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EFFECT OF *ALOE VERA* JUICE ON THE TOXICITY INDUCED BY ISONIAZID AND RIFAMPICIN DRUGS ON COMPLETE BLOOD COUNT IN MALE WISTAR RATS

P. P. Bhise [1] and G. V. Zodape [*2]

Departments of Zoology [1], S.S. & L.S. Patkar College of Arts and Science & V.P. Varde College of Commerce and Economics, S.V. Road, Goregaon (West), Mumbai- 400062, Maharashtra, India.
Departments of Zoology [2], Shivaji University, Kolhapur - 416004, Maharashtra, India.

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Correspondence to Author:
G. V. Zodape

Departments of Zoology,
Shivaji University, Kolhapur,
416004, Maharashtra, India.

E-mail: drgautamvz5@gmail.com

ABSTRACT: Male Wistar rats were procured from 'The Bombay Veterinary College', Parel, Mumbai. The animals were maintained and housed in cages and were acclimatized in laboratory conditions for ten days prior to the experiment. The experimental rats were fed orally with isoniazid 300 mg/ 70 kg body weight / day; rifampicin 450 mg / 70 kg body weight / day and *Aloe vera* extract 50 mg/ kg body weight / day for 30 days. Blood samples of the above groups (A to H) were taken after 30th day from cardiac puncture for estimation of mean concentration of complete blood Count. The present changes resulted from the antitubercular drugs in the hematological changes might be attributed to the toxic metabolites of the isoniazid and rifampicin. The toxic, reactive metabolites of the drugs bind to cellular macromolecules and release or form toxic free radicals intern caused the tissue damage. The present study was concluded that Supplementation of *Aloe vera* extract resulted in significant improvement in the hematological parameters might be due to its antioxidant, antistress, cytotoxic, antioxidant, hypoglycemic and anti-inflammatory properties.

INTRODUCTION: Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world including those in developed countries, turning to complementary and alternative medicine. Many traditional remedies employ herbal drugs for the treatment of liver ailments**.** [1, 2, 3, 4]. *Aloe vera* has been used for many centuries for its curative and therapeutic properties and although over 75 active ingredients from the inner gel have been identified, the therapeutic effects have not been correlated well with each individual component [5].

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Many of the medicinal effects of aloe leaf extracts have been attributed to the Polysaccharides found in the inner leaf parenchymatous tissue, [6, 7] but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance [8].

In the pharmaceutical industry, *Aloe vera* has been used for the manufacture of tropical products such as ointments and gel preparations, as well as in the production of tablets and capsules [9, 10]. Important pharmaceutical properties that have recently been discovered from both the *Aloe vera* gel and whole leaf extracts include the ability to improve bioavailability of co-administered vitamins in human subjects [11].

The biological activities include promotion of wound healing, antifungal activity, hypoglycemic

or anti diabetic effects, anti inflammatory, anticancer, hematologic, immunomodulatory and gastroprotective properties. In recent years study has shown that both *Aloe vera* gel and whole leaf extracts have been investigated for their drug absorption enhancing properties and some of these extracts have been associated with cytotoxic effect and some others were not efficient enough to ensure that therapeutic levels of poorly absorbable drugs are achieved [12].

Isoniazid drug is used for the treatment of tuberculosis and diabetes. Isoniazid induces generalized convulsions, coma and metabolic acidosis. Death may occur from acute respiratory failure or hypertension, liver and peripheral nervous and hematologic system is the main target organs of isoniazid chronic toxicity. Over dosage of Isoniazid has produced nausea, vomiting dizziness, slurred speech, blurred vision and visual hallucinations. Symptoms of over dosages usually occur within 30 minutes to 3 hours following ingestion of the drug [13, 14].

Rifampicin or rifampin (USAN) is a bacteriostatic antibiotic drug of the Rifampicin group. It is a semisynthetic compound derived from Streptomyces mediterranei. Rifampicin may be abbreviated RIF, RMP, RD or R. Rifampicin was introduced in 1967 as a major addition to the cocktail-drug treatment of tuberculosis and inactive meningitis, along with isoniazid, ethambutol, pyrazinamide and streptomycin. It must be administered regularly daily for several months without break; otherwise, the risk of drug-resistant tuberculosis is greatly increased [15].

Rifampicin is typically used to treat Mycobacterium infections, including tuberculosis and leprosy. It also has a role in the treatment of methicillin-resistant Staphylococcus aureus (MRSA) in combination with fusidic acid. It is used in prophylactic therapy against Neisseria meningitidis (meningococcal) infection. It is also used to treat Listeria species, Neisseria gonorrhoeae, Haemophilus influenzae and Legionella pneumophila. The most common effects are fever, gastrointestinal disturbances, rashes and immunological reactions. Liver damage, associated with jaundice, has also been reported and in some rare cases has led to death [16, 17].

MATERIALS AND METHODS:

Fresh *Aloe vera* leaves were brought from the botanical garden and sample was identified and was brought to the laboratory in the Department of Zoology S.S & L.S. Patkar College Goregaon (west), Mumbai India.

a)Extraction:

Aloe vera leaves were rinsed 2-3 times in the tap-water. 50 grams of leaves were then grounded with 50ml of distilled water in sterilized pestle and mortar. The homogenized mixture was filtered twice through a cotton cloth and centrifuged at 5,000 rpm for 10 minutes. Supernatants were collected and diluted with 50 ml of distilled water to obtain a concentration of 50mg /day / kg body wt. of male Wistar rat. As per the body weight, Isoniazid tablets (Macleods Pharmaceuticals, Andheri, Mumbai) and Rifampicin capsules (Lupin Ltd, Kartholi, Jammu & Kashmir) were dissolved in sterile distilled water and given orally.

b)Experimental Design:

Forty eight (48) male Wistar rats (age 60-100 days, weighing 175-260 mg) were purchased and procured from 'The Bombay Veterinary College', Parel, Mumbai. The animals were maintained and housed in cages in the Department of Pharmacology, The Bombay Veterinary College, departmental animal house and were fed on commercial rat pallets brought from the market. The rats were acclimatized in laboratory conditions for ten (10) days prior to the experiment. The rats were divided into eight (8) groups containing Six (6) rats in each group. The experiments were carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee (CPCSEA No. MCV/IAEC/19/2014) for the present experimental study.

Group A: Control i.e. male albino wistar rats fed with rat pellets and ordinary water.

Group B: Male albino wistar rats fed orally with *Aloe vera* extract 50 mg / kg body weight / day for 30 days.

Group C: Male albino wistar rats fed orally with *Aloe vera* extract 50 mg / kg body weight / day and Isoniazid drug (LD) the dose will be decided on the basis of human consumption, 300 mg / 70 kg body weight / day for 30 days

Group D: Male albino wistar rats fed orally with *Aloe vera* extract 50 mg / kg body weight / day and rifampicin drug (LD) the dose will be decided on the basis of human consumption, 450 mg / 70 kg body weight / day for 30 days

Group E: Male albino wistar rats fed orally with isoniazid drug (LD) the dose will be decided on the basis of human consumption, 300 mg / 70 kg body weight / day for 30 days.

Group F: Male albino wistar rats fed orally with rifampicin drug (LD) the dose will be decided on the basis of human consumption, 450 mg / 70 kg body weight / day for 30 days.

Group G: Male albino wistar rats fed orally with isoniazid + rifampicin drug (LD) the dose will be decided on the basis of human consumption, 300 mg (isoniazid) + 450 mg (rifampicin) / 70 kg body weight / day for 30 days

Group H: Male albino wistar rats fed orally with *Aloe vera* extract 50 mg / kg body weight / day and with isoniazid+ rifampicin drug (LD) the dose will be decided on the basis of human consumption, 300 mg (isoniazid) + 450 mg (rifampicin) / 70 kg body weight / day for 30 days.

c) Analysis of the blood samples:

Blood samples of the above groups were taken after 30th day from cardiac puncture and was analyzed at the Unique Bio Diagnostics Enterprises (UBE)Veterinary Pathology Laboratory, B-20, Bhuvaneshwar, Dr. V. K. Valimbe Road, Near Gururani Nagkanya Chowk, Parel Village, Mumbai, for estimation of Hemoglobin; R. B. C.; W. B. C. Plaelet Count; Packed Cell Volume; Mean corpuscular volume ; Mean corpuscular hemoglobin; Mean corpuscular hemoglobin concentration; Neutrophils; Eosinophils; Lymphocytes; and Monocytes.

RESULTS AND DISCUSSIONS:

TABLE: 1: SHOWING THE MEAN CONCENTRATION OF COMPLETE BLOOD COUNT IN MALE WISTAR RATS

Rats code Group	Hb gm %	RBC x 10^6 / cmm	WBC X 10^3 / cmm	PLT X 10^5/ cmm	PCV %	MCV fl	MCH pg	MCHC gm/dl	N. %	E. %	L. %	M. %
A	14.7	7.3	16.5	10.2	39.5	53.6	19.9	37.2	34.5	1.1	66.6	01
B	14.3	7.2	14.6	9.5	37.0	50.8	19.6	38.7	33.8	1.1	58.1	01
C	13.9	7.0	12.3	10.6	37.3	53.5	19.9	37.4	29.1	0.3	68.0	01
D	13.9	7.3	12.7	7.9	36.6	49.9	19.0	37.3	36.0	1.0	62.0	01
E	15.6	8.1	8.4	6.9	41.5	51.3	19.3	37.7	27.5	1.8	71.5	01
F	14.3	8.0	9.0	8.7	38.0	47.6	18.2	38.4	30.0	1.0	73.1	01
G	15.6	7.3	11.3	7.3	42.5	58.6	21.9	36.8	36.5	0.6	68.5	01
H	15.7	7.1	13.0	7.9	40.5	56.6	21.5	38.8	25.1	0.8	68.3	01

*Each value is the average of 6 determinations.

Hb: Hemoglobin g / dl
RBC: Red Blood Cells million / cmm
WBC: White Blood Cells thousands / cmm
PLT: Plaelet Count lakhs / cmm
PCV: Packed Cell Volume %
MCV: Mean corpuscular volume fl
MCH: Mean corpuscular hemoglobin pg
MCHC: Mean corpuscular hemoglobin concentration gm/dl
N: Neutrophils %
E: Eosinophils %
L: Lymphocytes %
M: Monocytes %

The mean concentration of Complete Blood Count in male wistar rats has been illustrated Table 1.

The level of haemoglobin in control group A was found to be (14.7 gm %). In comparison with the control group the lowest level of hemoglobin was recorded in group C&D (13.9 gm %) low haemoglobin concentrations usually indicate the presence of anaemia it may be because of isoniazid and rifampicin drug. These low haemoglobin and haematocrit has been attributed to destruction of sickle red blood cells by the

phagocytes of the body immune system in the circulating blood system [18]. Whereas the highest levels of hemoglobin was recorded in group H (15.7 gm %), the high haemoglobin concentrations usually indicate the presence of dehyand occasionally polycythaemia.

The RBC count in control group A was found to be (7.3 x 10^6 / cmm). The total RBC was found minimum in group C (7.0 x 10^6 / cmm) low red blood cell (erythrocyte) numbers usually indicates the presence of anaemia reduced RBC count but this blood parameter was not affected in rats because they were treated with isoniazid and *Aloe vera* combination. The level of RBC was found maximum in group E (8.1 x 10^6 / cmm). High red cell counts usually indicate the presence of dehydration - and only occasionally true polycythaemia.

Animals with obvious clinical signs of haemorrhage or with a regenerative anaemia on routine screening should have their platelet count monitored. However it is a useful examination to include in a routine screen because of the number of chronic, subclinical conditions which can affect circuplatelets. This may be occur because of isoniazid drug it might caused acute respiratory failure or hypertension, liver and peripheral nervous and hematologic system is the main target organs of isoniazid chronic toxicity significantly.

The WBC count in control group A was found to be (16.5 X 10^3 / Cmm). The total WBC was found minimum in group F (9.0 X 10^3/Cmm) where no significant increased in WBC were found in remaining groups. The observed decrease in total WBC count by rifampicin drug suggests that the drug may be immunosuppressive. This reduction could be due to their diminished production, redistribution from peripheral blood into the tissues or rapid destruction of WBC [19]. The most common effects are fever, gastrointestinal disturbances, rashes and immunological reactions and other proteins, [20] thus the reduction in WBC production could arise from the drug binding to some proteins which has been reported to regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBC [21]. This reduction in WBC by rifampicin is however beneficial to SCD patients because most of them have high WBC count which result in production of injurious cytokinins and may cause tissue damage.

The Platelet (PLT) count was found minimum in group E (6.9 X 10^5/ cmm) a reduced number of platelets (thrombocytopenia) occurs due to decreased production, increased destruction or loss from the body circuand is seen in bone marrow disease, uraemia, toxaemia, infection, hypoadrenocortidsm, DIC, immune-mediated disorders, myeloprolifera disorders, haemorrhage and splenomegaly. The PLT count was found maximum in group C (10.6 X 10^5/ cmm) an increased number of platelets (thrombocytosis) occurs due to excessive production rate or decreased removal from the circulation in acute or chronic infections, inflammatory disease, drug induced, some myeloproliferative disorders (most cause thrombocytopenia) or malignant neoplasia.

The amount PVC count was found minimum in group D (36.6%)**.** The significant decrease in PCV concentration by the action of Aloe *vera* and rifampicin suggests that a net combination *Aloe vera* and rifampicin drug found beneficial effect on erythropoiesis which may be of benefit to sickle cell patients. Sickle cell patients show a significant decrease in their haematocrit (PCV) concentration compared to normal individual resulting in severe anaemia [22]. The PVC count was found maximum in group G (42.5%) a high PCV usually indicates the presence of dehydration because of isoniazid and rifampicin combination. Many authors consider that older animals are in a state of relative dehydration -and this is certainly likely in the presence of polyuric syndromes such as diabetes, renal failure and hyperadrenocorticism.

The low MCV values were found in group F (47.6 fl) may be because of red cell size may be small such as is caused by chronic haemorrhage and occasionally iron deficiency or feline haemo. The MCV count was found maximum in group G (58.6fl) that may be appeared due to the drug absorption enhancement mechanism of isoniazid and rifampicin drug. The similar results were also suggest that the antisickling agents that increase

MCV and or decrease MCHC markedly delay the rate of polymerization of deoxyhaemoglobin and inhibit red blood cell sickling [23].

Mean corpuscular hemoglobin (MCH) is a calculation of the amount of oxygen-carrying hemoglobin inside RBC. The amount MCH count was found minimum in group F (18.2 pg) where as the MCH count was found maximum in group G (21.9 pg) since macrocytic RBC is larger than either normal or microcytic RBC, it would also tend to have higher MCH values.

The amount MCHC count was found minimum in group G (36.8 gm/dl). This was appeared because of the addition of isoniazid and rifampicin significantly decreased the amount of MCHC suggests that individual application of isoniazid and rifampicin had no significant effect on blood MCHC. The MCHC count was found maximum in group H (38.8 gm/dl) isoniazid and rifampicin significantly reduced RDW while it was elevated by addition of *Aloe vera*, isoniazid and rifampicin combination.

The amount neutrophils count was found minimum in group H (25.1%). The mean percentage neutrophils obtained in this study was found to be higher in group G (36.5%). In isoniazid and rifampicin than the control and other test groups. This was suggested the high degree of infection. This may appeared due to ingestion of isoniazid and rifampicin as a matter of fact, may have induced an increased in the metabolic rate, with the resultant increased in the generation of free radicals with the attendant of cellular damage. The immune system responds to this damages caused by production of oxidants during stressful conditions. During such responses, free radicals are produced by the neutrophils the first-responders to inflammatory cells to remove damage cells. Being highly mobile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages [24]. Neutrophils also recruit and activate other cells of the immune system.

The mean values of eosinophils recorded in control group A was found to be (1.1%) The amount eosinophils count was found minimum in group C (0.3%) The mean eosinophils obtained in this study were significantly higher in group E (1.8 %) fed with isoniazid drug. The increased eosinophils percentage in isoniazid drug ingested group above the normal range in this study was suggestive of a high level of infection the rats might have been exposed to couple with depressed immune system. The other authors have also suggested that the eosinophils primarily are associated with parasitic infections and an increase in their number may indicate depressed immune system [25]. Eosinophils along with basophils and mast cells are important mediators of allergic responses and associative pathogenesis in the development of asthma [26].

The mean values of lymphocytes recorded in control group A was found to be (66.6 %.) The amount lymphocytes count was found minimum in group B (58.1 %) where as the lymphocytes count was found maximum in group F (73.1 %) which was fed with rifampicin drug. In this study, it was possible that the membranes of these lymphocytes were oxidized as the rats were subjected to *Aloe vera* as a low normal to low absolute lymphocyte concentration is associated with increased rates of infection after trauma.

The amount monocytes count was found neither increase nor decrease in the percentage of monocytes in this study. This might be due to short circulation period of monocytes in blood stream; monocytes circulate for only about one to three days and then typically move into tissues throughout the body, it is likely most of the monocyte would have moved into tissue before the blood was obtained for analysis.

CONCLUSION: The present changes resulted from the antitubercular drugs in the hematological changes might be attributed to the toxic metabolites of the isoniazid and rifampicin. The toxic, reactive metabolites of the drugs bind to cellular macromolecules and release or form toxic free radicals intern caused the tissue damage. The present study was concluded that Supplementation of *Aloe vera* extract resulted in significant improvement in the hematological parameters might be due to its antioxidant, antistress,

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