ORIGINAL ARTICLE

Assessment of Anti-Anemic Effects of Saccharum Munja Roxb Roots Extract

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ABSTRACT
Anticancer agents often cause bone marrow suppression resulting in progressive anemia which may influence the therapeutic effects of different cancer treatment.
Objective: The objective of this study was to evaluate, effects of ethanolic extract of Saccharum munja roxb (Kaana or Sirkanda) on hemoglobin and red blood cells counts suppressed by carboplatin.
Methodology: Anemia was induced in mice with a single iv dose of 50 mg/kg carboplatin. Hematological responses including, RBC and Hb was measured at 3rd, 5th and 8th day. Saccharum munja roxbethanolicextract with dose of 25mg/kg, 50mg/kg and 100mg/kg were given to different treatment groups.
Results: In the low dose (R. ext 25mg/kg) group hemoglobin level was found to be 11g/dl on 8^{th}day. However (R.ext 50mg/kg) showed more improvement in hemoglobin levels i.e11.5 g/dl at 8th day. The mice treated with higher doses of extract (R.ext100mg/kg) showed major improvement in hemoglobin levels at 8th day which was 12.6g/dl.RBC count increased after oral administration of Saccharummunja extract (R.ext 25mg/kg) to 6.4 $\times 10^6$cells /mm^3 on 8^{th}day. However, in (R.ext 50mg/kg) extract treated group RBC count was found to be 6.9 $\times$ 10^6cells /mm^3 on 8^{th}day. In (R.ext 100mg/kg) treated group the RBC count was 7.4 $\times 10^6$ /mm^3 on 8^{th}day.
Conclusion: Thus, the results suggested that ethanolic-extract ofSaccharum munja roxb at different dosesshow beneficial effects in improving different parameters of blood.This study also appropriately described the time course of hematological changes after carboplatin induced anemia in mice. Therefore, this study method can be useful tool to explore potential strategies for the management of anemia caused by chemotherapeutic agents.
Keywords: Carboplatin, heamoglobin, Saccharummunja

INTRODUCTION
Anemia is the most widely recognized blood issue and is classified by decreasing red blood cell count and on the other hand, amount of hemoglobin (Hb) in the blood. Low HB levels defined anemia, although the male Hb threshold is below 130 g / L and the woman is below 120 g / L, even in the elderly. According to World Health Organization estimation, 1.62 billion people worldwide affected by low Hb levels and anemia(1).Anemia is a common disease and so far different types of anemia have been identified. This almost always results in a severe decrease in circulating erythrocytes and Hb. Destruction of intracellular or extra-scular RBCs (RBC), haemo-lytic anemia is a form of hereditary and / or acquired anemia. Up to 75% of cancer patients experience the ill effects of anemia either as a result of the disease or due to the myelo-suppressive effect of chemo-therapy and / or bone marrow irradiation. Anemia in patients having cancer chemotherapy related with a noteworthy decline in patients' quality of life due to decrease in HB. Anemia with erythropoietinuse can improve Hb, for example, recombinant human erythropoietin has been shown clinically effective to improve the quality of the patients (2).

It is estimated that around 50% anemia, cases globally are due to an iron insufficiency. The most common reasons for anemia are due to nourishing deficiencies, ceaseless aggravation, proceeding with kidney ailment, and sickness with unidentified causes.Anemia in adults may be different in African region where it may be caused by the soil, especially canine influenza infections, incessant contamination, for example, HIV, jungle fever diseases and nourishing inadequacies. More individuals in Africa might be progressively defenseless against iron inadequacy and Vitamin B12 insufficiency which are chiefly gotten from creature items which more individuals may not bear (3).

Medicinal plants have been used to control of many diseases in developing countries including anemia. Asia has great medicinal plant variety because of its unique geography and environment (8).Demand of herbal drugs is increasing all over the world and government of Pakistan has also made the laws to regulate the business of herbal product. is essential to evaluate their traditional claimed therapeutic use at scientific basis.There are more than 400 types of anemia, in which the most common type is hemolytic anemia (9).

Saccharummunjaroxb. (Kaana or Sarkanda) have been used to treat burning sensations, thirst, dyspepsia, herpes, eye diseases, and in urinary complaints. The roots of S.m.roxbhave been used to treat dysuria, blood disorders, giddiness and vertigo (10).There are various causes and types of anemia, including: sickle cell anemia, iron deficiency anemia, vitamin B$_{12}$ anemia, drug induced anemia, disease induced anemia, etc (11).

Regardless of the clinical noteworthiness of anemia in the older, the fundamental causes are not exactly known. Quite a bit of this is because of perpetual aggravation or renal abnormalities. Moreover, hunger can prompt nutrient B$_{12}$, folic acid or iron insufficiency are the major cuase anemia. Likewise, anemia might be an early sign of harmful disease, including myelodysplastic disorders (MDS). Finally, one third of aged anemic patients have unexplained anemia and no specific causes can be identified (12).

Clinical research studied been lead to awareness in adjusting the dose and programmed of hemato-poieticcares to advance administration of chemo-therapy

inducedanemia. The status of reference point HB standards at the start of erythropoietin treatment and the effectiveness of erythropoietin management qualified to the claim of chemo-therapy have recognized. These research studies can be enabled with advance of models-based on mechanism of the drug that can characterize course of development of anemia. Pharmaco-dynamics models for neutropenia and thrombocytopenia have been established. The hematopoietic system convincingly seized by cell life-span copies which had only restricted application to reproducemyelo-suppressive properties of medicinal agent(13).

Objectives
1. To investigate the anti-anemic effect of Saccharummunjaroxb. Roots extract.
2. To measure the effects of different doses of Saccharummunjaroxb. roots extract on RBC and HB count in mice model of Anemia

MATERIALS AND METHODS

3.1. Drugs and Chemicals:
3.1.1. Carboplatin
3.1.2. Sacchrummunjaroxb
3.1.3. Distilled water
3.1.4. Ethanol 70%
3.1.5. Normal Saline

3.2. Experimental animals: Adult albino mice weighing 210±10 g were divided into 5 groups, each group having 3 mice. The animals were provided with standard diet and water ad libitum. Animals were kept in standard environmental condition (temperature 21±2 ⁰C, relative humidity 60±10% and light with interval of 12 hrs.) Animals were allowed to acclimatize to lab environment for one week.

3.3. Preparation of crude extract: Roots powder was soaked in 70% ethanol and 30% distilled water for seven days. Extract was filtered through muslin cloth and then passed through what man's filter paper for getting clear filtrate. The excess solvent was removed with rotary evaporator at 40 °C.

3.4. Grouping of Animals and Treatment Protocols: Group 1 was served as normal. Anemia was induced by injecting intravenously carboplatin (50 mg/kg) in group 2, 3, 4 and 5 on day 0. Group 2 was disease (Carboplatin treated), Group 3 was served as treatment 1 (25 mg/kg treated), group 4 as treatment 2 (50 mg/kg treated) and Group 5 as treatment 3 (100 mg/kg treated). The plant roots extract was orally administered to Group 3, 4 and 5 from day 1 to day 8.

Blood sampling was done through cardiac puncture on 3rd, 5th, and 8th day of treatment from all treated groups for evaluation of CBC.

Table 01: Study groups with their respective treatments.

Sr. No.	Group Name	Treatment	Concentration	No. of animals
1	Group 1(-/-)	Normal Saline	10ml/kg	3
2	Group 2 (+/-)	Carboplatin	50mg/kg	3
3	Group 3	Root Extract(Saccharummunja)	25mg/kg	3
4	Group 4	Root Extract (Saccharummunja)	50mg/kg	3
5	Group 5	Root Extract (Saccharummunja)	100mg/kg	3

3.5. Statistical analysis: Data was analyzed using SPSS Version 22.0. Absolute factors like sex, therapeutic history, unfriendly occasions and so forth will be accounted for as frequencies and rates One route Analysis of change (ANOVA) test was connected with Tukey-go test to gauge the degree of essentialness among gatherings. The information was graphically plotted utilizing Graph Pad Prism. The degree of importance, (P < 0.05) was considered as noteworthy.

RESULTS

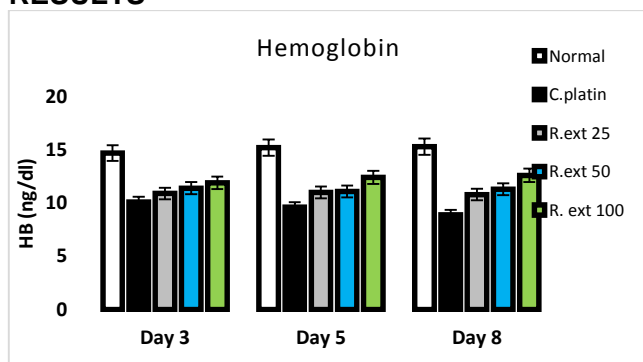Fig. 4.1: Comparison of HB levels before and after treatment using different concentration of ethanolicextract of (Saccharummunja) roxb at 3rd, 5th and 8th day

The purpose of this study was to compare the anemia in mice induced by C. platin effecting Hb levels and analysis of effects of different concentration ethanolic extract of (Saccharummunja) roxb on C. platin induced anemia, and Hb levels of mice at 3rd, 5thand 8thday, Five groups of mice were used in study. In four out of five groups (C. platin) were given to induced anemia in mice and different concentrations of extract were used as treatment.
1. Normal group,
2. Diseased group,(C.platin treated group).
3. (Saccharummunja) (R.ext 25mg/kg treated).
4. (Saccharummunja) (R.ext 50mg/kg treated), and
5. (Saccharummunja) (R.ext100mg/kg treated).

In test group, all mice were found to be anemic, and count of HB and mice of test group before treatment were decreased. The HB level wascompared with control mice. As shown in figure 1, in control group, HB level was found to be 15.07 g/dl on 3rd day. It decreased after i.v administration of carboplatin to 10.52 g/dl on 3rd day, 9.61g/dl at day 5thand 8.98 g/dl on 8th day. In low dose extract (R. ext 25) group HB level was found to be 10.8g/dl on 3rd day, 10.9g/dl on 5th and 11g/dl on 8thday. However the (R.ext 50) shows more improvement in HB levels at 3rd day of treatment 11.1g/dl, 11.3 g/dl on 5thday and 11.5 on 8thday. There is little improvement in HB after low doses of extract given to mice. The mice treated with higher doses

of extract (R.ext 100) showed major improvement in HB levels with HB levels at 3rd day, 11.9g/dl, 12.4g/dl at 5th day and 12.6g/dl at 8thday. The anemia resulted in administration of carboplatin 50mg/kg was replaced by the root extract of Saccharummunjaroxb.
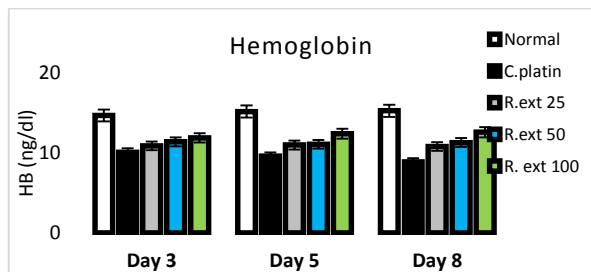

Fig. 4.2: Comparison of HB levels between C.platin and Extract (Saccharummunja) roxb treated groupsat 3rd, 5th and 8th day.

The severity of anemia in mice induced by C.platin by effecting HB levels and evaluation of effectiveness of different concentration ethanolicextract of (Saccharummunja) roxb on C.platin induced anemia, at day 3rd, 5thand 8thwere done in research study in order to ascertain whetheranemia could be due to a direct lytic effect of the drug on HB of mice. (The Fig.2) shows comparison between, HB levels of (C.platin), (R.ext 25), (R.ext 50) and (R.ext.100).

Test was repeated for three times at 3rd, 5th and 8th day of experiment to check and compare HB levels in different group. This is an immaculate test to observe effect of quality and efficacy of different concentration of (Saccharummunja) roxb.Root extract for the improvement of carboplatin induced HB loss in mice of different groups. Results of HB levels of Group V (R.ext-100 mg/kg treated) mice found be wise to results when contrasted with Group IV (R.ext-50 mg/kg treated) and Group III (R.ext-25 mg/kg treated) mice as appeared in Results were found factually critical for Group V (P= .000) in correlation with Group II. It was likewise seen during concentrate that Group II (Diseased) had demonstrated least action than Group I (p=.0001). Comparison between C.platin treated and various extract treated groups show (Figure 4.2).The comparison between C.platin and Group III (R.ext-25 mg/kg treated) showed little improvement in HB levels with (p= 0.0151) however the effect of Group IV (R.ext-50 mg/kg treated) and Group V (R.ext-100 mg/kg treated) showed much improvement in HB levels of mice with (p = 0.0048) and (p = 0.0004).
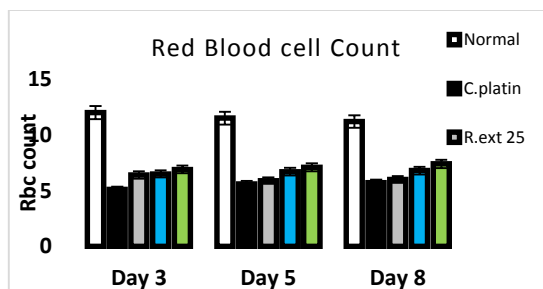

Fig.4.3: Comparison of RBC count before and after treatment using different concentration of ethanolic extract of (Saccharummunja) roxb at 3rd, 5th and 8th day

The purpose of this study was to measure temporally, the severity of anemia in mice induced by C.platin by effecting RBC's levels and analysis the effects of different concentration ethanolic extract of (Saccharummunja) roxb on C.platin analyzing anemia, and RBC's levels of mice at day 3rd, 5thand 8thwas done to evaluate whether the anemia could be due to a direct lytic effect of the drug on RBC's of mice. The five group of mice used in our study were (Normal group), (C.platin), (R.ext 25 mg/kg treated), (R.ext 50 mg/kg treated) and (R.ext.100 mg/kg treated). In four out of five groups (C.platin) were given to induced anemia in mice and different concentrations of extract were used as treatment.

In the C.platin treated group, all mice were observed to be anemic, and the check of RBC's & HB of model mice were diminished as contrasted and that in control mice. We completed routine assessment of blood in charge mice and model mice. We found that mice in test gatherings mice were anemic, and count of RBC's, test group mice were lower than that of control mice (P<0.05). As shown in figure 2, in the test groups, RBC's count was found to be 5.11 cells 10^6 /mm^3 on day 0. It increased after oral administration of (Saccharummunja) extract (R.ext 25 mg/kg treated)to 5.9 cells 10^6 /mm^3 on 3rd day, 6 cells 10^6 /mm^3 on 5thday and 6.4 cells10^6 /mm^3 on 8thday. However in the (R.ext 50 mg/kg treated) extract group RBC's count was found to be 6.4 cells 10^6 /mm^3 on 3rd day, 6.7 cells 10^6 /mm^3 on 5th and 6.9 cells 10^6 /mm^3 on 8thday. In (R.ext 100 mg/kg treated) treated group RBC's count was 6.9 cells 10^6 /mm^3 on 3rdday, Raised to 7.1 cells 10^6 /mm^3 on 5thand 7.4 cells 10^6 /mm^3 on 8thday. To decrease inter individual differences, samples were taken on 3rd, 5th and 8th day of experiment.

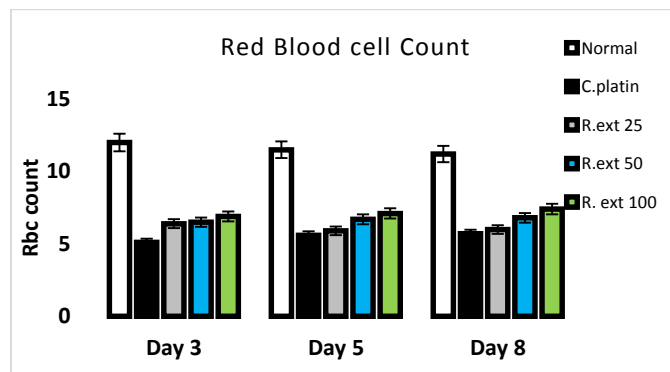Fig.4.4: Comparison of RBC count between C.platin treated and (Saccharummunja) roxb Extract treated groupsat 3rd, 5th and 8th day

The severity of anemia in mice induced by C.platinRBC's counts and evaluation of effectiveness of different concentration ethanolic extract of (Saccharummunja) roxb on C.platin induced anemia, at 3rd, 5th and 8thday was done in research study in order to ascertain whether the anemia could be due to a direct lytic effect of the drug on RBC's count of mice. (The Fig.4.4) shows the comparison between the RBC's levels of (C.platin), (R.ext 25 mg/kg treated), (R.ext 50 mg/kg treated) and (R.ext.100 mg/kg treated).

It was observed during experimentation that Group V (R-ext 100) showed more promising (p = 0.001) results than Group IV (R.ext-50 mg/kg treated) (p = 0.001) and Group III (R.ext-25 mg/kg treated) (p = 0.001) in comparison with the normal Group I as shown in fig.(4.4). However, results of group III were not statistically much significant (P<0.05) in comparison with Group V. the comparison between C.platin vs (R.ext-25 mg/kg treated) showed, extract treated group improvement in RBC count compare to C.platin treated group (p = 0.0008), the other extract treated group shows effectiveness of extract in improvement of RBC levels (R.ext-50 mg/kg treated) (p = 0.0001), while (R.ext-100 mg/kg treated) showed (p = 0.0001) .

DISCUSSION

Anemia is a condition in which red blood cells count decrease and causes hypoxia due to a failure to meet tissue oxygen demand. It is a disease due to a decrease in hemoglobin (Hb) inside erythrocytes (RBCs) or a deficiency of RBCs, which provide oxygen to peripheral tissues (Hye et al. 2014). Hemolytic anemia is acquired anemia resulting from either intravascular or extra vascular RBC destruction. Hemoglobin is the iron-containing respiratory protein inside red blood cells that carries oxygen (14).

Traditional herbal remedies are being used for years to rectify the disease, but they have not been investigated. Medicinal plants have been the source of aid in the control of many diseases in developing countries including anemic diseases. Asia has great medicinal plant variety because of its unique geography and environment (15). Demand of herbal drugs is increasing all over the World and government of Pakistan has also made the laws to regulate the business of herbal product. It is essential to evaluate their traditional claimed therapeutic use at scientific basis. All over the world there are more than 400 types of anemia, in which mostly occurred is hemolytic anemia (16).

Saccharummunjaroxb (Kaana or Sarkanda) is used in burning sensations, thirst, dyspepsia, herpes, eye diseases, and in urinary complaints. The roots of S.m. roxbare used to treat dysuria, blood disorders, giddiness and vertigo. This study complies with (10) study showing increased the RBC's and improvedHb level after the administration of Saccharummunjaroxb depicting its beneficial effects on blood disorders. The RBC's rate could be purpose behind anemia in this examination; however blood misfortune and RBC's annihilation are almost certain potential reasons for the anemia model. As indicated by our outcomes, mice Hb focus expanded more than that of infected mice.Hb levels of Group V (R.ext-100 mg/kg treated) in mice was wise to results when contrasted with Group IV (R.ext-50 mg/kg treated) and Group III (R.ext-25 mg/kg treated) mice as compared in results were found difference for Group V (p = .000) as compared with Group II. It was additionally seen during concentrate that Group II (Diseased) had indicated least action than Group I (p=.0001). The comparison between C.platin treated and various extract treated groups shown in(Figure 4.4).The comparison between C.platin and Group III (R.ext-25 mg/kg treated) showed little improvement in HB levels with (p= 0.0151) however the effect of Group IV (R.ext-50 mg/kg treated) and Group V (R.ext-100 mg/kg

treated) showed much improvement in Hb levels of mice with (p = 0.0048) and (p = 0.0004).The Group V showed more recovery with the treatment of (R.ext 25, 50 and 100 mg/kg treated) induced by carboplatin after loss of HB(17). HB loss is one important reason leading to anemia (Kim et al., 2014). The disease group show lesser RBC count as compared to treated groups with (R.extracts 25, 50 and 100 mg/kg treated).

There are homeostatic changes during chronic administration of carboplatin(18). Whether these can be captured in animal model showed that HB count severely decrease in disease group (p= 0.0001) as compared to other groups treated with (Saccharummunja) (R.ext-25 mg/kg treated), (R.ext-50 mg/kg treated) and (R.ext-100 mg/kg treated). The R-ext-100 shows more promising results as compared to rest of the groups showing significant results in comparison with other groups (p= 0.0001). The group treated with carboplatin shown hemato-toxicity after repeated exposures to carboplatin in mouse model(19). The RBC's test consists of applying using blood of the animal and measuring at day. To decrease inter individual differences, samples were taken on 3rd, 5th and 8th day of the experiment.

Anemia model was adequate to describe single-cycle chemo-therapy effects on RBC's, the comparison between C.platin vs (R.ext-25 mg/kg treated) showed, extract treated group improvement in RBC's count compare to C.platin treated group (p = 0.0008), other extract treated group showed effectiveness of extract in improvement of RBC's levels (R.ext-50 mg/kg treated) (p = 0.0001), while (R.ext-100 mg/kg treated) showed (p = 0.0001).

Evaluation of model can reflect anemia development after multi-cycle chemo-therapy(20). In case of myelo-suppressive drugs developing anemia, with platelets and erythroid profiles desired since thrombocytopenialinked with a source of anemia as a result of hemorrhage. Different animal models useful to define time progression of anemia from chemo-therapy and further evaluate optimization of different treatmentdrugsforanemia(21).

CONCLUSION

This study emphasizes the relevance of anemia and contributes in the understanding the effects of carboplatin on HB and RBCs count in mice model. It was observed during the study that ethanolic-extract ofSaccharum munja roxb root provided beneficial results in improving different parameters of blood at different doses. Future studies roots extracton other parameters such as erythropoietin, reticulocytes will help us to understand better the effects of Saccharum munja roxb Roots Extract and its effect of different blood parameters.

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