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EFFECT OF GREEN LEAFY VEGETABLES (SPINACH AND SORREL) ON HAEMATOPOIESIS IN MALE WISTAR RATS

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ABSTRACT

Spinach (*Spinacia oleracea* L.) is an edible flowering plant in the Amaranthaceae family and Sorrel (*Hibiscus sabdariffa* Linn.) is a shrub belonging to the Malvaceae family. It is thought of native to Asia (India to Malaysia) or Tropical Africa. The present study was aimed at investigating the effect of Green leafy vegetables (Spinach and Sorrel) on Hematopoiesis in male Wistar rats to know the effect on hematological parameters and oxidative stress in male rats. The study reveal that Spinach and sorrel leaves increased the RBC count, WBC count Hb%, and MCHC when compared control. The sorrel leaves exhibited prominent effect when compared to spinach leaves. However didn't exhibit prominent effect on platelet count. In addition, both the plant extracts showed significant effect on in decreased MDA levels when compared to control.

KEYWORDS: Green Leafy Vegetables, *Spinacia Oleracea,* Hibiscus Sabdariffa, Haematopoiesis.

INTRODUCTION

Haematopoiesis is the process of production, multiplication, and specialization of blood cells in the bone marrow. It begins with the most basic blood cell, the stem cell or "Pluripotent hematopoietic stem cell" (PHSC). The end products of this process are mature white blood cells, mature red blood cells, and platelets (Ogawa *et al*., 1993).

PHSCs have the ability to either divide and create other PHSCs, or to commit into one of several "differentiation" pathways. These pathways eventually result in the production of a type of blood cell (Ogawa *et al*., 1993).

Steps involved in the Haematopoesis

1. **Erythropoiesis**: Synthesis of erythrocytes (red blood corpuscles) is called as erythropoiesis.
2. **Lymphopoiesis**: Synthesis of white blood cells (lymphocytes) is called lymphopoiesis.
3. **Myelopoiesis**: Synthesis of granulocytes, megakaryocytes and macrophages from myeloid progenitors is called myelopoesis (ogawa *et al*., 1993).

BLOOD

Blood is a fluid connective tissue consisting of red blood cells, lymphocytes, platelets and various elements (Rang *et al*., 2007).

FUNCTIONS OF BLOOD

Transportation. As you just learned, blood transports oxygen from the lungs to the cells of the body and carbon dioxide from the body cells to the lungs for exhalation. It carries nutrients from the gastrointestinal tract to body cells and hormones from endocrine glands to other body cells. Blood also transports heat and waste products to various organs for elimination from the body.

Regulation. Circulating blood helps maintain homeostasis of all body fluids. Blood helps regulate pH through the use of buffers. It also helps adjust body temperature through the heat absorbing and coolant properties of the water in blood plasma and its variable rate of flow through the skin, where excess heat can be lost from the blood to the environment. In addition, blood osmotic pressure influences the water content of

cells, mainly through interactions of dissolved ions and proteins (Rang *et al*., 2007).

Protection. Blood can clot, which protects against its excessive loss from the cardiovascular system after an injury. In addition, its white blood cells protect against disease by carrying on phagocytosis. Several types of blood proteins, including antibodies, interferon's, and complement, help protect against disease in a variety of ways (Rang *et al*., 2007).

Factors effecting Haematopoesis

1. Age
2. Sex
3. Ethnic background
4. Body build
5. Social
6. Nutritional
7. Environmental facts

Age: Age is the main factor affecting haematopoesis. As blood cells are more produced in bone marrow, more blood cells are produced in the growing bones in children.

Most of the bones in children produce blood cells. In case of adults only few bones like ribs, pelvis, spine etc produce blood cells. So comparatively adults produce less blood cells than children.

In aged people very less amount of the blood cells are produced because yellow bone marrow is more than red bone marrow (Roger *et al*., 2003).

- **Sex:** Males have more blood cells than females. Males tend to produce more RBC and haemoglobin compared to females (Roger *et al*., 2003).
- **Nutritional:** Haematopoesis primely depend upon the type of nutrition we intake. green leafy vegetables, high iron content vegetables, fruits and vegetables containing β carotene increase the blood production. Junk foods and foods that contain quinine (tonic water, bitter lemon, bitter melon), aspartame (diet soda, sugar-free and low-fat candy and cakes) or alcohol (beer, wine, hard liquor).
- **Environmental facts**: Exposure to pollution, heavy exposure to lead and other heavy metals causes iron deficiency anaemia. other environmental factors like source of water, sewage also alter blood cell production (Roger *et al*., 2003).
- **Social:** social factors like education, employement, family, safety, food habbits also alter haematological parameters (Roger *et al*., 2003).
- **Ethnic background:** Ethnic factors also affects Haematopoesis. For ex : Most of the Americans suffer with sickle cell anaemia when compared to Indians**.**
- **Bodybuild:** Persons with with good body build and fitness have good haematopoetic ranges. Persons with lean body masses and suffering with hormonal disturbances may suffer with anaemia (Roger *et al*., 2003).

SPINACH LEAVES

Spinach belongs to *Amaranthaceae* family. Its scientific name ***Spinacia oleracea*** is a wonderful green-leafy vegetable often recognized as one of the *functional foods* for its nutritional, antioxidants and anti-cancer constituents. Its tender, crispy, dark-green leaves are favorite ingredients of chefs all around planet (Johanna *et al.,* 1994).

SPINACH LEAVES

Figure 1: Spinach leaves

- Spinach leaves contains rich vitamins of A and K

SPINACH JUICE NUTRITION

- Spinach is high in vitamin A and C, and is one of only four vegetables that are also high in vitamin E. It's also a good source of choline, calcium, iron, potassium and folic acid. Spinach is surprisingly high in protein per calorie. raw vegetables as good sources of protein. But when looking at raw vegetables as a protein source, it should be noted that leafy greens are very light in calories, therefore it would take an enormous amount

of chewing to chalk up your daily required intake of protein. For instance, the 30 percent protein in spinach seems impressive; however, a cup only has one gram of protein and a mere seven calories. You would have to eat ten cups to make up ten grams. But this is where the juice machine shines. Juicing ten cups of spinach will produce about a cup of liquid spinach, which then can be easily added to a daily ration of veggie juice. Talk about a glass of concentrated nutrition (Johanna *et al.,* 1994)

- Spinach has 14 times the iron per calorie than red meat. It is true that the iron in meat has a greater bioavailability than plant foods, but for most of us, eating lots of raw green veggies will solve any iron deficiency, especially when released from their fibers in juice form and with the help of added vitamin C-rich lemon.
- Spinach is also important due to its chlorophyll content, and also supplies an abundance of nutrients such as oxalic acid, which is beneficial in the cleansing and healing of the intestinal tract.
- Spinach is one of the highest sources of eye-protecting, cancer-fighting lutein. Lutein has been shown to play a crucial role in protecting against age-related macular degeneration, which is the leading cause of blindness among the elderly (Johanna *et al.,* 1994).
- Spinach Juice is an excellent source of Vitamin C, vitamin A, protein, lutein, chlorophyll, vitamin E, Glutathione, alpha lipoic acid, calcium, potassium, Fibre, choline, folic acid (Louise *et al*., 1993).
- Biological effects of Spinach Juice Anti cataracts, anti-inflammatory, good for complexion, promotes healing, protects from macular degeneration and reduces risk of cancer.

Hibiscus sabdariffa

- Sorrel scientific name Hibiscus sabdariffa belongs to family Malvaceae. small, annual shrub with sour leaves, which are used as vegetable or for making pickle. Red sepals are used for making sherbet or syrup (kokate et al., 2005).
- Sorrel is a very popular green vegetable in Chakma community and it is known as "Aamelli". Gongura is a very rich source of iron and vitamins, folic acid and anti-oxidants essential for human nutrition.
- Sorrel or the red sorrel is known by various names like the ambadi or pulicha keerai in various parts of India which comes in two varieties namely the green leaf and the red variety. The red variety is sourer than the green leaves. The leaves are bitter, mildly astringent and acidic in taste. (Harold and Graham *et al*., 1992)

***Hibiscus sabdariffa* (Sorrel) leaves**

Figure 2: Sorrel leaves

- The sorrelis known as Puntikura in the Telengana region of Andhra Pradesh and is a very rich source of Iron, vitamins, folic acid and anti-oxidants essential for human nutrition. Similarly it is called as Pulichakeerai in Tamilnadu and is very popular over the state.
- Sorrel as the main ingredient are sorrel Pappu (Lentils), Gongura mamsam (goat/mutton) and Gongura royyalu (shrimp). In recent times, Gongura Chicken is also being served in restaurants. Gongura and calabash is extremely popular with the Telugu community in South Africa. Apart from the curries there are many varieties of pickles made with gongura such as Gongura pachadi, Gongura pickle ambadi (Harold and Graham *et al*., 1992)

The nutritional values per 100g of cooked sorrel leaves (sorrel) are includes

Energy: 24 kcal

Proteins: 1. 8 g

Carbohydrates: 2. 9 g

Fat: 0. 6 g

Fibers: 0. 7 g

Potassium: 321 mg

Iron: 2. 1 mg

Vitamin C: 26mg (Harold and Graham *et al*., 1992).

- Biological effects of Sorrel leaves helps to cool any inflammation and heat in the blood. Fresh leaves of the sorrel help to stimulate the stomach and to sharpen the appetite. Moreover it is an herb that helps to cools the liver.
- These leaves are sour in taste and have lot of medicinal values. These leaves and flowers have a cooling effect and act as an appetizer. They are very useful in relieving symptoms of fever. You can use these leaves to treat jaundice. The juice extracted from these leaves mixed with butter milk is highly recommended once daily to treat jaundice. Sorrel greens relieve indigestion, cold and cough. It cleanses the intestines. It also helps in removing infections as well (Harold and Graham *et al*., 1992).

LITERATURE REVIEW

Literature of selected plant

- ✓ Abraham *et al.,* (2000) reported that leaves of *Spinach oliracea* have been numerous beneficial effects, such as chemo and central nervous system protection and anti cancer and anti aging functions.
- ✓ Wang *et al*., (2004) reported that leaves *Spinach oliracea* of Free radicals are involved in neurodegenerative disorders, such as ischemia and aging. We have previously demonstrated that treatment with diets enriched with blueberry, spinach, or spirulina have been shown to reduce neurodegenerative changes in aged animals. The purpose of this study was to determine if these diets have neuroprotective effects in focal ischemic brain.
- ✓ Essa *et al*., (2005) reported that Antioxidant activity of leaves of *Hibiscus sabdariffa.* In this study Ammonium chloride treated rats showed a significant increase in the levels of circulatory ammonia, urea, AST, ALT, ALP, TBARS and HP. These changes were significantly decreased in rats treated with HSEt and ammonium chloride.
- ✓ Haywards *et al*., (2009) reported that *Hibiscus sabdariffa* is a flowering plant shows study in Effect of *Hibiscus sabdariffa* Dried Calyx Ethanol Extract on fat Absorption-Excretion, and BodyWeight Implication in Rats.
- ✓ Pooja *et al*., (2009) reported that leaves of *Hibiscus sabdariffa.* In this studyAntioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. Leaves and calyces extracts in rats.

Aim and Objectives

`The present study was aimed at investigating the effect of Green leafy vegetables (Spinach and Sorrel) on Hematopoiesis in male Wistar rats.

Objectives of the work

- To know the effect of selected Green leafy vegetables (Spinach andSorrel) on hematological parameters in male rats.
- To know the effect of selected Green leafy vegetables on oxidative stress.

MATERIALS AND METHODS

DRUGS AND CHEMICALS

1. *Spinacia oleracea* (Spinach)
2. *Hibiscus sabdariffa* (Sorrel)

Chemicals

1. Thiobarbituric acid (Himedia India Ltd)
2. Hydrogen peroxide (SD Fine Chemicals Ltd)
3. Trichloroacetic acid (Himedia India Ltd)
4. Formalin 10% (SD Fine chemicals Ltd)
5. DTNB (5, 5-Dithiobis (2-nitrobenzoic acid) reagent (Himedia India Ltd)
6. DPPH (1, 1-diphenyl-2-picrylhydrazyl) reagent (Himedia India Ltd)

Equipments

1. Cobus u 411 - CBP.
2. Micro centrifuge tubes - Tarsons
3. Ultra Centrifuge - Remi Industries, Mumbai.
4. UV-Vis spectrocolorimetry - SHIMADZU UV-1800.
5. Refrigerator
6. Electronic weight balance

Animals

Pathogen free adult male albino rats weighing 200-250 gm were used. Male rats were chosen in order to avoid fluctuations due to oestrous cycle. The rats were housed in poly propylene cages and maintained at (24 ± 1°C) with

relative humidity ≤45-55% and 12/12 hours light and dark cycle rats were fed with a balanced diet (standard chew pellets) and tap water ad libitum. The study protocol was approved by institutional animal ethical committee of Vaagdevi College of Pharmacy, Hanamkonda, Warangal Register No. (IAEC NO: 1047/ac/07/CPCSEA).

Experimental design

Animals (30) were weighed and kept in cages accordingly and randomly divided into 5groups (n=6). Drugs were prepared freshly and given suspended in distilled water daily for 21days. On day 1, the training sessions for all the animals were given. Drugs were administered, after 1hr the retention time (RT) was calculated.

STUDY PROTOCOL

The animals were divided in to 5 groups, each group contain 6 animals.

The treatment as follows

- Group-1 served as control and received vehicle only.
- Group- 2 received acqueous suspension of SPINACH LEAVES (100mg/kg oral)for 21 days.
- Group -3 received acqueous suspension of SORREL LEAVES (100mg/kg oral) for 21 days.
- Group-4 received acqueous suspension of SPINACH LEAVES (300mg/kg oral) for 21 days.
- Group-5 received acqueous suspension of SORREL LEAVES (300mg/kg oral) for 21 days.

Spinach, Sorrel leaves was dissolved in distilled water for oral administration. All leaves juice were prepared fresh daily.

Evolution parameters

1. Anti-oxidant activity
2. Hematological parameters

METHODS FOR ASSESMENT OF ANTIOXIDANT ACTIVITY

Thio barbituric acid reactive substance assay (Vogel *et al*., 2002)

Lipid peroxidation (LPO) was assayed according to the method of Okhawa *et al*. 1991

Principle

The reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA), a secondary product of lipid peroxidation has been widely adopted as a sensitive assay method for measurement of lipid peroxidation in biological fluids. It is widely used as a index of the extent to which lipid peroxidation has progressed. Since the assay procedure estimates the amount of TBA reactive substances e. g. MDA, it is also referred to as TBARS (Thiobarbituric acid reactive substance) test.

Procedure

To 0. 5 ml of Liver homogenase, 0. 5 ml of 30% trichloro acetic acid (TCA) was added to precipitate the proteins and vortexed for 30 sec. Clear supernatant was taken after centrifuging at 3000 rpm for 10 min. To the supernatant, 500µl of 1%TBA solution and 500µl of water was added and this solution was heated for 1hr at 98°C. Cool the solutions to room temperature and kept them in ice for 5 minutes. Then read the pink color at 532 nm using spectrophotometer. Standard graph was plotted using TEP (1, 1, 3, 3-tetra ethoxy propane).

ESTIMATION OF COMPLETE BLOOD PICTURE (CBP)

A complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) or blood panel**.**

The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes) Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdictions.

Automated blood count

The blood is well mixed (though not shaken) and placed on a rack in the analyzer (Cobus u 411). This instrument has many different components to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood.

RESULTS AND DISCUSSION

Heamatopoesis studies to effects of administration of Spinach and Sorrel:

Table 1: Acute toxicity studies in rats after 48 hrs of administration of spinach and sorrel leaves

Plant name	Dose (mg/kg)	Period of signs observation (hrs)	Signs of toxicity observed
Spinach	360	48	No toxic changes observed
Sorrel	300	48	No toxic changes observed

Effects of the graded doses of spinach and sorrel on the body weights of rats

Table 2: Body weight changes in different groups of rats

Parameter	Control	Spinach (100mg/kg)	Spinach (300mg/kg)	Sorrel (100mg/kg)	Sorrel (300mg/kg)
Weight before administration	126±4. 2	192±7. 2	185±5. 6	180±4. 7	182 ±6. 8
Weight after administration of 21 days	160±13. 7	174±4. 3	132±8. 2	206±3. 8	250±5. 7

The mean changes in body weight of different groups of rats is shown in Table no. 2 The results indicate that spinach (100mg/kg), sorrel (100mg/kg and 300mg/kg) increased body weight to extent of 1. 08, 1. 28 and 1. 56 however spinach 300mg/kg decreased body weight by 0. 82 times compared to control.

Effects of the graded doses of spinach and sorrel on the heamatological parameters rats.

Table no:3 Heamatological changes in different groups of rats.

Parameters	Control	Spinach (100mg/kg)	Spinach (300mg/kg)	Sorrel (100mg/kg)	Sorrel (300mg/kg)
RBC ($\times 10^6/\mu l$)	6. 30±0. 56	7. 32±0. 1^b	8. 28±0. 3^a	8. 76±0. 1^b	9. 56±0. 1^a
WBC ($\times 10^3/\mu l$)	5. 41±0. 43	7. 45±0. 4	8. 65±0. 1^b	7. 62±0. 1	8. 46±0. 3^b
Hb (gm/dl)	12. 8±0. 99	13. 5±0. 24^b	14. 7±0. 25^a	14. 2±0. 3^b	15. 3±0. 4^a
MCHC (gm/dl)	32. 07±1. 76	32. 02±0. 94^b	31. 4±0. 626^c	31. 8±0. 764^b	33. 9±0. 4^a
PLT ($\times 10^3/mm^3$)	630. 61±1. 93	525. 54±3. 21^a	601. 87±2. 61^b	603. 63±1. 87^b	573. 56±10^a

Values were expressed as Mean ± SD of Complete Blood Picture of different treated groups (RBC, WBC, HB, HCT, MCV, MCH, MCHC, PLT) are estimated compared with AlCl3 group taking as control. Data was analyzed by one-way ANOVA followed by Dunnet's test for multiple comparisions (n=6 in each group).

$^a p<0.001$, $^b p<0.01$, $^c p<0.05$ indicates significant values between different goups when compared to control.

Spinach (100mg/kg) the increased to RBC count by 1. 43 times when compared to control. Spinach (300mg/kg) the increased RBC count by 1. 62 times when compared to control. Sorrel (100mg/kg) the RBC count by 1. 71 times increased when compared to control. Sorrel (300mg/kg) the RBC count by 1. 87 times increased when compared to control.

Spinach (100mg/kg) the increased Hb levels by 1. 05 times when compared to control. Spinach (300mg/kg) the increased Hb levels by 1. 14 times when compared to control. Sorrel (100mg/kg) the increased Hb levels by 1. 10 times when compared to control. Sorrel (300mg/kg) the Hb levels increased 1. 19 times when compared to control.

Spinach (100mg/kg) the increased WBC levels by 1. 16 times when compared to control. Spinach (300mg/kg) the increased WBC levels by 1. 34 times when compared to control. Sorrel (100mg/kg) the increased WBC levels by 1. 18 times when compared to control. Sorrel (300mg/kg) the WBC levels increased 1. 38 times when compared to control.

Spinach (100mg/kg) the increased MCHC levels by 0. 99 times when compared to control.

Spinach (300mg/kg) the increased MCHC levels by 0. 98 times when compared to control. Sorrel (100mg/kg) the increased MCHC levels by 1. 02 times when compared to control. Sorrel (300mg/kg) the MCHC levels increased 1. 05 times when compared to control.

Spinach (100mg/kg) the increased PLT levels by 0. 83 times when compared to control. Spinach (300mg/kg) the increased PLT levels by 0. 95 times when compared to control. Sorrel (100mg/kg) the increased PLT levels by 0. 96 times when compared to control. Sorrel (300mg/kg) the PLT levels increased 0. 90 times when compared to control.

Effects of the graded doses of spinach and sorrel leaves on MDA levels of anti oxidant activity in serum.

LIPID PREOXIDATION

For the estimation of malondialdehyde levels, standard graph of TEP (1, 1, 3, 3-tetra ethoxy propane) was plotted between the concentration and absorbance values.

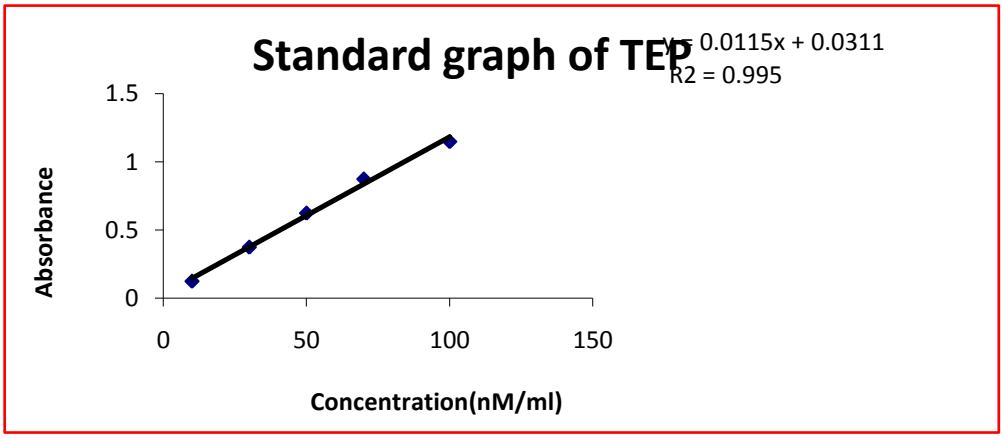

Fig 3: Standard graph of 1, 1, 3, 3-tetra ethoxy propane (TEP)

The standard graph of constructed using absorbence and concentration was linear with a regression equation of Y=0. 0115X-0. 0311 and r value of 0. 99.

Effects of the graded doses of spinach and sorrel leaves on MDA levels of anti oxidant activity in serum.

Table no. 4 MDA levels in different groups of rats.

Group (n=5)	DRUG	MDA (nM/ml)
I	Normal control	186. 07±5. 05
II	Spinach (100mg/kg)	103. 19±1. 89[a]
III	Spinach (300mg/kg)	98. 07±0. 71[a]
IV	Sorrel (100mg/kg)	128. 62±3. 98[a]
V	Sorrel (300mg/kg)	112. 44±3. 58[a]

All the values are expressed in Mean±SD of malondialdehyde levels. p<0. 001 compared with corresponding values of control.

Antioxidant activity has been expressed in serum MDA levels (Nmoles/mg of serum). Spinach (100mg/kg) the decreased MDA levels by 0. 55 times when compared to control. Spinach (300mg/kg) the decreased MDA levels by 0. 52 times when compared to control. Sorrel (100mg/kg) the decreased MDA levels by 0. 68 times when compared to control. Sorrel (300mg/kg) the MDA levels decreased 0. 60 times when compared to control.

CONCLUSION

In conclusion, the present study reveal that Spinach and sorrel leaves increased the RBC count, WBC count Hb%, and MCHC when compared control. The sorrel leaves exhibited

prominent effect when compared to spinach leaves. However didn't exhibit prominent effect on platelet count. In addition, both the plant extracts showed significant effect on in decreased MDA levels when compared to control.

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