HAEMATOLOGICAL CHARACTERIZATION IN N-NITROSO-N ETHYLUREA INDUCED TUMOUR BEARING RATS ON ORAL ADMINISTRATION OF PLEUROTUS PULMONARIUS AND PLEUROTUS OSTREATUS METABOLITES

[1]Akanni E.O, [2]Oloke J.K & [3]Fakunle E.E

[1]Department of Biomedical Science, Ladoke Akintola University of Technology, Osogbo. Nigeria.

[2]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Nigeria.

[3]Department of Haematology and Blood Transfusion, Ladoke Akintola University of Technology, Osogbo. Nigeria.

E-mail: olufemiakanni@yahoo.com

ABSTRACT

The effects of oral administration of the aqueous solution of metabolites of *Pleurotus pulmonarius* and *P. ostreatus* on the haematological parameters and haematimetric indices pre and post tumour induction by N-Nitroso-N-ethylurea in Wister rats were studied. Solid tumour was induced on Wister rats by painting the shaved interscapular region of the experimental rats with 25µL of 0.04M N-Nitroso-N-ethylurea in acetone daily for 5 consecutive weeks. The solution of the metabolites in water was administered by gavage (0.2ml of 20mg/ml) pre, during and post tumor induction. The tumour burden was estimated and scored with the antitumour effects of the metabolites evaluated by assessing changes in tumour size, number and some haematological parameters**.** Automation method using Sysmex KX-21N Instrument and Leishman staining technique were employed in the analysis of complete blood count, leukocytes differential count and cells morphology of various treatment groups. Results revealed that the tumour control group (C) rats developed anaemia with significant differences "$P<0.05$" also noted in the erythrocytes, platelet and white blood cell count (total) as well as haemoglobin and haematocrit levels. The haematimetric indices such as MCH and MCHC presented no significance "$P>0.05$". No statistical differences were noted in the differential leukocyte counts when tumour control group and control/placebo group were compared. Results indicated the cancer chemo preventive potentials of the metabolites as there is no significant difference "$P>0.05$" in the haematological parameters of the group induced with tumour and were administered *Pleurotus pulmonarius* and *P. ostreatus* metabolites simultaneously compared with the group that fed on commercial diet and water only. There is however a significant difference "$P<0.05$" in the erythrocyte count, haematocrit and MCH of the group bearing solid tumour that were administered placebo solution when compared with the parameters in the group given commercial feed and the metabolites. Also, no significant differences "$P>0.05$" were observed in the baseline haematological parameters of the group that were induced with tumour and administered Pleurotus pulmonarius simultaneously when compared with their post-analytical parameters. Study reveals that both metabolites ameliorated anaemia in cancerous states of the rats and are non-toxic to healthy animals as they are well tolerated. However, metabolites of *Pleurotus pulmonarius* were observed to demonstrate a better cancer chemoprevention potential than *Pleurotus ostreatus* metabolites as evidenced by the results of the parameters studied although the difference is not statistically significant "$P> 0.05$".

Key words*: Cancer chemoprevention, tumour suppressor, N-Nitroso-N-ethylurea, Pleurotus pulmonarius and P. ostreatus metabolites, Haematological parameters and Anaemia.*

1. INTRODUCTION

Cancer (malignant neoplasm) is a class of diseases in which a group of cells display *uncontrolled growth* (division beyond the normal limits), *invasion* (intrusion on and destruction of adjacent tissues), and sometimes *metastasis* (spread to other locations in the body via lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, do not invade or metastasize. Most cancers form a tumor but some, like leukemia, do not (Cancer Research UK; 2007).

The disease causes about 13% of all deaths, according to the American Cancer Society, 7.6million people died from cancer in the world in 2007 (American cancer society, 2006). Cancer can affect other animals besides humans and plants too.

Cancer is fundamentally a disease of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, genes which regulate cell growth and differentiation must be altered (Croce, 2008). Genetic changes can occur at many levels, from gain or loss of entire chromosomes to a mutation affecting a single DNA nucleotide.

There are two broad categories of genes which are affected by these changes. Oncogenes may be normal genes which are expressed at inappropriately high levels, or altered genes which have novel properties. In either case, expression of these genes promotes the malignant phenotype of cancer cells. Tumor suppressor genes are genes which inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes. Typically, changes in many genes are required to transform a normal cell into a cancer cell (Knudson, 2001).

In developed countries, adult cancer is presently responsible for about 25% of all deaths and 0.5% of the population is diagnosed with cancer on yearly basis (Jemal *et al*., 2005). Cancer can also occur in children, but it is rare. Statistics from the SEER of the US NCI demonstrate that childhood cancer increased 19% between 1975 and 1990, mainly due to increased incidence in leukaemia (James *et al*., 1999).

There is reasonable doubt that children having near nuclear facilities face an increased risk of cancer (Global research, 2005). The age of peak incidence of cancer in children occur during the first year of life (infant). The average annual incidence in the United States, 1975-1995 was 233 per million infants (James *et al*., 1999). White infants have higher cancer rates than black infants. Leukaemia accounted for a substantial proportion of this difference, the average annual rate for white infant (48.7 per million) was 66% higher than black infants (Jemal *et al*., 2005).

HIV is associated with a number of malignancies, including Kaposi's sarcoma, non-Hodgkin's lymphoma, and HPV-associated malignancies such as anal cancer and cervical cancer. AIDS-defining illnesses have long included these diagnoses. The increased incidence of malignancies in HIV patients points to the breakdown of immune surveillance as a possible etiology of cancer (Wood and Harrington, 2005). Certain other immune deficiency states (e.g. common variable immunodeficiency and IgA deficiency) are also associated with increased risk of malignancy (Mellemkjaer *et al.,* 2002).

The term 'mushroom' is used here according to the description of (Chang and Miles, 1992) as 'a macrofungus with a distinctive fruiting body, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand. Mushrooms constitute at least 14 000 and perhaps as many as 22 000 known species. The number of mushroom species on the earth is estimated to be 140 000, suggesting that only 10% are known. Assuming that the proportion of useful mushrooms among the undiscovered and unexamined mushrooms will be only 5%, which implies 7000 yet undiscovered species will be of possible benefit to mankind. Even among the known species the proportion of well investigated mushrooms is very low.

The medicinal use of mushrooms has a very long tradition in the Asian countries, whereas their use in the Western hemisphere has been slightly increasing only since the last decades.

Hot water extracts of many mushrooms used in traditional Chinese medicine and other folk medicines have long been said to be efficacious in the treatment of various diseases including many forms of cancer. The use of medicinal mushroom extracts in the fight against cancer is well known and documented in China, Japan, Korea, Russia and now increasingly in the USA (Mizuno *et al*., 1995). However, it is only within the last three decades that chemical technology has been able to isolate the relevant compounds and use them in controlled experiments. They have been extensively screened for medical properties especially for anticancer application (Mizuno, 1999). Many species of mushrooms have been found to be highly potent immune system enhancers, potentiating animal and human immunity against cancer (Wasser and Weis, 1999a, Borchers *et al*., 1999, Kidd, 2000; Ikekawa, 2000., Feng *et al*., 2001)

2. MATERIALS AND METHODS

The Metabolites: *Pleurotus pulmonarius and Peurotus ostreatus* metabolites were produced by submerged fermentation at the Biotechnology Laboratory of Ladoke Akintola University of Technology, Ogbomoso. Nigeria.

Administration of metabolites: 0.2ml of 20mg/ml of the metabolites of *Pleurotus pulmonarius and P. ostreatus* were administered by gavage pre, during and post tumor induction as designed in the respective treatments.

The experimental rat: 48 Wister rats of about 150 g each were purchased from the animal house of Obafemi Awolowo University Ile-Ife, Nigeria. The rats were randomly arranged in separate wooden cages in 8 per group of 2 replicates of 4 each were allowed to acclimatize for 7 days before the commencement of the experiment. The animal room temperature was 28 ± 2^0C with 12h light/dark cycle.

The experimental (control) diet: The rats' commercial (control) diet was purchased from the animal house of the Ladoke Akintola University of Technology, Osogbo. The rats were fed with the rat pellets and water *ad libitum*.

Tumour induction: Tumour inducing agent, carcinogen N-Nitroso-N- ethylurea ($C_3H_7N_3O_2$) Sigma-Aldrich ID N 3353, Mol.wt 117.1; was dissolved in acetone to prepare 0.04M concentration. 25µL was used to paint the shaved interscapular (region) skin of the rats daily for 5 weeks as designed in the experimental protocol.

Experimental protocol:

Group A: Metabolites /Tumor (n=8). The rats were treated with *Pleurotus pulmonarius* and *P. ostreatus* metabolites (in separate groups) through oral administration and induced with tumour using N-Nitroso-N-ethylurea every 24hrs daily for one month. These groups were evaluated according to the efficacy of the metabolites in providing chemopreventive measures.

Group B: Tumor/metabolites (n=8). The rats were induced with N-Nitroso-N-ethylurea and after 2 weeks treated with the metabolites. These groups were evaluated according to efficacy of metabolites to serve as chemotherapeutic agent.

Group C: Tumor/placebo (n=8). Rats were induced with N-Nitroso-N-ethylurea daily and administered placebo with normal diet and water *ad libitum* for one month. This is the tumour test group.

Group D: Tumor/metabolites (n=8). The animals were treated with the metabolites every 24hrs a day through oral route and after 2weeks induced with N-Nitroso-N-ethylurea daily. This group was evaluated according to the efficacy of the metabolites in providing chemo preventive measure.

Group E: Control/placebo (n=8). The animals were not induced with N-Nitroso-N-ethylurea but received a placebo solution every 24hrs. The haematological parameters of this group were designated as my reference values.

Group F: Control/metabolites (n=8). The rats were not induced with N-Nitroso-N-ethylurea but received the metabolites every 24hrs. These groups were evaluated for possible adverse reaction associated with metabolites.

Hematological Analysis Using Automation: (Sysmex KX – 21N Instrument, Sysmex Corporation, Kobe, Japan, 2003)

Sysmex KX – 21N is a quantitative automated hematology analyzer for in-vitro diagnostic use in determining 19 haematological parameters. It directly measure the WBC, RBC, haemoglobin, haematocrit, platelets, Absolute lymphocytes count, Absolute mixed count and Absolute Neutrophil count while the remaining parameters are calculated or derived, Mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution weight etc.

Principle: Blood sample collected in EDTA anticoagulant (50ul) is diluted with cell-pack in the WBC counting Chamber. A fixed volume or stromatolyser - WH solution is added to the automatic machine to obtain a final dilution of 1.500. The addition of stromatolyser – WH lyses the RBC and the remaining cell stoma is at a level undetectable by the instrument.

At the same time the WBC membrane is preserved and the WBC are stabilized at level detectable by the instrument. They are then counted by Direct current method. Haemoglobin is released during RBC lyses, and is converted to the red methaemoglobin automatically to the haemoglobin detector where the absorbance of the red pigment is measured to give blood haemoglobin level.

3. RESULTS

The results of the haematological and haematimetric parameters in the various treatment groups of the experimental rats are as presented in the tables.

Table 1a: ***Comparison of Baseline and Post-analytical data of studied haematological parameters in the treatment groups using P. pulmonarius metabolites.***

	PCV (%)	HAEMOGLOBIN (g/dl)	RBC ($\times 10^6/\mu L$)	MCV (fl)	MCH(pg)	ICHC(dl)	PLATELETS	WBC ($10^3/\mu L$)
	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
A BASE Vs A POST	38.73 ±3.33 47.80 ± 2.82*	13.00 ± 1.90 13.90 ± 0.36	5.87 ± 1.61 8.20 ± 0.87	69.25 ±16.44 58.43 ± 2.61	24.43 ± 11.94 17.0 ± 1.60	38.78 ± 7.84 29.13 ± 1.50	659.50 ± 399.78 591.67 ± 66.43	18.88 ± 6.87 8.37 ± 1.72
B BASE Vs B POST	42.50 ± 2.65 47.75 ± 3.58	11.95 ± 0.95 13.70 ± 0.49*	7.18 ± 0.31 8.04 ± 0.57*	59.15 ± 1.81 59.45 ± 2.78	16.63 ± 0.78 17.10 ± 1.09	28.15 ± 1.65 28.78 ± 1.80	379.25 ± 218.16 597.50 ± 53.23	18.15 ± 4.04 9.90 ± 4.97*
C BASE Vs C POST	24.38 ±18.54 47.93 ± 3.38	6.23 ± 6.20 14.33 ± 1.20*	3.98 ± 3.38 7.84 ± 0.58	49.73 ± 35.6 61.23 ± 2.27	10.53 ± 8.09 18.28 ± 0.81*	17.23 ± 14.8 29.88 ± 0.51	365.00 ± 253.94 616.00 ± 109.10	8.88 ± 10.25 10.60 ± 4.22
D BASE Vs D POST	42.85 ± 5.31 52.40 ± 4.42	12.78 ± 0.75 15.37 ± 1.50*	6.54 ± 1.37 8.93 ± 0.51*	66.65 ± 7.45 58.57 ± 1.71	20.15 ± 4.18 17.17 ± 0.75	30.05 ± 2.68 29.10 ± 0.95	517.75 ± 203.47 489.00 ± 128.05	15.58 ± 3.57 7.47 ± 1.95*
E BASE Vs E POST	31.13 ± 15.24 39.80 ± 14.12	11.83 ± 2.91 14.03 ± 2.16	5.17± 2.31 6.17 ± 1.84	59.23 ± 5.10 59.35 ± 1.20	24.68 ± 5.99 17.50	42.48 ± 13.3 30.00	343.50 ± 198.53 121.00 ± 7.07	12.85 ± 3.50 8.10
F BASE Vs F POST	37.23± 1.08 61.13 ± 4.17*	10.90 ± 0.59 13.93 ± 1.44*	6.11 ± 0.14	60.95 ± 0.89 60.05 ± 1.48	17.85 ± 0.70 14.55 ± 2.47	29.28 ± 0.78 24.30 ± 4.81	835.75 ± 210.53 371.50 ± 293.45	12.40 ± 2.96 14.68 ± 2.59

***** indicate "P<0.05" ('t' test significant)

POST ANAL = Post-analytical, M = Mean, SD = Standard Deviation, PCV = Packed Cell Volume (Haematocrit), RBC = Red Blood Cell Count (Erythrocyte Count); WBC = White Blood Cell Count (Leukocyte Count), MCV = Mean Cell Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

Table 1b: ***Comparison of Baseline and Post-analytical data of studied haematological parameters in the treatment groups using P. ostreatus metabolites*.**

	PCV (%)	HAEMOGLOBIN (g/dl)	RBC ($\times10^6$/µL)	MCV (Fl)	MCH (pg)	MCHC(dl)	PLATELETS	WBC 10^3/µL)
	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
A BASE Vs	33.80 ± 11.33	11.05 ± 1.97	5.59 ± 1.89	60.55 ± 2.92	21.13±5.39	34.95 ± 9.15	564.50 ± 315.47	20.55 ± 7.01
A POST	47.48 ± 5.91	13.90 ± 1.88	7.42 ± 0.73	64.00 ± 4.27	18.96±1.42	29.18 ± 1.56	247.25 ± 24.69	13.90 ± 1.09
B BASE Vs	38.20 ± 5.29	11.25 ± 1.79	6.29 ± 0.91	61.00 ± 5.10	17.93±1.48	29.38 ± 1.12	820.00 ± 404.82	18.40 ± 8.56
B POST	44.45 ± 7.89	12.83 ± 2.41	7.11 ± 1.08	61.48 ± 4.55	18.44±0.85	29.38 ± 1.12	128.50 ± 3.51*	6.30 ± 1.73*
C BASE Vs	41.75 ± 2.85	12.33 ± 0.91	7.23 ± 0.50	57.78 ± 1.79	17.05±0.71	29.53 ± 0.41	687.75 ± 562.33	13.45 ± 4.73
C POST	43.83 ± 4.37	13.00 ± 1.40	7.09 ± 1.06	58.83 ± 2.63	17.83±0.83	29.50 ± 1.44	163.75 ± 61.26	6.95 ± 2.48
D BASE Vs	40.60 ± 7.26	12.17 ± 2.15	6.91 ± 1.17	58.70 ± 2.33	17.60±0.66	29.97 ± 0.06	791.00 ± 290.81	10.57 ± 1.21
D POST	46.48 ± 5.22	14.10 ± 1.44	8.29 ± 0.10	57.53 ± 3.01	17.83±1.68	30.63 ± 0.96	507.75 ± 291.64	7.63 ± 2.15
E BASE Vs	38.95 ± 2.64	11.73 ± 0.81	6.40 ± 0.64	61.10 ± 3.14	18.45 ±1.69	30.13 ± 1.32	577.75 ± 480.73	11.23 ± 2.86
E POST	51.95 ± 4.43*	15.23 ± 0.70*	8.95 ± 0.61*	55.28 ± 0.84*	17.28±0.31	31.43 ± 1.09	246.75 ± 5.56	21.13 ± 1.31*
F BASE Vs	23.63 ± 4.13	7.83 ± 2.44	3.64 ± 0.89	66.20 ± 14.96	22.38 ±1.02	32.65 ± 7.63	573.50 ± 384.81	9.60 ± 5.98
F POST	46.85 ± 5.16*	14.85 ± 1.64*	8.07 ± 0.52*	56.28 ± 2.73	16.94±1.34	32.13 ± 1.70S	326.50 ± 129.56	11.39 ± 1.36

***** indicate "P<0.05" ('t' test significant)

POST ANAL = Post-analytical, M = Mean, SD = Standard Deviation, PCV = Packed Cell Volume (Haematocrit), RBC = Red Blood Cell Count (Erythrocyte Count); WBC = White Blood Cell Count (Leukocyte Count), MCV = Mean Cell Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

In Tables 1 (a) *P. pulmonarius* and (b) *P. ostreatus*, haematological parameters of Group A baseline (tumour induction and *Pleurotus pulmonarius* and *ostreatus* administration simultaneously) were compared with the post-analytical parameters of the same Group A. No statistically significant differences "P>0.05" were noted in the erythrocyte count, haemoglobin, platelet count, white cell count and haematimetric indices, because the metabolites may have prevented anemia that is usually present in many patients with cancer at the time of diagnosis (i.e. anaemia of chronic diseases). The metabolites are thought to have preventive effects against inflammation and infection usually associated with cancer since it was administered simultaneously. Statistically significant differences "P<0.05" were observed in haematocrit level when Group A baseline and Group A post-analytical results were compared, as a result of initial cancer growth notice before it reverted.

Table 2a: ***Comparison of Post-analytical data of some haematological parameters in treatment groups treated with Pleurotus pulmonarius metabolites or Placebo.***

POST ANAL	PCV (%)	HAEMOGLOBIN (g/dl)	RBC ($\times10^6$/µL)	MCV (fl)	MCH (pg)	MCHC (dl)	PLATELETS	WBC (10^3/µL)
	M± SD	M± SD	M± SD	M± SD	M± SD	M± SD	M± SD	M± SD
A Vs	47.80 ± 2.82	13.90 ± 0.36	8.20 ± 0.87	69.25 ± 16.44	58.43 ± 2.61	29.13 ± 1.50	591.67 ± 66.43*	8.37 ± 1.72
E	39.80 ± 14.12	14.03 ± 2.16	6.17 ± 1.84	59.35 ± 1.20	59.35 ± 1.20	30.00	121.00 ± 7.07	8.10
C Vs	47.93 ± 3.38	14.33 ± 1.20	7.84 ± 0.58	61.23 ± 2.27	61.23 ± 2.27	29.88 ± 0.51	616.00 ± 109.10*	10.60 ± 4.22
E	39.80 ± 14.12	14.03 ± 2.16	6.17 ± 1.84	59.35 ± 1.20	59.35 ± 1.20	30.00	121.00 ± 7.07	8.10
C Vs	47.93 ± 3.38*	14.33 ± 1.20	7.84 ± 0.58*	61.23 ± 2.27	61.23 ± 2.27*	29.88 ± 0.51	616.00 ± 109.10	10.60 ± 4.22
F	61.13 ± 4.17	13.93 ± 1.44	10.09 ± 0.61	60.05 ± 1.48	60.05 ± 1.48	24.30 ± 4.81	616.00 ± 109.10	14.68 ± 2.59
A Vs	47.80 ± 2.82*	13.90 ± 0.36	8.20 ± 0.87*	69.25 ± 16.44	58.43 ± 2.61	29.13 ± 1.50	591.67 ± 66.43	8.37 ± 1.72*
F	61.13 ± 4.17	13.93 ± 1.44	10.09 ± 0.61	60.05 ± 1.48	60.05 ± 1.48	24.30 ± 4.81	616.00 ± 109.10	14.68 ± 2.59
E Vs	39.80 ± 14.12	14.03 ± 2.16	6.17 ± 1.84*	59.35 ± 1.20	59.35 ± 1.20	30.00	121.00 ± 7.07	8.10
F	61.13 ± 4.17*	13.93 ± 1.44	10.09 ± 0.61	60.05 ± 1.48	60.05 ± 1.48	24.30 ± 4.81	616.00 ± 109.10	14.68 ± 2.59
B Vs	47.75 ± 3.58	13.70 ± 0.49	8.04 ± 0.57	59.45 ± 2.78	59.45 ± 2.78	24.30 ± 4.81	597.50 ± 53.23	9.90 ± 4.97
D	52.40 ± 4.42	15.37 ± 1.50	8.93 ± 0.51	58.57 ± 1.71	58.57 ± 1.71	30.05 ± 2.68	489.00 ± 128.05	7.47 ± 1.95

***** indicate p<0.05 ('t' test significant)

POST ANAL = Post-analytical, M = Mean, SD = Standard Deviation, PCV = Packed Cell Volume (Haematocrit), RBC = Red Blood Cell Count (Erythrocyte Count); WBC = White Blood Cell Count (Leukocyte Count), MCV = Mean Cell Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

Table 2b: ***Comparison of Post-analytical data of some haematological parameters in treatment groups treated with Pleurotus ostreatus metabolites or Placebo.***

POST ANAL	PCV (%)	HAEMOGLOBIN (g/dl)	RBC ($\times 10^6$/µL)	MCV (fl)	MCH (pg)	MCHC	PLATELETS	WBC (10^3/µL)
	M± SD	M± SD	M± SD	M± SD	M± SD	M± SD	M± SD	M± SD
A Vs	47.48 ± 5.91	13.90 ± 1.88	7.42 ± 0.73*	64.00 ± 4.27*	18.96 ± 1.42	29.18 ± 1.56	247.25 ± 24.69	13.90 ± 1.09*
E	51.95 ± 4.43	15.23 ± 0.70	8.95 ± 0.61	55.28 ± 0.84	17.28 ± 0.31	31.43 ± 1.09	246.75 ± 5.56	21.13 ± 1.31
C Vs	43.83 ± 4.37*	13.00 ± 1.40*	7.09 ± 1.06*	58.83 ± 2.63*	17.83 ± 0.83	29.50 ± 1.44	163.75 ± 61.26*	6.95 ± 2.48*
E	51.95 ± 4.43	15.23 ± 0.70	8.95 ± 0.61	55.28 ± 0.84	17.28 ± 0.31	31.43 ± 1.09	246.75 ± 5.56	21.13 ± 1.31
C Vs	43.83 ± 4.37	13.00 ± 1.40	7.09 ± 1.06	58.83 ± 2.63	17.83 ± 0.83	29.50 ± 1.44	163.75 ± 61.26	6.95 ± 2.48*
F	47.48 ± 5.91	14.85 ± 1.64	8.07 ± 0.52	56.28 ± 2.73	16.94 ± 1.34	32.13 ± 1.70	326.50 ± 129.56	11.39 ± 1.36
A Vs	47.48 ± 5.91	13.90 ± 1.88	7.42 ± 0.73	64.00 ± 4.27*	18.96 ± 1.42	29.18 ± 1.56*	247.25 ± 24.69	13.90 ± 1.09*
F	47.48 ± 5.91	14.85 ± 1.64	8.07 ± 0.52	56.28 ± 2.73	16.94 ± 1.34	32.13 ± 1.70	326.50 ± 129.56	11.39 ± 1.36
E Vs	46.85 ± 5.16	15.23 ± 0.70	8.95 ± 0.61	55.28 ± 0.84	17.28 ± 0.31	31.43 ± 1.09	246.75 ± 5.56	21.13 ± 1.31*
F	47.48 ± 5.91	14.85 ± 1.64	8.07 ± 0.52	56.28 ± 2.73	16.94 ± 1.34	32.13 ± 1.70	326.50 ± 129.56	11.39 ± 1.36
B Vs	44.45 ± 7.89	12.83 ± 2.41	7.11 ± 1.08	61.48 ± 4.55	16.94 ± 1.34	29.65 ± 1.04	128.50 ± 3.51*	6.30 ± 1.73
D	46.48 ± 5.22	14.10 ± 1.44	8.29 ± 0.10	57.53 ± 3.01	17.83 ± 1.68	30.63 ± 0.96	507.75 ± 291.64	7.63 ± 2.15

***** indicate $p<0.05$ ('t' test significant)

POST ANAL = Post-analytical, M = Mean, SD = Standard Deviation, PCV = Packed Cell Volume (Haematocrit), RBC = Red Blood Cell Count (Erythrocyte Count); WBC = White Blood Cell Count (Leukocyte Count), MCV = Mean Cell Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

Haematologic parameters of the animals with solid tumour that were administered placebo solution (Group C) were compared with the parameters in animals given commercial feed and *P. pulmonarius(Table 2a) and P. ostreatus(Table 2b)* only (Group F). Significant difference was "P<0.05" was noted in the erythrocyte count, haematocrit and MCH. The haematimetric indices such as MCV, MCHC and parameters like platelet counts, white cell count and haemoglobin level presented no significance "P>0.05". This information established tumor formation in Group C and the effect of tumour such as anemia, on the animals in this group.

The healthy animals treated with oral administration of *P. pulmonarius (Table 2a), P. ostreatus (Table 2b)* metabolites and commercial feed only (Group F) presented no significant alteration "P<0.05" in haemoglobin levels, leukocyte count, platelet count and haematimetric indices when compared to healthy animals administered water and commercial diet only (Group E). This finding indicates that the mushroom is not toxic to blood cells.

Also, haematologic parameters of the animals that were induced with cancer and administered *P. pulmonarius* simultaneously (Group A) were compared with the parameters in animals given commercial feed and water only (Group E). There is no significant difference "P<0.05" in their erythrocyte count, haematocrit, haemoglobin, leukocyte count and haematimetric indices such as MCV, MCH and MCHC. This observation is indicative of the cancer/tumour chemo-preventive and immunomodulating potentials of *P. pulmonarius and P ostreatus metabolites*
Table 2 a and b respectively.

4. DISCUSSION AND CONCLUSION

In this study, anaemia was evident in the tumour positive group (tumour plus placebo). The rats developed tumours and showed reduced haemoglobin level, packed cell volume and red blood cells levels (Group C). This is a common feature in cancer condition, that is, anaemia of chronic disease due to increased levels of pro-inflammatory cytokines that have inhibitory effect on erythroid precursors (Moraes *et al.,* 2000). Significant differences "P<0.05" were observed in the number of erythrocytes, platelet counts, white blood cell count (total) as well as haemoglobin, and haematocrit levels. The haematimetric indices such as MCH and MCHC presented no significant difference "P>0.05". No statistical differences were also observed in the differential leucocyte counts when groups C and group E were compared.

There was no statistically significant difference "P>0.05" in the erythrocyte count, haemoglobin, platelet count, white cell count and haematimetric indices of Group A baseline (tumour induction and administration of *P. pulmonarius* simultaneously) when compared with their post-analytical parameters (Table 1a). This appears to be due to the metabolites being able to prevent anemia that is usually present in many subjects with cancer at the time

of diagnosis (i.e. anaemia of chronic diseases). The metabolites are therefore thought to exhibit preventive effects against inflammation and infection usually associated with cancer since it was administered simultaneously.
All rat groups where tumour was induced and administered with the metabolites' solution orally exhibited only mild anaemia compared with control group on normal diet only. However, the experimental group on normal feed with the oral administration of metabolites demonstrated normal haematological parameters with slight improvements on the baseline values. The metabolites do not exhibit any degree of cytotoxicity on the cellular components of the blood as compared with the blood cells in the control group on normal rat feed and water. These observations compared favorably with the report of Vanessa *et al.,* 2008 when they studied haematologic and metabolic effects of dietary supplementation with *Agaricus sylvaticus* fungi on rats bearing solid Walker 256 tumour. It was concluded that *A. sylvaticus* is able to reduce anaemia and improve biochemical parameters in animals with cancer and has no adverse effects on the blood cells of healthy animals.

5. REFERENCES

[1] Borchers AT, Stern JS, Hackman RM, Keen CL, Gershwin EM (1999): Mushrooms, tumors, and immunity. *Soc Exp Biol Med* 221:281–293
[2] Cancer Research UK (2007): UK cancer incidence statistics by age. Retrieved on 2007-06-25.
[3] Chang ST, Miles PG (1992): Mushroom biology – a new discipline. Mycologist 6:64–65
[4] Croce, CM (2008): Oncogenes and Cancer. N Engl J Med. 31:358 (5): 502-511.
[5] Feng, W., Nagai, J. and Ikekawa, T (2001): A clinical pilot study of EEM for advanced cancer treatment with EEM for improvement of cachexia and immune function compared with MPA. *Biotherapy* **15**, 691-696.
[6] Ikekawa T (2001): Beneficial effects of edible and medicinal mushrooms in health care. *Int J Med Mushrooms* 3:291–298
[7] James G. Gurney, Malcolm A. Smith, Julie A. Ross (1999): Cancer Incidence and Survival among Children and Adolescents. *United States SEER program 1975-1995, Cancer Statistics Branch, National Cancer Institute.*
[8] Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ (2005): Cancer statistics, 2005. CA Cancer J Clin ***55***(1): 10–30.
[9] Kidd PM (2000): The use of mushroom glucans and proteoglycans in cancer treatment. Altern Med Rev 5:4–27.
[10] Knudson, AG (2001). Mutation and Cancer. Nature reviews Cancer. 1, 157–162
[11] Mellemkjaer L, Hammarstrom L, Andersen V (2002): Cancer risk among patients with IgA deficiency or common variable immunodeficiency and their relatives: a combined Danish and Swedish study. Clin. Exp. Immunol. 130 (3): 495–500
[12] Mizuno, T., Sakai, T. and Chihara, G. (1995): Health foods and medicinal usages of mushrooms. *Food Reviews International* **11**, 69-81.
[13] Mizuno, T. (1999); The extraction and development of antitumor active polysaccharide from medicinal mushrooms in Japan. *International Journal on Medicinal Mushroom*, **1**:9-29.
[14] Wasser S P, Weis A, (1999): Therapeutic effects of substances occurring in higher basidiomycetes mushrooms, a modern perspective (review). Crit. Rev. Immunol. 19: 65 – 96
[15] Wood C, Harrington W (2005): AIDS and associated malignancies. Cell Res. *15 (11-12): 947-52.*