



## **COMPARING THE ROLE BRASSICA OLECEREA WITH FOLIC ACID ANDFERROUS SULFATE IN THE TREATMENT OF ANEMIA INDUCED EXPERIMENTALLY BY LEAD ACETATE**

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### **Abstract**

The work aimed to compare the role of Broccoli, folic acid or ferrous sulfate in treatment of anemia caused by lead acetate. 25 female rabbits were divided into 5 randomized groups: 1st group (control), 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> groups were received lead acetate 10 mg/kg.Bw/daily for 4 weeks, 3<sup>rd</sup>, 4<sup>rd</sup>, 5<sup>rd</sup> groups also treated as following with folic acid, ferrous sulfate and alcoholic extract of the Brassica oleracea, blood samples were taken to calculate blood parameters. The results recorded a decrease in level of RBCs indices, PCV, PPOX, iron and ferritin after administration of lead and increase in bilirubin concentration and TIBC. From other side after treatment by extraction plant, folic and iron the results seen revealed increase in erythrocyte indices, PCV, PPOX with a decrease in mean of bilirubin and elevated in value of iron, ferritin with decrease in TIBC. These results support the hypothesis that broccoli excrete beneficial effect in treating anemia and lessen effects on the hematological system.

**Keyword:** PCV, Hb, protoporphyrinogen oxidase, bilirubin RBCs indices, Ferritin, TIBC

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## 1. Introduction

Brassica oleracea L. var. italica has obtained abig accord of heed for the assist of more health people. Especially broccoli ,which has health benefits that may not be found in many other plants(Mushtak *et al.*,2021).It has great amount of feeder wealthy in vitamins , minerals and bio-active phyto-chemicals. Broccoli is included in genus Brassica and is known to have a lot of bioactive non-enzymatic compounds. Broccoli is rich in nutritional antioxidant such as vitamin C and E as well as nonnutritional antioxidant such as carotenoids, a phenolic compound mainly flavonoids. Thus, a diet rich in broccoli plays a turn in the protection of diseases, like CVD and some tumors of body (Omid and Araghi,2021).likewise broccoli activate the antioxidant against stress of ROS in many diseases (Yoko *et al.*,2019). It perhaps number-1 on the roll of anti-cancer vegetables with broccoli being the subject to the highest amount of scientific and medical research. Broccoli is considered a major deterrent to lung, stomach, mouth, ovarian, breast, cervix, colon and prostate cancer. Since broccoli is high in beta carotene and iron, it may help prevent anemia especially in people who follow a vegetarian diet (Batol *et al.*,2022). Folic acid or vitamin B9, is a form water soluble vitamin point in fortified food and supplements. Folate is naturally derived from food, particularly from dark green leafy vegetables , and the biologically active ,which is converted to

Wash the broccoli leaves neatly and arid at 45 °C. leaves were desiccated and land to a shape thick powder, 50 gm of the it was mixed in twenty hundred fifty milliliter of ethyle-alcohol in a neat glass . Put and covered the beaker in bath of water 37c . Next twenty four hr., the admixture was mingled for 1 hour with a magnetic stirrer. Then airing graze it off and place in the fridge until supplemental use (Anesini and Perez,1993).

### Design of Experiment

Animals within different treatment groups were maintained on their respective diets, followed for

### Red blood cells indices

Red blood cells indices(total RBCs, MCV MCH,MCHC), Hb and pcv determined by an automated hematology (Genex, USA).

### Biochemical test

Total bilirubin concentration was resolved by colorimetric of enzyme methods using commercial laboratory kit purchased from (Biolabo-

dihydrofolic acid in the liver, and essential in meeting the requirements of the function of the human body. Folate is used to synthesize, repair methylate Deoxyribonucleic acid DNA; therefore it is chiefly serious in people for continuous cell division and growth (Khalid *et al.* ,2021). Folate deficiency has many consequences, such as megaloblastic anemia, resulting from ineffective erythropoiesis. Anemia is defined as diminish hemoglobin level or circulating red blood cells and it is the most common hematological disorder.In adequate intake or absorption of iron in conjunction with anemia (Jafer *et al.*,2015). Poor nutritional status such iron and folic acid deficiency is the main causes of maternal anemia in supplement to physiological etiologies (Rasheed and Al-Nuaimmi, 2022).Fe one of the generally plentiful fundamental for the normal function erythrocytes and entire bit of some enzyme with proteins interested in O2 carry in organism.iron sulfate, accordingly treatment of anemia caused by lead acetate it toxic to tissue (Mustafa *et al.* ,2022) , required in addition to iron replacement, other agents for hematopoietic tissue regeneration, the Broccoli can be a choice for such purpose (Wąsik and Ślota, 2022).

## 2. Materials and Methods

### Plant extract planning

30 days and received the: treatment follows :1<sup>st</sup> group (control group) :no treatment (which given food and water),2<sup>nd</sup> group (pb- Anemic): Animals will be exposed by lead acetate 10mg/kg/Bw/day orally(4) .3<sup>rd</sup> Group (T1F+pb): Animals were treated with folic acid 0.07mg/kg/Bw orally (12) for 4weeks. 4<sup>th</sup> Group( T2FS+pb) :Animals were treated with ferrous sulfate 33mg/kg/Bw(21)orallyfor4week,5th group(T3B+pb):.Animals were treated orally with alcoholic extract of the Brassica oleracea var. italica300mg/kg/Bw..

France).Protoporphyrinogen oxide enzyme (PPOX) was examined by used Rabbit PPOX(Protoporphyrinogen Oxidase) ELISA kit ( ELIK BIOTECHNOLOGY ).

### Iron status

Total serum iron, Ferritin and TIBC were measured by using chemistry analyzer smart 120 kit (giesse italy).

### Statistical analysis

The Statistical Analysis System- SAS (23) program was used to detect the effect of difference groups and week in study parameters. LSD-Least significant difference and T-test was used to significant compare between means in this study.

A satirical analysis of hematological parameters in (Table 1) showed that mean of RBCs, Hb, and PCV were significantly ( $p \leq 0.05$ ) lowered in animals that exposed of lead acetate compared with the control, on the other hand these parameters significantly increased ( $p \leq 0.05$ ) in treated groups with the highest value in plant.

### 3. Results and Discussion

Table 1. Comparison of Brassica with folic and iron in RBCs, PCV and Hb in pb anemic rabbits

Groups	RBCs $10^6$	Pcv%	Hb g/dl
	After 4weeks of treatment	After 4weeks of treatment	After 4 weeks of treatment
Control	5.23 $\pm$ 0.08 b	36.96 $\pm$ 0.11 a	12.30 $\pm$ 0.17 a
Pb- anemic	4.10 $\pm$ 0.21 c	31.56 $\pm$ 0.74 b	8.64 $\pm$ 0.65 b
T1F+PB	5.44 $\pm$ 0.17 b	37.32 $\pm$ 0.20 a	12.36 $\pm$ 0.23 a
T2FS+Pb	5.58 $\pm$ 0.15 b	37.76 $\pm$ 0.09 a	12.89 $\pm$ 0.190 a
T3B+Pb	6.07 $\pm$ 0.11 a	37.57 $\pm$ 0.19 a	13.07 $\pm$ 0.09 a
LSD	0.457 *		0.984 *

Means having with the various letters in same column differed significantly ( $P \leq 0.05$ ).

Results in regard to RBCs indices (MCV, MCH, MCHC) were significantly ( $p \leq 0.05$ ) reduced in animals that exposed of lead acetate (Table 2) compared with the control group, whereas they increased in treated groups with the highest value in Broccoli group.

Table 2. Comparison of Brassica with folic and iron in MCV, MCH and MCHC in pb anemic rabbits

Groups	MCVfl	MCH pg	MCHC g/dl
	After 4weeks of treatment	After 4weeks of treatment	After 4 weeks of treatment
Control	82.00 $\pm$ 0.89 a	27.10 $\pm$ 0.28 a	33.97 $\pm$ 0.24 a
Pb-anemic	63.20 $\pm$ 0.86 c	19.20 $\pm$ 0.53 b	30.74 $\pm$ 0.22 b
T1F+Pb	78.00 $\pm$ 1.00 b	26.97 $\pm$ 0.27 a	34.03 $\pm$ 0.05 a
T3B+PB	81.80 $\pm$ 0.86 a	27.21 $\pm$ 0.08 a	34.14 $\pm$ 0.13 a
LSD	2.475 *	0.901 *	0.509 *

Means having with the different letters in same column differed significantly. \* ( $P \leq 0.05$ ). Likewise the data in (Table 3) indicated decrease  $p \leq 0.05$  in protoporphyrinogen oxidase enzyme comparing with T1F and T3B, on other hand seen

significant increase  $p \leq 0.05$  in Bilirubin concentration groups that treated with lead acetate, compared with other groups that treatment with folic acid, ferrous sulfate and extraction of plant.

Table 3. Comparison of Brassica with folic and iron in bilirubin and ppoX in pb anemic rabbits

Groups	Bilirubin mg/dl	PPOX nmol/ml
	After 4weeks of treatment	After 4weeks of treatment
Control	0.220 $\pm$ 0.03 b	0.784 $\pm$ 0.03 b
Pb- anemic	1.20 $\pm$ 0.24 a	0.183 $\pm$ 0.02 c
T1F+PB	0.320 $\pm$ 0.04 b	0.860 $\pm$ 0.02 ab

<b>T2FS+Pb</b>	<b>0.400 ±0.05 b</b>	<b>0.844 ±0.02 ab</b>
<b>T3B+Pb</b>	<b>0.240 ±0.05 b</b>	<b>0.904 ±0.03 a</b>
<b>LSD</b>	<b>0.347 *</b>	<b>0.082 *</b>

Means having with the diverse letters in same column differed significantly. \* (P≤0.05).

Results of (Table 4) showed that the value of ferritin ,iron was observed elevated in mean value p≤0.05 after treatment by folic acid ,Fs and plant extraction compared with control and anemic

groups , In contrast at the same time ,TIBC increased significantly p≤0.05 in anemic group of experiment compared with post treatment and control groups

Table 4. Comparison of Brassica with folic and iron in iron and ferritin and TIBC in pb anemic rabbits

	<b>iron μmole/L</b>	<b>Ferritin μg/L</b>	<b>TIBC mg/l M</b>
<b>Groups</b>	<b>After4weeksof treatment</b>	<b>After4weeks of treatment</b>	<b>After 4 weeks of treatment</b>
<b>Control</b>	<b>128.60 ±0.51 a</b>	<b>9.00 ±0.44 ab</b>	<b>310.00 ±0.54 b</b>
<b>Pb- anemic</b>	<b>59.40 ±5.39 d</b>	<b>4.40 ±0.92 c</b>	<b>344.40 ±2.76 a</b>
<b>T1F+PB</b>	<b>100.60 ±1.21 c</b>	<b>8.00 ±0.89 b</b>	<b>315.20 ±1.93 a</b>
<b>T2FS+Pb</b>	<b>119.40 ±1.36 b</b>	<b>10.00 ±0.31 ab</b>	<b>313.80 ±2.83 b</b>
<b>T3B+Pb</b>	<b>128.80 ±1.16 a</b>	<b>10.80 ±0.58 a</b>	<b>313.20 ±1.62 b</b>
<b>LSD</b>	<b>7.69 *</b>	<b>2.01 *</b>	

Means having with the different letters in same column differed significantly. \* (P≤0.05).

Anemia is a worldwide health problem that affects many people in each socioeconomic status, all over the world. According to the causes, anemia take place several patterns. The lead acetate adversely affect on RBCs indices; RBCs count, Hb concentration and consequently other red blood cells indices referred to the type of anemia, the most common form was microcytic-hypochromic. Normally RBCs filled with hemoglobin and it reflect the size of the cell, reduction in MCH & MCV of Lead acetate exposed rabbits referred to a defect in the hemoglobin production. Reduction in RBCs indices reflect the multi factorial effects of Lead at the site of RBCs production in bone marrow and on the other hand in intravascular morphological alteration of RBCs. At bone marrow , Pb caused decrease ferrochelatase, enzyme that catalyzes the source of iron into the porphyrin ring leading to lowered iron in Hb (Casas and Sordo,2006). Other factors could contribute to low hemoglobin production is the iron availability caused by Lead (Wąsik and Słota, 2022) The noting in this study regarding RBC count and MCV, suggesting that bone marrow sub-chronic exposure , suppressed the production of Hb (Golalipour et al.,2007 ) ,unless, it would mark lack erythropoietin stimulus or impaired marrow function (Nagaraja et al.,,2010).The defective in hemoglobin synthesis resulted form other types of heavy metals like lead(Sadat and Alqayim. 2013; .Sultan et al.,2018). Has big leverage on pathways ,such as hem synthesis, there by leading

to the outset of anemia . Displacement of other metals interferes with proteins that hold gene expression and nervous system disruption (Jwad Al-Wan,2012)disturbances of hemoglobin biosynthesis and anemia, that lead to decrease in ppx enzyme. The decreased serum ferritin and increase in TIBC may be due reduced systemic iron in the circulation with suggestion for iron cellular sequestration as a hemosiderin resulting in hemosiderosis (,Arneson and JBrickell. 2007;. Laith, et al.,2020 )Adequate iron status is compined with lead toxicity for many reasons. Firstly, most of ecological Lead absorption from intestine, standard Fe intake iron contest with Pb versus DMT1 transporter, due to the present enmity of these metals in the steps of absorption , also lead has been capable to connect and overlap with other enzymes,( Wąsik and Słota. 2022). Several new studies in animal model about pb proved a causative role of Pb in induced ROS and its lead to iron deficiency. Absorption of Fe lower in the mid gut of Drosera melanogaster. exposure of the Lead impaired iron metabolism by elevated of ROS formation, which thus decreased Fe2+ carrier to ferritin via affecting its excretion and trafficking ,then that are important for the uptake, carry and imposition of iron proteins transferrin mucin mobilferrin hemoglobin and specific enzymes of the hem (Liu et al.,2020) ,Macrophages play a key role in the development of the aforementioned disorders induced by Pb, so macrophages constitute a good experimental model

to study Pb-induced inflammation, Pb accumulation by macrophages can have serious cytotoxic and pro-inflammatory consequences (Emilia and Patrycja, 2020). We suggest here a although the hepicidin was not estimated in the present study. The riskiness of these findings were lowered in groups administered extraction of plant, ferrous sulfate and folic acid reflecting again the protective role of plant against lead toxic effects. Broccoli is one of the healthiest cruciferous vegetables (Abdelmonnem and Fathel, 2022). The health as strong sulfur-containing endogenous antioxidant against the free radical scavenger (Yoko et al., 2017) In addition, broccoli is important nutrient to the Hb biosynthesis and RBC synthesis in bone marrow. because have folic acid and iron in organic compounds. This protective mechanism of broccoli against harm full effects of pb could be explained by the broccoli may be attributed to the presence of nutrients like- vitamins, minerals and direct effect of broccoli on beneficent the

macrophages –hepicidine role in reducing iron release from storage cells, inflammation induced by Pb could mediate increase in hepicidin by IL-6 mechanisms (Srole and Ganz, 2021) compound. Ferrous sulphate treatment proved to a change the activity of GPx and SOD enzymes and GSH concentration with lower in Pb<sup>2+</sup> concentrations of blood. The iron reduction is one of the reasons in cases of anemia food and other essential materials (Zeki and Al-Warid, 2019). pool to the practicability of red blood cell formation, including vitamin B12 and B9. This components of transfer- methylation reactions play important role in synthesize DNA and RNA and maturation of RBCs (Mohammed and Hedef, 2021) immune system, the health benefits of morphology of RBCs by its ability to settle benefits of broccoli include strengthening of their membrane (Batol et al., 2022) and activities of sulphuran.

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