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EFFECT OF JUNK FOODS (RICE MEAL CHIPS) AND (POTATO CHIPS) ON HAEMATOPOESIS IN MALE WISTAR RATS

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ABSTRACT

Aim: The present study was aimed at investigating the effect of junk foods (potato chips and rice meal chips) on Hematopoiesis in healthy Wistar rats. **Subjects and Methods:** The study was carried in 30 male wistar rats which were given with different doses of junk food through oral gauge for 21 days. haematological parameters are determined before and after administration of junk foods. After 21 days animals are sacrificed and sent for histopathological studies of liver spleen and intestine. **Results:** Potato chips(100mg/kg) has decreased red blood cells 1.47 times when compared to control group and rice meal chips (300mg/kg) has decreased red blood cells 1.5 times compared to control group. Potato chips (100mg/kg) has decreased red blood cells 1.39 times and potato chips(300mg/kg) has decreased RBC 1.43 times when compared to control group. **Conclusion:** In conclusion, the present study demonstrates that the junk foods rice meal chips and potato chips induced oxidative stress, thrombocytopenia and anaemia in the animals.

KEYWORDS: Haematopoesis, wistar rats, anemia, thrombocytopenia.

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(Tortora *et al*., 2008). 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 (Tortora *et al*., 2008).

1. Erythropoesis: Synthesis of erythrocytes (red blood corpuscles) is called as Erythropoesis.

2. Lymphopoesis: Synthesis of white blood cells (lymphocytes) is called Lymphopoesis.

3. Myelopoesis: Synthesis of granulocytes, megakaryocytes and macrophages from myeloid progenitors is called Myelopoesis. (Tortora et al., 2008)

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 (Dickson et al., 1995).

Sex: Males have more blood cells than females. Males tend to produce more RBC and haemoglobin compared to females(Dickson et al., 1995).

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 (Dickson et al., 1995).

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(Dickson *et al*., 1995).

Social: Social factors like education, employement, family, safety, food habbits also alter haematological parameters(Dickson *et al*., 1995).

Ethnic background: Ethnic factors also affects Haematopoesis. For ex: Most of the Americans suffer with sickle cell anaemia when compared to Indians (Dickson *et al*., 1995).

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(Dickson *et al*., 1995).

JUNK FOOD

Junk food is a derisive slang term for food that is of little nutritional value and often high in fat, sugar, salt, and calories. Junk foods typically contain high levels of calories from sugar or fat with little protein, vitamins or minerals. Foods commonly considered junk foods include salted snack foods, gum, candy, sweet desserts, fried fast food, and sugary carbonated beverages(Junk food, Google search)

The consumption of junk foods is associated with the following disadvantages

i. Overeating becomes habituated to the students.
ii. These junk food fried chicken or fish, bacon, French fries, mayonnaise, and special sauces contain high fat ingredients, plentiful of cheese which can cause health problems like Hypertension, Type - 2 Diabetes, Hyperlipidaemia and peptic ulcer etc.
iii. Baked and fried items at high temperatures contain acrylamide, which is proven to be a carcinogenic substance.

MATERIALS AND METHODS

DRUGS AND CHEMICALS

1. Rice meal chips
2. Potato chips

Chemicals

1. Thiobarbituric acid (Himedia India Ltd)
2. Hydrogen peroxide (SD Fine Chemicals India Ltd)
3. Sodium chloride (Himedia India Ltd)
4. Trichloroacetic acid (Himedia India Ltd)
5. Phosphatebuffer solution

Equipments

1. Cobus u 411(CBP analyzer)
2. Colorimeter
3. Ultra centrifugator (Remi Industries, Mumbai.)
4. UV-Spectrometer (Remi Industries, Mumbai.)
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 propylene cages and maintained at (24 ± 1°C) with relative humidity 45-55% and 12/12 hours light and dark cycle and 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 in to 5 groups (n=6). Initial blood samples were collected from animals and checked for complete blood picture and the serum samples are analyzed for antioxidant activity. Initial body weights of animals were recorded.

Animals were given with drug prepared freshly and given daily for 21days. Doses of the drugs are fixed by performing limit test for 24 hrs. Two doses of each product 100mg and 300mg are prepared by solublising in distilled water and administerd to the animals according to the following study protocol. Body weights of animals are taken on 7^{th} day and 21^{st} day. Change in body weights are also noted.

After 21^{st} day the final blood samples were collected by retro orbital puncturing from eye and checked for complete blood picture. Serum samples were analysed for antioxidant activity and compared with initial control samples. After 21^{st} day animals are sacrificed according to animal ethical rules. liver, Spleen, intestine are isolated and examined for gross histopathological changes by comparing with control group.

DRUG 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(D/W) only
- Group- 2 received rice meal chips100mg/kg) in distilled water orally for 21days
- Group -3 received rice meal chips (300mg/kg) in distilled water orally for 21 days
- Group-4 received potato chips(100mg/kg) in distilled water orally for 21 days
- Group-5 received potato chips (300mg/kg) orally for 21 days in distilled water.

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.

ANTI OXIDANT ACTIVITY

Estimation of lipid peroxidase

The level of Lipid peroxides was estimated by Thio barbituric acid reaction method described by (Ohkawa *et al.*, 1979*)*

Reagents

Trichloro Acitic acid (30%)

Thiobarbituricacid(TBA)(1%)

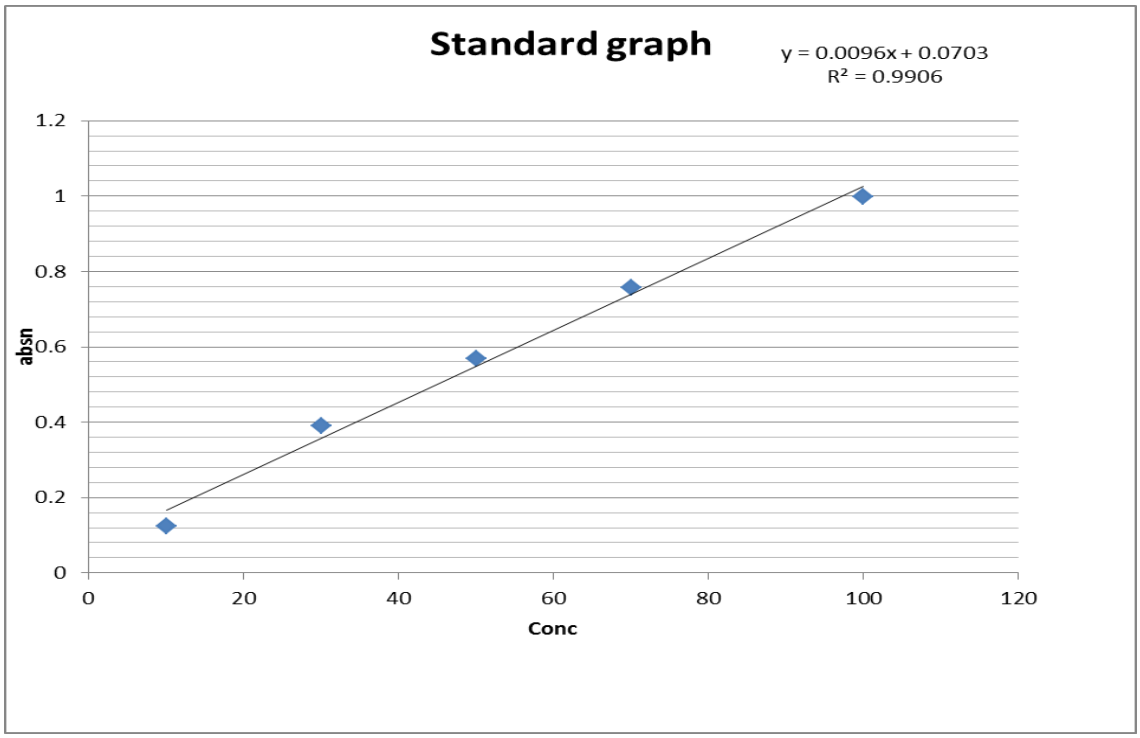
Procedure

To 0.5 ml of test sample, 0.5 ml of 30% Trichloro acitic acid, 100 µl of 1% TBA were added. The mixture was made up to 4 ml with water then cover with aluminium foil and then heated in a water bath at 90-97°C for 60 minutes. After cooling, the solution was filtered and the OD of filtrate was measured at 540nm in colorimetric.

The MDA values were measured by using standard graph.

Standard graph of MDA

Concentration	Absorbance
10	0.124
30	0.389
50	0.568
70	0.756
100	0.998



Statistical analysis: the data was expressed as mean ± SD values andchecked for significance using one way anova followed by multiple comparisions test. If p value is p<0.05*, p<0.1**, p<0.001*** are considered statistically significant results.

JUNK FOOD TAKEN FOR STUDY

1. Rice meal chips (Masala munch)
2. potato chips (Magic masala)

Rice meal chips

This junk food product is Manufactured by local brand and is a fried item above 100ºC. It contains the ingredients like Rice Meal, Edible Vegetable Oil, Corn Meal, Spices and Condiments (Onion Powder, Chilli Powder, Coriander Powder, Ginger Powder, Garlic powder, Black Pepper Powder, Turmeric Powder, Fenugreek Powder), Salt, Back Salt, Tomato powder, Sugar Acid, Tartaric Acid (Pepsicoindia.co.in). potato chips (Magic masala) This junk food is Manufactured by local brand. This product is fried item above 100ºc. It contains the ingredients like Potato, Edible Vegetable Oil, Spices & Condiments used as natural flavouring agents (Chilli Powder, Onion Powder, Dry Mango Powder, Coriander Powder, Ginger Powder, Garlic Powder, Black Pepper Powder, Turmeric Powder, Cumin), Salt, Black Salt, Sugar, Tomato Powder, Citric Acid and Tartaric Acid.

Significance of High and low white blood cells

Low WBC indicates leukocytopenia. It occurs in some viral infections and immunosupression and kidney cancer. Increased white blood cells indicate diseases like leukaemia and some bacterial infections.(www.mayoclinic.com).

Significance of High and low platelets

Low platelets indicate Thrombhocytopenia. Some drugs like quinine decrease platelets. Conditions like viral infections and leukaemia also decreases platelet count. Increase in platelet count indicates Thrombocytosis. This condition prevails incase of lung, gastro intestinal and breast cancers. It also occurs in IDA and some viral infections(www.mayoclinic.com).

Significance of high and low red blood cells

Decreased red blood cells indicate anaemia, kidney disease, bone marrow failure (for instance, from radiation or a tumor), malnutrition, or other causes. A low count may also indicate nutritional deficiencies of iron, folate, vitamin B12, and vitamin B6. (www.mayoclinic.com)

Increased red blood cells indicates polycythemia. Polycythemia occurs in case of anabolic steroids, Carbon monoxide poisoning, Congenital heart disease in adults, COPD and Kidney cancer (www.mayoclinic.com).

Significance of high and low Haemoglobin

High haemoglobin levels are observed very rarely. They may occur during Dehydration and bone marrow disorders. Low haemoglobin indicates anaemia. (www.mayoclinic.com).

Effect of Junk foods rice meal chips and Potato chips on RBC

Table 1: Showing Mean±SD values of RBC of control and test group.

Control(n=6)	Rice chips(100mg/kg)	Rice chips(300mg/kg)	Potato chips100mg/kg)	Potato chips(300mg/kg)
9.19 ± 0.56	6.25 ± 1.51***	6.08 ± 1.35***	6.6 ± 0.672***	6.41± 0.82***

Values were expressed as Mean ± SD of counts in RBC., ***p<0.001 as compared to control group. Data was analyzed by one-way ANOVA followed by Dunnet's test for multiple comparisions (n=6 in each group).

Rice chips(100mg/kg) has decreased red blood cells 1.47 times when compared to control group and rice chips (300mg/kg) has decreased red blood cells 1.5 times compared to control group.

Potato chips (100mg/kg) has decreased red blood cells1.39 times and potato chips(300mg/kg) has decreased RBC 1.43 times when compared to control group.

Effect of Junk foods Kurkure and Lays on WBC

Table 2: Showing Mean±SD values of WBC of control and test groups.

Control	Rice chips(100mg/kg)	Rice chips(300mg/kg)	Potato chips(100mg/kg)	Potato chips(300mg/kg)
12.81± 1.1	12.93 ± 1.46	14.78± 0.95***	8.48 ± 1.21***	6.95± 0.99***

Values were expressed as Mean ± SD of counts in WBC,***p<0.001 as compared to control group. Data was analyzed by one-way ANOVA followed by Dunnet's test for multiple comparisions (n=6 in each group).

Rice chips (100mg/kg) has increased white blood cells 1.03 times when compared to control group and rice chips (300mg/kg) has increased white blood cells 0.86 times compared to control group.

Potato chips (100mg/kg) has decreased white blood cells 1.51 times and potato chips (300mg/kg) has decreased RBC 1.84 times when compared to control group.

Effect of Junk foods rice meal chips and potato chips on HGB

Table 3: Showing Mean±SD values of HGB of control and test groups.

Control	Rice chips(100mg/kg)	Rice chips(300mg/kg)	Potato chips(100mg/kg)	Potato chips(300mg/kg)
15.21 ± 0.54	8.96 ± 2.2***	9.01 ± 2.57***	9.38 ± 2.14***	7.4 ± 2.31***

Values were expressed as Mean ± SD of counts in HGB,***p<0.001 as compared to control group. Data was analyzed by one-way ANOVA followed by Dunnet's test for multiple comparisions (n=6 in each group).

Rice chips (100mg/kg) has decreased haemoglobin 1.69 times when compared to control group and rice chips (300mg/kg) has decreased haemoglobin 1.68 times compared to control group.

Potato chips (100mg/kg) has decreased haemoglobin 1.62 times and Lays(300mg/kg) has decreased HGB 2.05 times when compared to control group.

Effect of Junk foods rice chips and potato chipson MCHC

Table 4: Showing Mean±SD values of MCHC of control and test groups.

Control	Rice chips(100mg/kg)	Rice chips(300mg/kg)	Potato chips(100mg/kg)	Potato chips(300mg/kg)
26.33 ± 3.1	25.01± 2.13***	22.96 ± 1.97***	16.7 ± 1.25***	14.16 ±1.16***

Values were expressed as Mean ± SD of counts in MCHC,***p<0.001 as compared to control group. Data was analyzed by one-way ANOVA followed by Dunnet's test for multiple comparisions (n=6 in each group).

Rice chips (100mg/kg) has decreased MCHC 1.04 times when compared to control group and rice chips(300mg/kg) has decreased MCHC 1.14 times compared to control group.

Potato chips (100mg/kg) has decreased MCHC 1.57 times and potato chips(300mg/kg) has decreased MCHC 1.85 times when compared to control group.

Effect of Junk foods rice chips and potato chips on Platelets

Table 5: Showing Mean±SD of PLT of control and test groups.

Control	Rice chips(100mg/kg)	Rice chips(300mg/kg)	Potato chips(100mg/kg)	Potato chips(300mg/kg)
462.83± 51.9	377.5± 33.11	222.5± 53.09***	302 ±86.44***	298.16 ±45.17***

Values were expressed as Mean ± SD of counts in PLT,***p<0.001 as compared to control group. Data was analyzed by one-way ANOVA followed by Dunnet's test for multiple

comparisions (n=6 in each group). rice chips(100mg/kg) has decreased platelets 1.22 times when compared to control group and rice chips (300mg/kg) has decreased platelets 2.08 times compared to control group. Potato chips (100mg/kg) has decreased platelets 1.53 times and potato chips (300mg/kg) has decreased platelets 1.55 times when compared to control group.

Antioxidant activity

Lipid Peroxidase(TBARS ASSAY)

Standard graph of MDA

Concentration	Absorbance
10	0.124
30	0.389
50	0.568
70	0.756
100	0.998

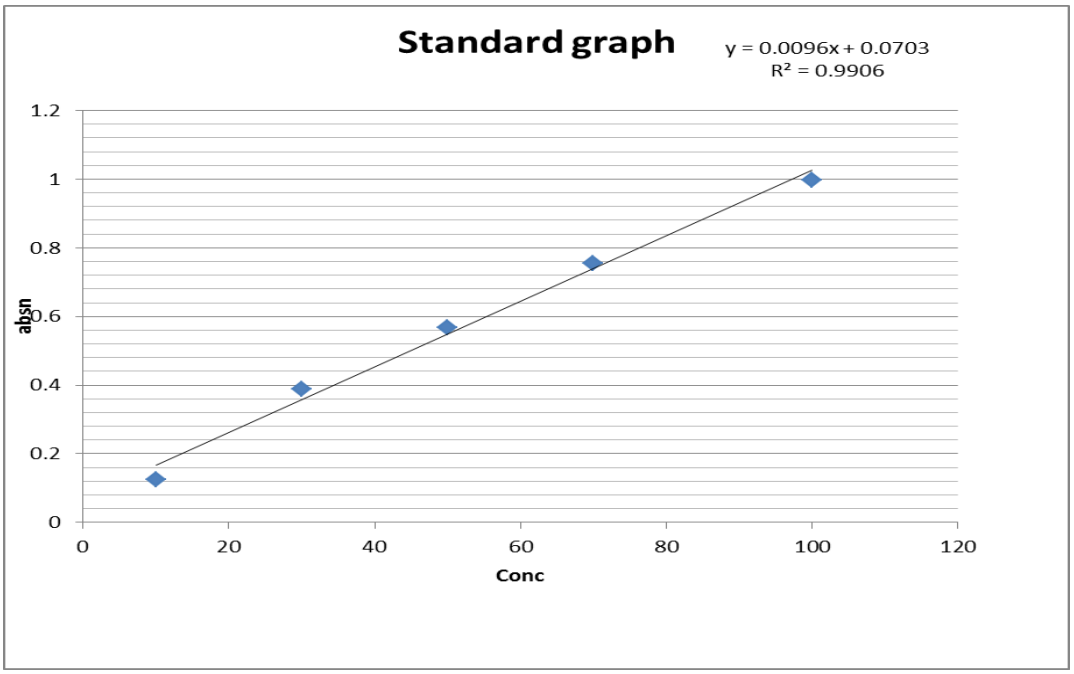


Table 6: Shows Mean ± SD values of MDA levels of control and test groups.

Groups	% MDA Levels (n moles/mg of serum)
Control	20.62 ± 1.60
Rice chips(100mg/kg)	25.52 ±5.34***
Rice chips(300mg/kg)	36.36 ± 2.99***
Potato chips(100mg/kg)	39.82 ± 3.34***
Potato chips(300mg/kg)	40.12 ±1.90***

Values were expressed as Mean ± SD; ***p<0.001 as compared to control group. Data was analyzed by one-way ANOVA followed by Dunnet's test for multiple comparisions (n=6 in each group).

MDA levels in serum has been increased significantly ($p<0.001$). This indicates oxidative stress caused by junk food.

Effect of Junk foods rice chips and potato chips on Body weight

Table 7: Showing effect of junk food on body weight of rats.

Groups	1st day(gm)	7th day(gm)	21st day(gm)
Control(C1)	180	182	183
Control(C2)	195	196	198
Control(C3)	210	211	213
Control(C4)	185	187	190
Control(C5)	174	176	180
Control(C6)	180	183	186
Rice chips(k1)	210	215	214
Rice chips (k2)	150	170	168
Rice chips k3)	175	179	174
Rice chips (k4)	165	170	154
Rice chips k5)	187	192	176
Rice chips (k6)	150	160	148
Rice chips (k7)	175	203	152
Rice chips (k8)	152	178	130
Rice chips (k9)	186	204	132
Rice chips (k10)	194	212	121
Rice chips (k11)	173	193	104
Rice chips (k12)	185	209	98
Potato chips(L1)	150	182	148
Potato chips L2)	165	195	120
Potato chips (L3)	172	192	156
Potato chips (L4)	163	210	142
Potato chips (L5)	158	195	132
Potato chips (L6)	172	210	153
Potato chips (L7)	177	215	142
Potato chips (L8)	153	220	80
Potato chips (L9)	184	215	76
Potato chips (L10)	194	218	95
Potato chips (L11)	179	226	105
Potato chips (L12)	185	230	102

The above results indicate weight gain initially due to junk food. Up to 7th day the animals have increased weight. After 21days there was significant decrease in weight.

CONCLUSION

In conclusion, the present study demonstrates that the junk foods rice chips and potato chips induced oxidative stress, thrombocytopenia and anaemia in the animals. Initially the body weight of the animals have been increased and after 21 days the body weight of the animals

were gradually decreased. Junk food (rice chips) have increased leukocytes and lays have decreased leukocytes. Junk food(rice chips) have damaged liver and spleen by causing degenerative tissue loss in liver and spleen. rice chips (300mg/kg) has degenerated more tissue in liver and spleen compared to rice chips (100mg/kg). Junk food (potato chips) have damaged spleen, observed with infilterates in the spleen. Liver tissue was also degenerated by potato chips. More tissue damage in spleen and liver is observed in potato chips (300mg/kg) when compared to potato chips (100mg/kg). High doses of drug(junk food) significantly altered the haematological parameters when compared to low dose. There was gross change in haematological parameters and histology of liver and spleen when compared to control animals. Junk foods rice meal chips and potato chips significantly altered haematological parameters.

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