidly proliferating hematopoietic progenitors and their mature progeny leading to decline in the number of peripheral blood cells [6]. Cytotoxicity towards normal host tissue is the primary dose limiting factor in cyclophosphamide therapy that reduces quality of life and restricts treatment protocol [6]. Accordingly, several new strategies are being developed to control and treat cancer. One such approach could be a combination of an effective phytochemical with chemotherapeutic agents, which when combined would enhance efficacy while reducing toxicity to normal tissues. There is a continued interest and need for the identification and development of nontoxic and effective chemopreventive

compound that can reduce the side effects of cyclophosphamide. Plants afford us such an opportunity. A number of medicinal plants have attracted the interest of scientists and plant extracts used in traditional therapy are being investigated for their chemopreventive activities [5-7]. Two Kenyan plants namely *Asparagus setaceus* Kunth and *Caesalpinia volkensii* Harm are such plants that could be investigated on their chemopreventive activity. *Asparagus setaceus* belong to the family Liliaceae while *Caesalpinia volkensii* belongs to Caesalpinaceae. Many medicinal uses of the various parts of plants from these two families have been reported in traditional folklore medicines. These have reportedly been used in the treatment of inflammatory diseases, bronchitis, pneumonia, syphilis and other venereal diseases, malaria and anti-helminthic [8,9]. The extracts of these two plants have been investigated and demonstrated to possess immunomodulatory activities in experimental animals [10-12]. In current study, we investigated extracts of the two plants for protection in cyclophosphamide induced myelosuppression in albino rats since little is known about the ability of these extracts to reverse cyclophosphamide induced myelosuppression in experimental animals.

Materials and Methods

Experimental Animals

Male and female albino rats weighing between 140-210 g used in the study were obtained from the animal house in the Department of Zoology, JKUAT. They were housed five per cage, maintained in an animal house under a 12:12-h light-dark cycle at a temperature of 25°C and fed on rat pellet and tap water *ad libitum*.

Plant Materials and their Collection

Plant materials were collected from Gatundu [1°3'0'S: 36°54'0'E] located in Central province of Kenya during the month of January 2013. Gatundu is approximately 40 km north of Nairobi. Aerial part and root of *Asparagus setaceus* and the leaves, stem and root of *Caesalpinia volkensii* were collected. The plants were identified in the herbarium, Department of Botany JKUAT, where voucher specimens were deposited. The plant materials were dried under shade at temperature below 30°C and pulverized in a hammer mill fitted with a sieve of 0.5 mm pore

Preparation of Plant Extracts

Preparation of Organic Extracts

The grounded plant material was extracted twice with organic solvents, hexane, dichloromethane, methanol and 90% ethanol at room temperature. Hundred grams of plant powder were extracted by mixing with 300 ml of the extracting solvent. The slurry of solvent and plant powder was stirred and left to stand for 48 hours, after which the supernatant was decanted. The decanted supernatants were filtered through Whatman® GF/C glass microfibre filter paper and the filtrate concentrated under vacuum at 40°C in Buchii rotary evaporator and dried in a freeze drier. The dry extracts were weighed and kept desiccated at 4°C until use.

Preparation of Aqueous Extracts

Plant powders weighing 300 g were boiled for twenty minutes in 800 ml of distilled water. After cooling to room temperature, the supernatants were decanted, centrifuged at 5400 × gravity for 10 min after which the supernatants were filtered through Whatman® GF/C glass microfibre filter paper, frozen at -15°C and then dried in a freeze-drier. The extracts were weighed and kept desiccated at 4°C.

Preparation of Extracts for Administration to Rats

The dichloromethane, hexane, methanol, ethanol and aqueous extracts of *C. volkensii* leaf, stem and root, and *A. setaceus* aerial part

and root were prepared by dissolving in dimethyl sulfoxide. All the plant extracts were dissolved so that the final volume of the solutions did not exceed 1 ml.

Experimental Groups

Rats were divided into 27 groups of 5 individuals each with two groups serving as controls. The groups were categorized as follow: Group 1 to 5 rats treated with dichloromethane extracts of *C. volkensii* leaf, stem and root and *A. setaceus* aerial part and root respectively and coded as DCVL, DCVS, DCVR, DASA, and DASR. Groups 6 to 10 were treated with methanolic extracts and coded as MCVL, MCVS, MCVR, MASA and MASR while groups 11 to 15 were treated with ethanolic extracts of the two plant parts and coded as follow, ECVL, ECVS, ECVR, EASA and EASR. Groups 18 to 22 were treated with hexane extracts of the two plant parts (HCVL, HCVS, HCVR, HASA and HASR) whereas groups 23 to 27 were treated with aqueous extracts of *C. volkensii* and *A. setaceus* and coded as ACVL, ACVS, ACVR, AASA and AASR. Group 16 categorized as the dry control was not manipulated in any way while Group 17 which was taken as treated control was administered with the solvent used to dissolve the extracts, dimethyl sulfoxide. All the other remaining groups were treated with the various crude extracts of *C. volkensii* and *A. setaceus*.

Bleeding of the rats

Blood was obtained from the tails of the rats. The tails were first sterilized by swabbing with 70% ethanol and then the tip of the tails snipped with sterile scissors. Bleeding was enhanced by gently "milking" the tail from the body towards the tip. After the operation, the tip was sterilized again by swabbing with 70% ethanol.

Evaluation of the Effect of Crude Extracts on Total WBC Counts in Cyclophosphamide Treated Rats

Animals were divided into the groups as mentioned in experimental group section. Each group consisted of a minimum of 5 animals. Total WBC were conducted in these groups of rats to establish their total WBC levels. Total

white blood cell counts in tail blood of rats was determined by the method of Srikumar *et al.* [13] using a Neubauer haemocytometer. Leukopenia was then induced by treating the rats with 200 mg cyclophosphamide per kilogram body weight intra-peritoneally. Once a drop in the total WBC level was ascertained, treatment with the crude extracts was conducted for a total of three days. The treated groups were dosed orally with the extracts at a dosage of 500 mg/kg body weight for three consecutive days. The plant crude extracts were administered through intra gastric route using the stomach tube to ensure the safe ingestion of the extracts and the vehicle. Total WBC counts were then conducted to determine if there was reversal of the leukopenic effect of cyclophosphamide. Control groups in all cases received physiological saline.

Results

Effects of the Extracts of *C. volkensii* and *A. setaceous* on Reversing WBC Reduction in Cyclophosphamide Treated Rats

Four days after CP treatment, there was a significant drop in the mean WBC count to about 6000 cells/mm³ in all the groups of rats (P=0.000). A significant increase in mean WBC counts was observed 9 days later after the administration of dichloromethane extract of the *C. volkensii* leaves (P=0.002). A significant reduction in WBC counts was seen 4 days after CP treatment (P=0.000) but following treatment of rats with methanol, ethanol and aqueous extracts of *C. volkensii* leaves, WBC counts was observed to increase significantly 9 days later following administration (P=0.000). With hexane extract, a significant drop was seen after CP injection ($t_{4(1)}=21.414$, P=0.000) whereas the extract was ineffective in reversing the decrease in WBC counts following CP injection (P=0.438) (**Figure 1**).

Four days following CP treatment, there was a significant drop in the WBC count (P=0.000). There was no change in WBC counts 9 days later after the administration of dichloromethane extract of the *C. volkensii* stem (P=1.000). With the methanol, ethanol and aqueous extracts, significant reduction in WBC counts were observed 4 days after CP

treatment (P=0.001). Methanol, ethanol and aqueous extracts of *C. volkensii* stem caused the WBC counts to significantly rise after 9 days following their administration (P=0.001). With hexane extract, a significant drop was seen after CP injection (P=0.000) whereas the extract was ineffective in reversing the decrease in WBC counts following CP treatment (P=0.885) (**Figure 2**).

WBC count decreased significantly 4 days after CP treatment (P=0.000). There was no change in WBC counts 9 days later after the administration of dichloromethane extract of the *C. volkensii* roots (P=0.843). With the methanol, ethanol and aqueous extracts, significant reduction in WBC counts were seen 4 days after CP treatment (P=0.000). The three extracts caused the WBC counts to significantly rise after 9 days following administering (P=0.001). With hexane extract, a significant drop was seen after CP injection (P=0.000) whereas the extract was ineffective in reversing the decrease in WBC counts following CP injection (P=0.275) (**Figure 3**).

Four days following CP treatment, there was a significant drop in the WBC count (P=0.000). There was no alteration in WBC counts 9 days later after the administration of dichloromethane extract of the aerial parts of *A. setaceous* (P=0.138). With the methanol, ethanol and aqueous extracts, significant reduction in WBC counts were seen 4 days after CP treatment (P=0.000). The three extracts caused the WBC counts to significantly rise after 9 days following their administering (P=0.000). With hexane extract, a significant drop was seen after CP injection (P=0.000) whereas the extract was ineffective in reversing the decrease in WBC counts following CP injection (P=0.636) (**Figure 4**).

After 4 days following CP treatment there was a significant drop in the WBC count (P=0.000). There was no change in WBC counts 9 days later after the administration of dichloromethane extract of the roots of *A. setaceous* (P=0.398). With the methanol, ethanol and aqueous extracts, significant reduction in WBC counts were observed 4 days after CP treatment (P=0.000). The three extracts caused the WBC counts to significantly rise after 9 days (P=0.000). With

hexane extract, a significant drop was seen after CP injection ($P=0.000$) whereas the extract was ineffective in reversing the decrease in WBC counts following its injection ($P=0.069$) (**Figure 5**).

CP treatment was observed to cause a significant drop in the WBC count 4 days after administering ($P=0.000$). There was a rise in WBC counts 9 days later after administration of dichloromethane extract of the leaves which was highly significant ($P=0.002$). With the dichloromethane extracts of *C. volkensii* stem and root, significant reduction in WBC counts were seen 4 days after CP treatment ($P=0.000$). The two extracts did not have any effect on the WBC counts 9 days after administration ($P=1.000$ and $P=0.843$). With the dichloromethane extract of *A. setaceous* aerial parts, a significant drop was seen after CP injection ($P=0.000$) whereas the extract was ineffective in reversing the decrease in WBC counts ($P=0.138$). The dichloromethane extract of *A. setaceous* root was also ineffective in reversing the decrease in WBC count ($P=0.398$) after a significant drop ($P=0.000$) following CP treatment (**Figure 6**).

A significant drop in the WBC count was observed 4 days after CP treatment, ($P=0.000$). There was a rise in WBC counts 9 days later after the administration of methanol extracts of the *C. volkensii* leaf which was highly significant ($P=0.000$). With the methanol extracts of *C. volkensii* stem and root, significant reductions in WBC counts were seen 4 days after CP treatment ($P=0.000$). The two extracts caused an increase in WBC counts 9 days after administration ($P=0.001$). With the methanol extract of *A. setaceous* aerial part, a significant drop was seen after CP injection ($P=0.000$) whereas the extract was very effective in reversing the decrease in WBC counts following CP injection ($P=0.000$). The methanol extract of *A. setaceous* root was also effective in reversing the decrease in WBC count ($P=0.000$) after a significant drop ($P=0.000$) following CP treatment (**Figure 7**).

Four days after CP treatment, there was a significant drop in the WBC count ($P=0.000$). There was a rise in WBC counts 9 days later after the administration of ethanol extracts of the *C. volkensii* leaf which was highly

significant ($P=0.000$). With the ethanol extracts of *C. volkensii* stem and root, significant reduction in WBC counts were seen 4 days after CP treatment ($P=0.001$). The root extracts caused the WBC counts to rise significantly 9 days after their administration ($P=0.001$) while the stem extract did not have an effect ($P=0.142$). With the ethanol extract of *A. setaceous* aerial part, a significant drop was seen after CP injection ($P=0.000$) whereas the extract was very effective in reversing the decrease in WBC counts following CP injection ($P=0.000$). The ethanol extract of *A. setaceous* root was also effective in reversing the decrease in WBC count ($P=0.000$) after a significant drop ($P=0.000$) following CP treatment (**Figure 8**).

Four days following CP treatment, there was a significant drop in the WBC count ($P=0.000$). There was no effect on WBC counts 9 days later after administration of hexane extracts of *C. volkensii* leaf ($P=0.438$). With the hexane extracts of *C. volkensii* stem and root, significant reduction in WBC counts were seen 4 days after CP treatment ($P=0.000$). The two extracts did not cause any rise in the WBC counts 9 days after administration ($P=0.885$ and $P=0.275$). With the hexane extracts of *A. setaceous* aerial part, a significant drop was seen after CP injection ($P=0.000$) whereas the extract was very ineffective in reversing the decrease in WBC counts following CP injection ($P=0.636$). The hexane extract of *A. setaceous* root was also ineffective in reversing the decrease in WBC count ($P=0.069$) after a significant drop ($P=0.000$) following CP treatment (**Figure 9**).

A significant drop in the WBC count was observed 4 days after CP treatment, ($P=0.000$). There was a rise in WBC counts 9 days later after the administration of aqueous extracts of the *C. volkensii* leaf which was highly significant ($P=0.000$). With the aqueous extracts of *C. volkensii* stem and root, significant reductions in WBC counts were seen 4 days after CP treatment ($P=0.000$). The two extracts caused an increase in WBC counts 9 days after administration ($P=0.001$). With the aqueous extract of *A. setaceous* aerial part, a significant drop was seen after CP injection ($P=0.000$) whereas the extract was very effective in reversing the decrease in WBC

counts following CP injection ($P=0.000$). The aqueous extract of *A. setaceous* root was also effective in reversing the decrease in WBC count ($P=0.000$) after a significant drop ($P=0.000$) following CP treatment (**Figure 10**).

Assessing the Time Taken by Methanolic, Ethanolic and Aqueous Extracts of *C. volkensii* and *A. setaceous* in reversing the Effects of Cyclophosphamide

WBC count was done before any treatment, then on the fourth day after CP treatment. WBC counts were again performed starting on the 3rd day, and then on the 6th, 9th, 12th and 15th day after extracts treatment. One group was injected with lithium carbonate and acted as positive control. CP injection caused the WBC counts to drop to 6000 cells/mm³ in all groups of experimental animals and this was found to be statistically significant (ANOVA, $F_{6,42}=6.4594$, $P=0.0001$). It was observed that the methanolic extracts of *C. volkensii* and *A. setaceous* were effective in reversing a decline in WBC counts induced by CP injection. Most of the extracts had reversed the negative effect of CP drugs by day 9 which was also found to be statistically significant (ANOVA, $F_{6,42}=5.7807$, $P=0.0002$). Lithium carbonate took a few days in reversing the suppressive effect of CP. The control group injected with CP only experienced decline in WBC counts with levels going below 2500 cells/mm³ by day 12 (**Figure 11**).

CP injection in groups of rats resulted in a significant drop in WBC counts to about 6000 cells/mm³ after 4 days and this reduction was observed to be statistically significant (ANOVA, $F_{6,42}=6.86105$, $P=0.0000$). The ethanolic extracts of the two plants and lithium carbonate were very effective in reversing the decline in WBC counts following CP injection. Lithium carbonate took few days in reversing the suppressive effect of CP whereas most of the extracts had reversed the negative effect of CP drugs by day 9 which was also found to be statistically significant (ANOVA, $F_{6,42}=6.40513$, $P=0.0001$). There was a rapid decline in WBC counts of the negative control group of rats which never recovered from CP treatments with their WBC counts going below 2500 mm³ by day 12 (**Figure 12**).

A drop in WBC counts was seen following CP injection (ANOVA, $F_{6,42(1)}=5.772427$, $P=0.0002$). From **Figure 13**, it can be seen that the aqueous extracts and lithium carbonate were very effective in reversing a decline in WBC counts and lithium carbonate took a few days in reversing the suppressive effect of CP whereas most of the extracts had reversed the negative effect of CP drugs by day 9 which was also found to be statistically significant (ANOVA, $F_{6,42(1)}=7.080849$, $P=0.000049$). There was a rapid decline in the negative control group with WBC counts levels going below 2500 mm³ by day 13.

The extracts of aqueous, ethanol and methanol of *C. volkensii* leaves, stem and roots and *A. setaceous* aerial parts and roots were also evaluated on their ability to protect pretreated rats against cyclophosphamide induced myelosuppression and to determine whether pretreatment is necessary. The methanolic extracts of *C. volkensii* and *A. setaceous* caused a rise in WBC counts by day 9 following their administration. Cyclophosphamide treatment caused a drop in the WBC counts three days later in all groups of experimental animals which then remained almost at the same level at day 6, 9 and 12. When compared to the negative control, the extracts performed well in protecting the rats from the effects of CP. Lithium carbonate proved to be superior to all the extracts (**Figure 14**).

All the ethanolic extracts caused an increase in WBC counts by day 9 (**Figure 15**). Cyclophosphamide treatment caused a drop in the WBC counts 3 days after its administration. For most ethanolic extracts WBC counts then stabilized by day 6 and remained at the same level at day 9 and 12. WBC counts decreased to very low levels in the negative control group with the count dropping to about 3000 cells/mm³.

All the aqueous extracts caused a rise in WBC counts by day 9. Cyclophosphamide treatment caused a drop in the WBC counts 3 days after its injection and this continued to drop slightly but had stabilizes by day 6 and then remained stable at day 9 and 12. WBC count at day 12 after cyclophosphamide injection was almost at the same level of WBC counts at the beginning of the experiments before the rats were treated

with any extracts. This was an indication that the aqueous extracts could actively protect the rats from the suppressive effects of CP (**Figure 16**).

Discussion

As expected, cyclophosphamide caused a significant drop in WBC in rats, but upon treatment of the rats with the methanol, ethanol and aqueous extracts of *C. volkensii* (leaf, stem and root) and *A. setaceous* (aerial part and root), an increase in total WBC was observed, when compared to the negative control treated with cyclophosphamide. Lithium carbonate was superior to extracts in reversing the effects of cyclophosphamide induced leukopenia. In pre-treated rats, methanol, ethanol and aqueous extracts of *C. volkensii* (leaf, stem and root) and *A. setaceous* (aerial part and root), were found to have an active protective effect in rats, when administered before cytostatic. Injection of a single dose of 200 mg/kg cyclophosphamide produced a fall in total WBC on the third day. A rebound increase in total WBC count occurred on the 9th day. The leukopenia which occurred in control animals following cyclophosphamide was less marked in the treated animals. A point to note is that WBC counts in all extract treated animals never went below 6000-8000 cells/mm after injection of cyclophosphamide. Thatte *et al.* [14] reported that *A. racemosus* could be producing leucocytosis probably by activating macrophages. Activated macrophages are known to secrete a large number of colony stimulating factors and IL-1 which in turn stimulate other immunocytes like neutrophils [4]. IL-1 and CSF are substances known to cause leucocytosis and it appeared that activation of macrophage produced protection of the rats against cyclophosphamide induced leukopenia. It may be that the extracts of *A. setaceous* and *C. volkensii* also act in this manner to confer protection. The methanolic, ethanolic and aqueous extracts showed an increase in WBC. These results are also in agreement with other studies. Administration of the ethanolic extract of *A. paniculata* in cyclophosphamide treated mice was found to enhance total WBC count which was drastically reduced in the cyclophosphamide alone treated control animals suggesting that cyclophosphamide induced myelosuppression

was reversed or inhibited by extract administration through its immunomodulating activity [6]. Preclinical studies investigating the effect of *A. racemosus* against the myelosuppressive effects of single and multiple doses in mice showed that it prevented to a significant degree, leukopenia produced by cyclophosphamide. It is now accepted that *A. racemosus* is a potent immune-stimulant with effects comparable to lithium and glucan producing leucocytosis with predominant neutrophilia [14]. Nayak and Abhilash [5] demonstrated that alcoholic extract of *Pimenta dioica* leaves increased WBC counts in mice treated with cyclophosphamide after 10 days of treatment. The increase in WBC was attributed to the immune stimulant properties of the *P. dioica*. In another study whose result conflicts with the result of this study, Khoo and Ang [7] demonstrated that the aqueous extracts of *Astragalus membranaceus* and *Ligustrum* did not prevent cyclophosphamide induced myelosuppression. It did not delay the onset, hasten the recovery or shorten the duration of Leukopenia. Geidam *et al.* [15] demonstrated that aqueous stem extract of *Momordica balsamina* linn elevated WBC counts in normal rats but did not affect other hematological parameters. Administration of *Rubia cordifolia* was demonstrated to increase total WBC count in immunocompromised albino rats indicating that the extract was able to stimulate hemotopoetic system. n- butanol soluble and ethyl acetate soluble fractions of methanolic extract of *Lagenaria siceraria* fruits significantly increased the WBC and lymphocyte counts. Increase in WBC count was observed in *Withania somnifera* treated mice compared to untreated control [16]. In a review by Sagrawat and Khan [17], *A. racemosus* was reported to protect rat and mice against experimentally induced abdominal sepsis while oral administration of decoction of powdered roots of *A. racemosus* produced leucocytosis along with enhanced phagocytic activity of the macrophages and polymorphs. Sheeja and Kuttan [6] demonstrated that intraperitoneal administration of the extracts of *Andrographis paniculata* significantly increased the total WBC count and bone marrow cellularity.

Conclusion

The study has demonstrated that aqueous, ethanolic and methanolic extracts of *A. setaceous* and *C. volkensii* are effective in protecting rats against cyclophosphamide induced leukopenia. It showed that the extracts offer protection against cyclophosphamide induced myelosuppression and hence holds promise as candidates to overcome this problem. These results open up exciting possibilities for the use of plant products in the prevention of adverse bone marrow effects associated with cyclophosphamide treatment.

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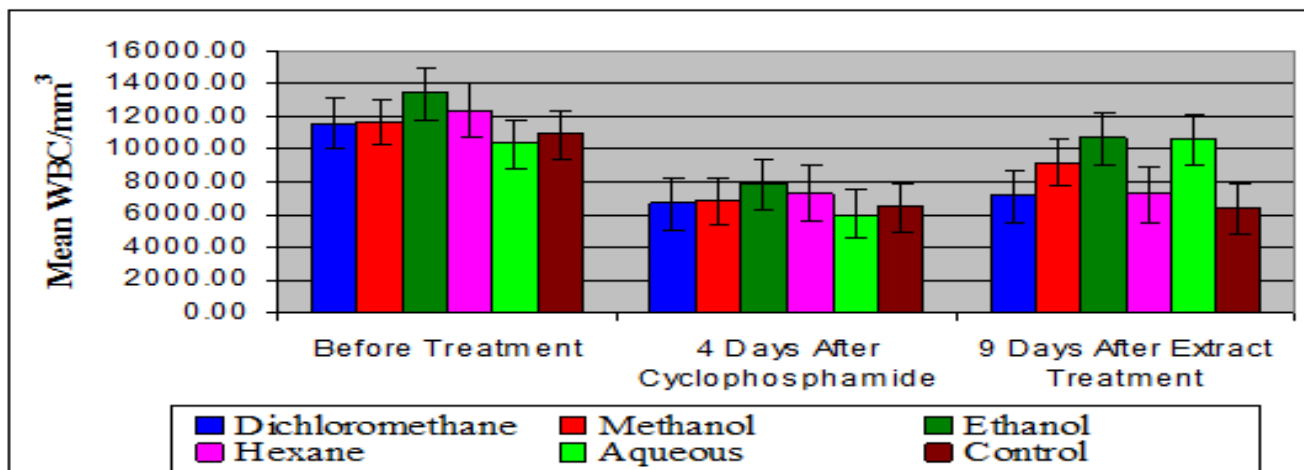


Figure 1: Effect of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* leaves on WBCs counts in rats 4 days after cyclophosphamide treatment and 9 days later after extracts treatment.

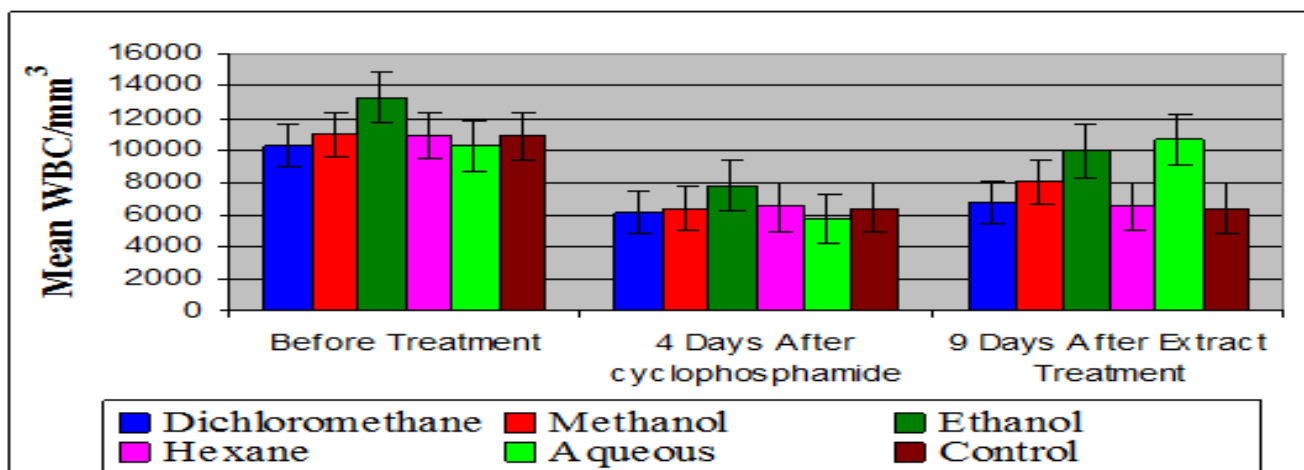


Figure 2: Effect of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* stem on WBCs counts in rats treated with cyclophosphamide.

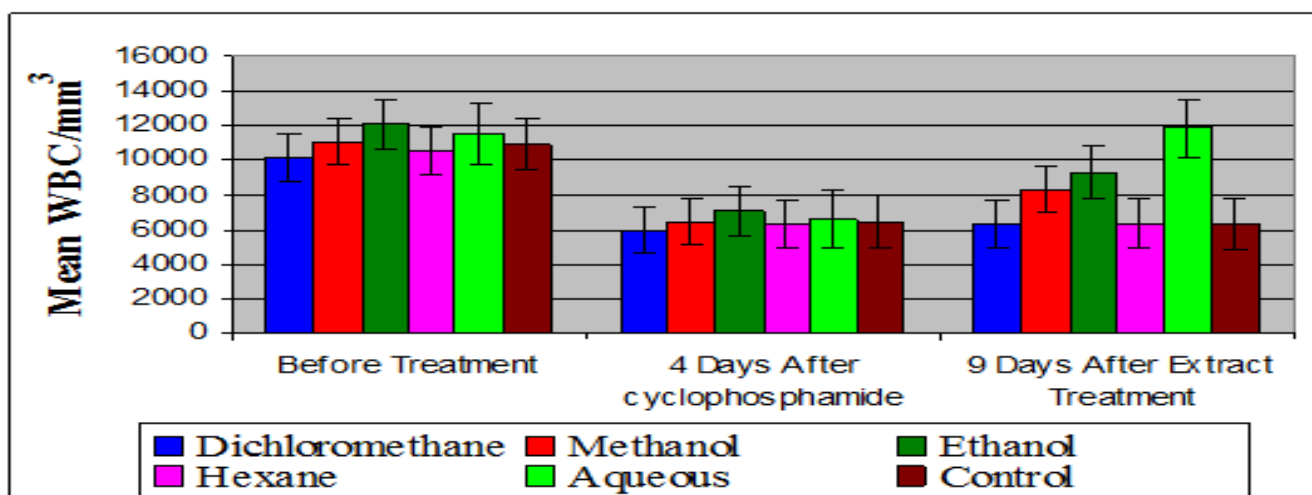


Figure 3: Effect of dichloromethane methanol, ethanol and hexane extracts of *C. volkensii* root on WBCs counts in rats treated with cyclophosphamide. *Control group was administered with dimethyl sulfoxide.

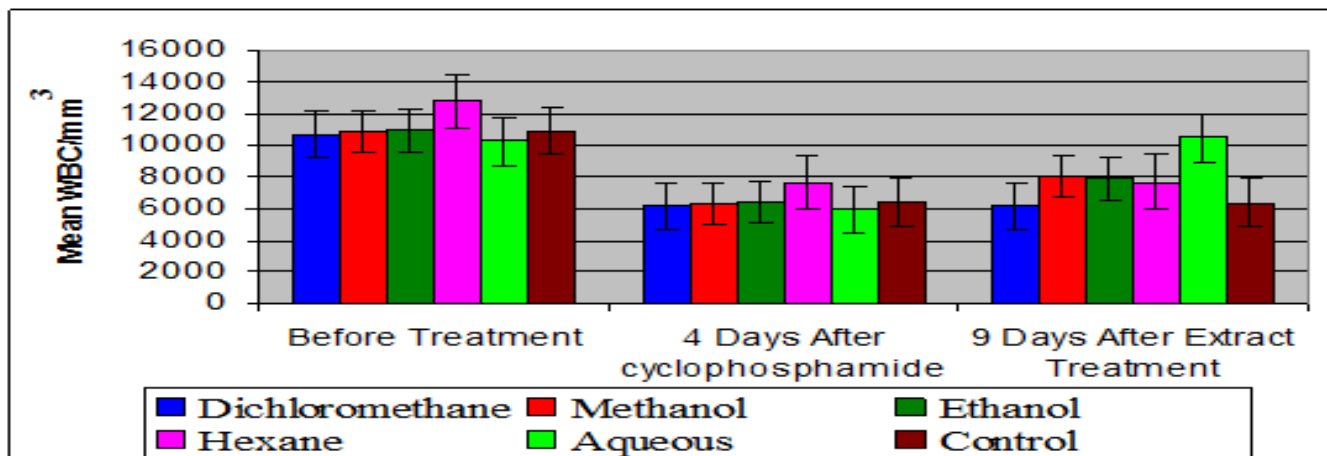


Figure 4: Effect of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *A. setaceous* aerial part on WBCs counts in rats treated with cyclophosphamide. *Control group was administered with dimethyl sulfoxide.

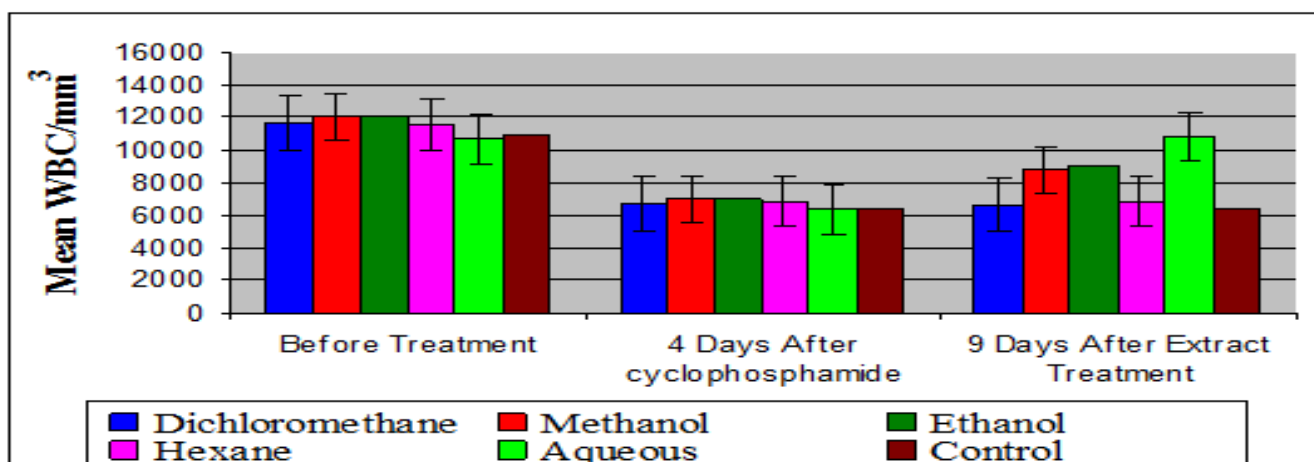


Figure 5: Effect of dichloromethane methanol, ethanol hexane and aqueous extracts of *A. setaceous* roots on WBCs counts in rats treated with cyclophosphamide. *Control group was administered with dimethyl sulfoxide.

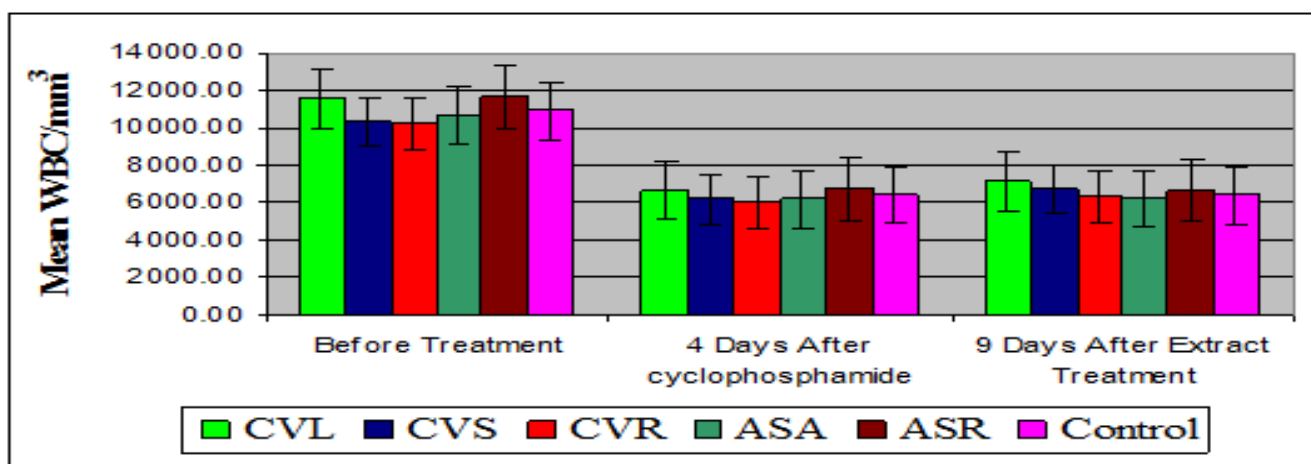


Figure 6: Effects of dichloromethane extracts of *C. volkensii* and *A. setaceous* on WBC counts in rats treated with cyclophosphamide*Control group was administered with dimethyl sulfoxide.

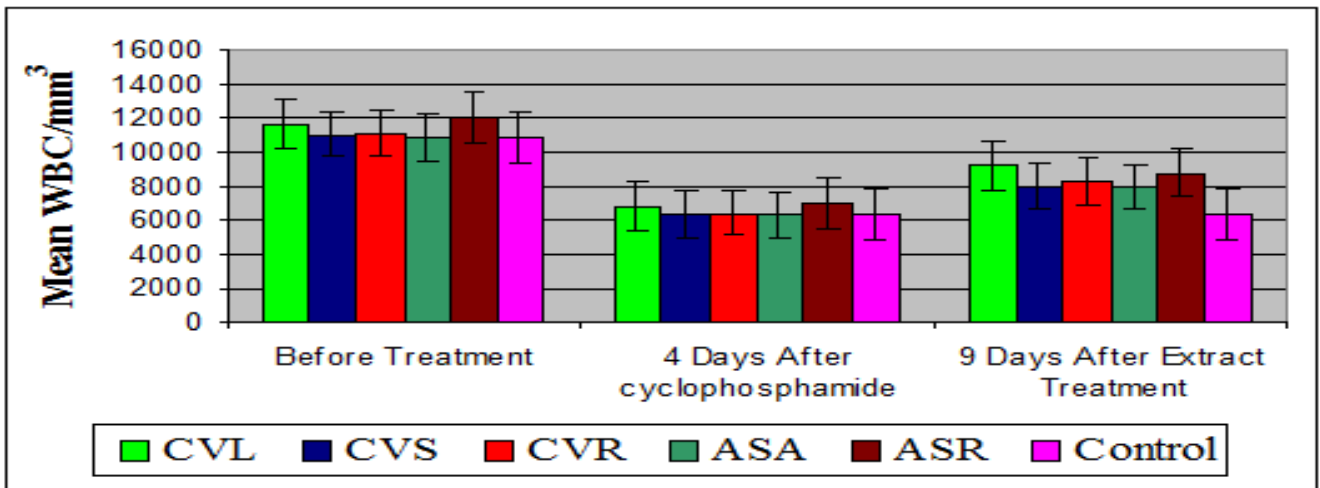


Figure 7: Effects of methanol extracts of *C. volkensii* and *A. setaceous* on WBCs counts in rats treated with cyclophosphamide.

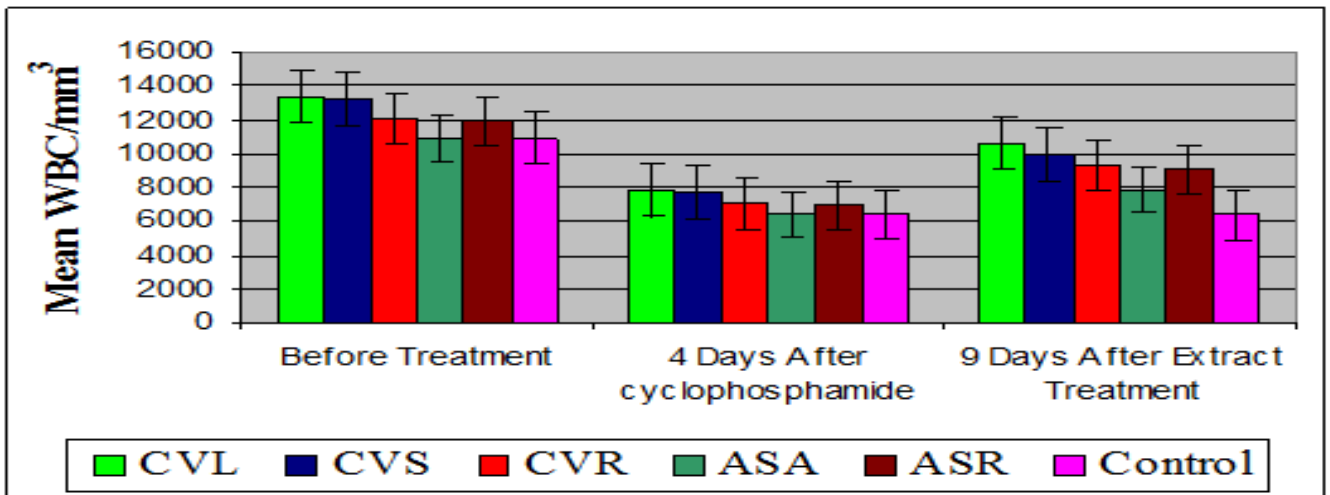


Figure 8: Effects of ethanol extracts of *C. volkensii* and *A. setaceous* on WBCs counts in rats treated with cyclophosphamide.

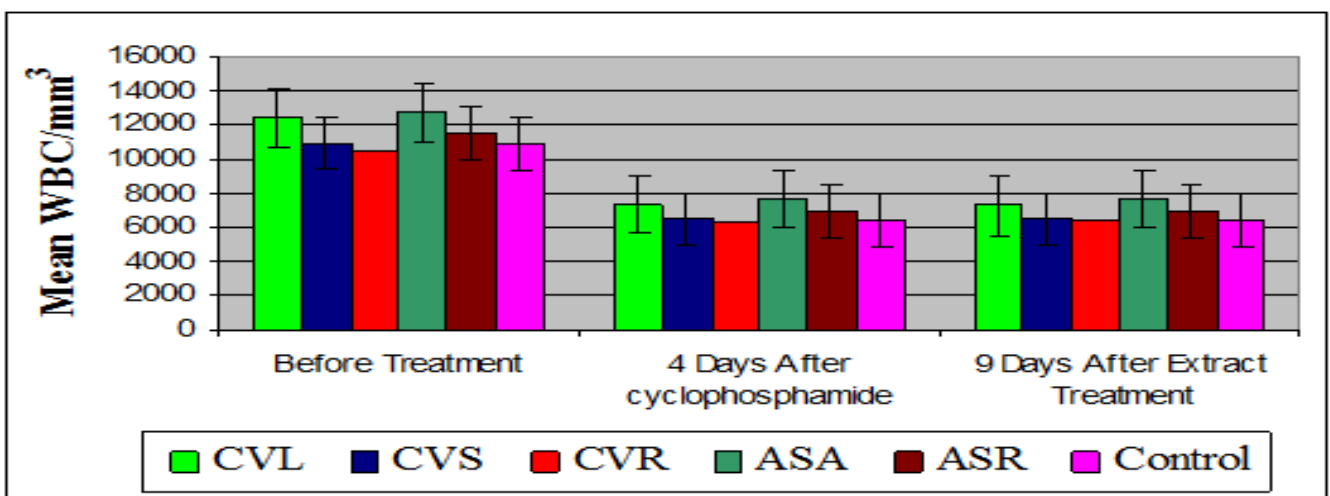


Figure 9: Effects of hexane extracts of *C. volkensii* and *A. setaceous* on WBC counts in rats treated with cyclophosphamide.

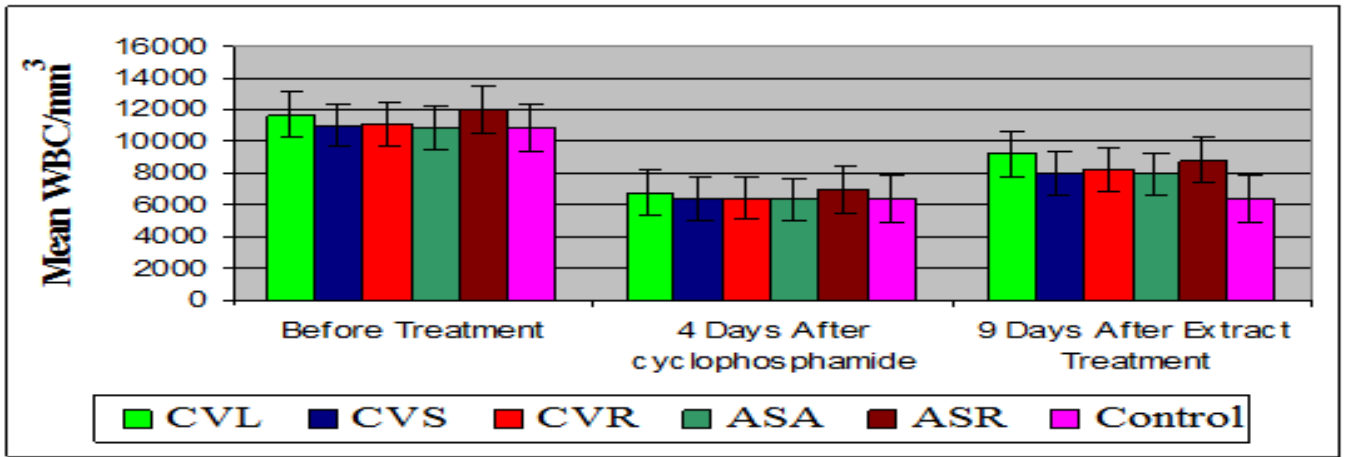


Figure 10: Effects of Aqueous extracts of *C. volkensis* and *A. setaceous* on WBCs counts in rats treated with cyclophosphamide. *Control group was administered with dimethyl sulfoxide.

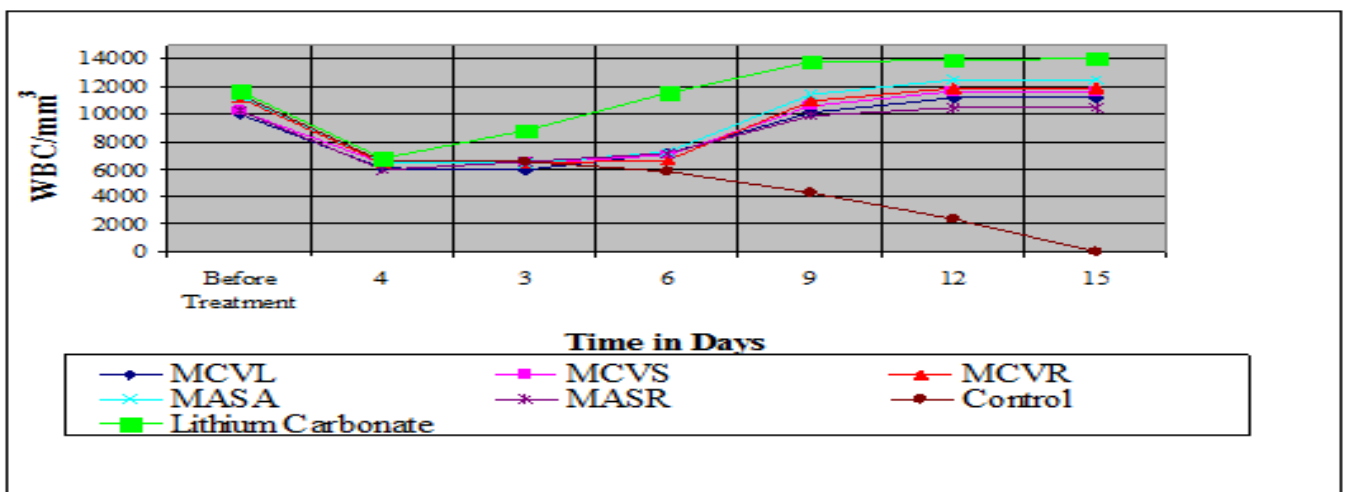


Figure 11: Effects of methanol extracts of *A. setaceous* and *C. volkensis* on cyclophosphamide treated rats. *Control group was administered with dimethyl sulfoxide while group administered lithium carbonate acted as positive control.

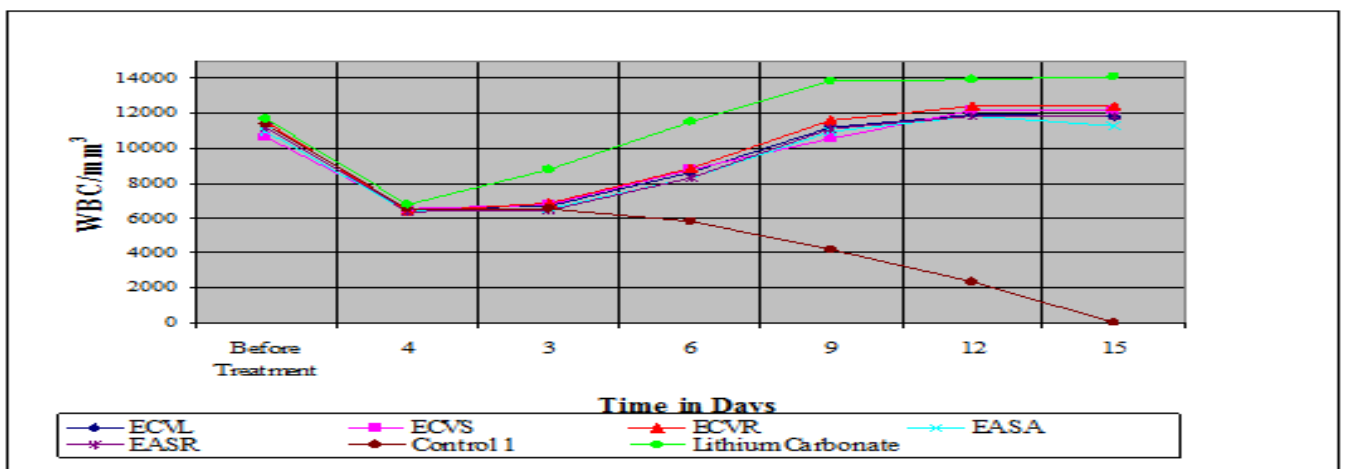


Figure 12: Effects of ethanol extracts of *A. setaceous* and *C. volkensis* in cyclophosphamide treated rats. *Control 1 group was administered with dimethyl sulfoxide while group administered lithium carbonate acted as positive control.

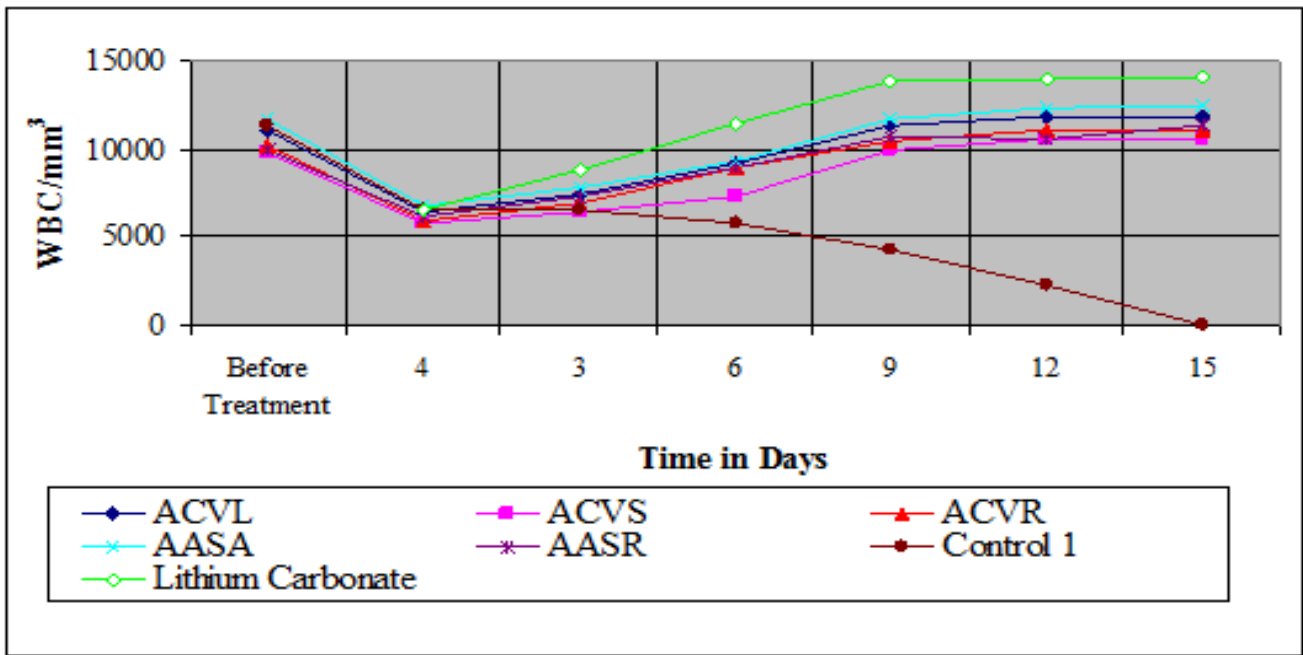


Figure 13: Effects of aqueous extracts of *A. setaceous* and *C. volkensis* in cyclophosphamide treated rats.

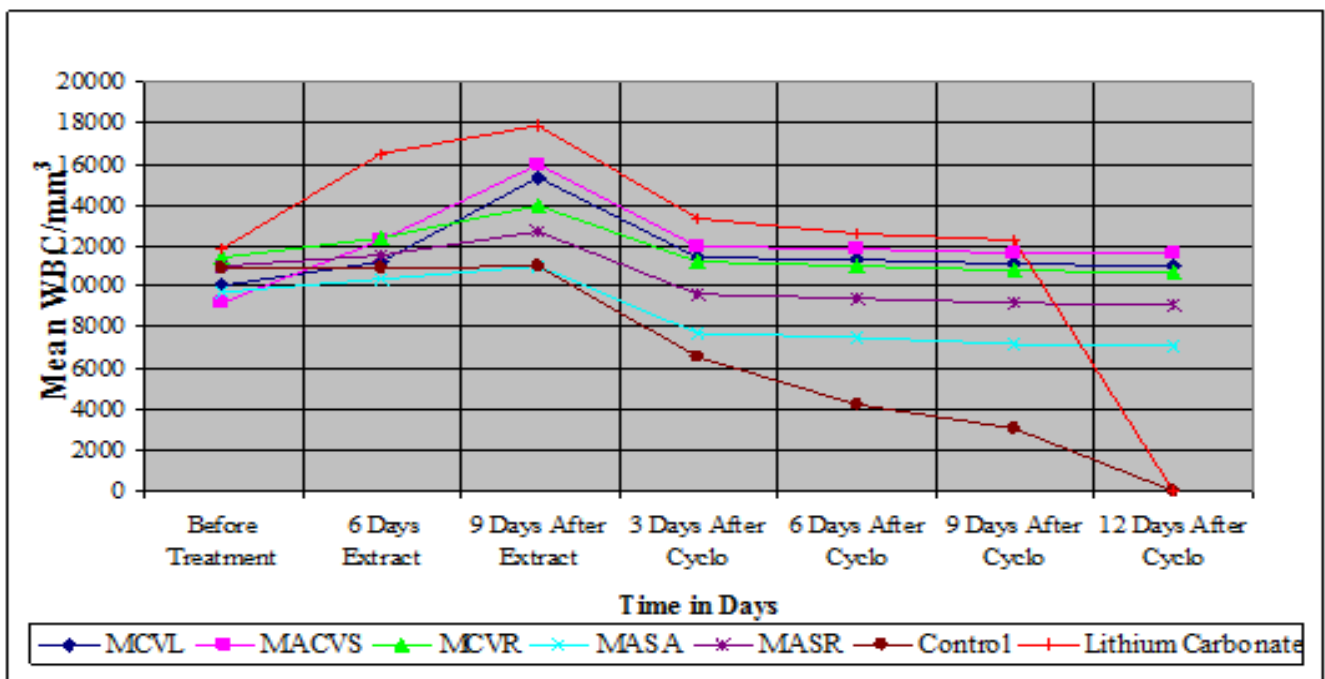


Figure 14: The effect of methanolic extracts of *C. volkensis* and *A. setaceous* against myelosuppression induced by cyclophosphamide in pre-treated rats. *Control group was administered with dimethyl sulfoxide while group administered lithium carbonate acted as positive control.

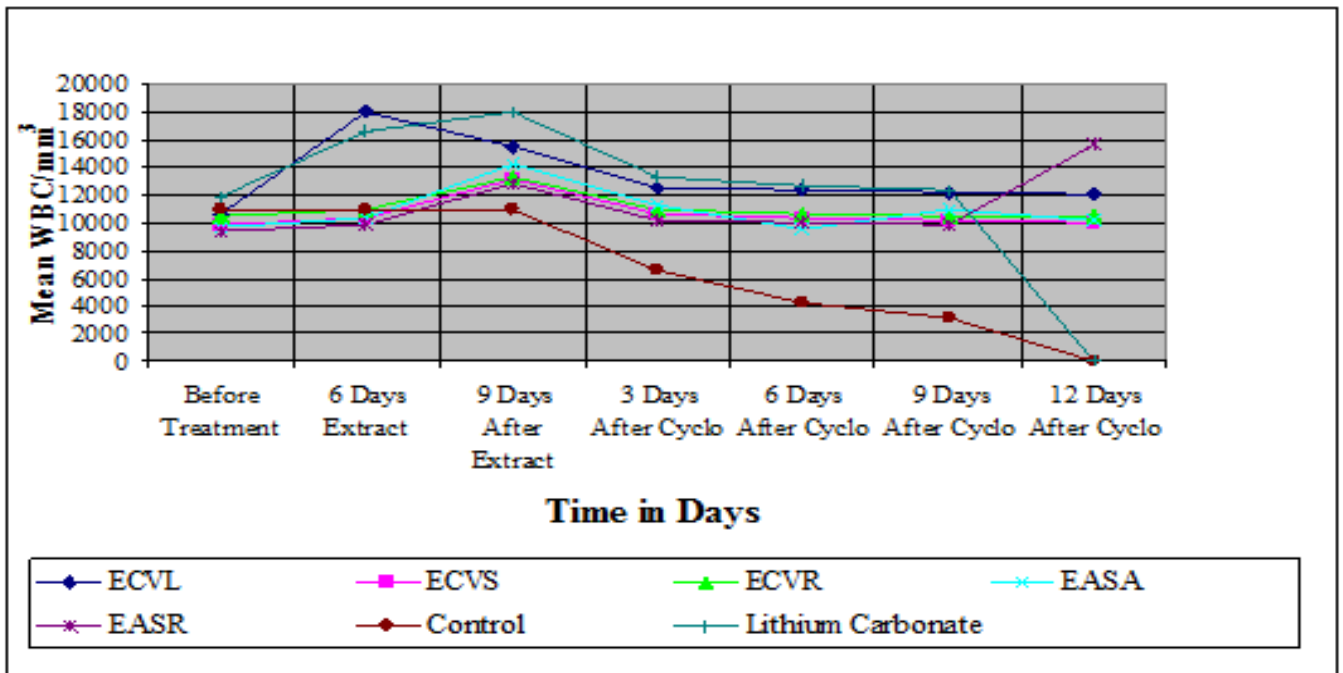


Figure 15: The effect of ethanolic extracts of *C. volkensii* and *A. setaceous* against myelosuppression induced by cyclophosphamide in pre-treated rats. *Control group was administered with dimethyl sulfoxide while group administered lithium carbonate acted as positive control.

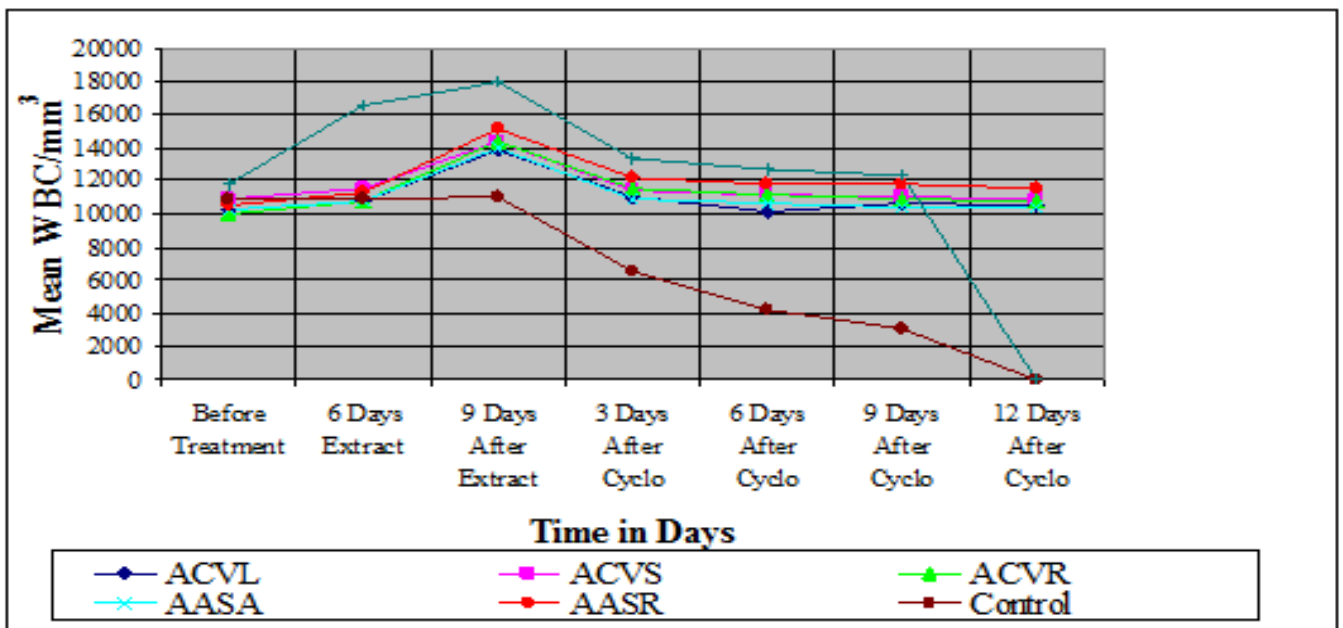


Figure 16: The effect of aqueous extracts of *C. volkensii* and *A. setaceous* against myelosuppression induced by cyclophosphamide in pre-treated rats. *Control group was administered with dimethyl sulfoxide while group administered lithium carbonate acted as positive control.

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