

Quality, quantity and hematological disorders in blood under Ethanol analyte an *in vitro* study

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Abstract: The purpose of this manuscript is to analyze the quantity, quality and disorders of blood components and blood parameters before and after addition of five different concentrations i.e. (0μl, 10μl, 20μl, 30μl and 40μl) of ethyl alcohol (ethanol) in the blood of Breast cancer patient. We diagnose the disorders in cells count, shape of cells, size of cells and many blood parameters like Hemoglobin, Red blood cells distribution width, Mean corpuscular volume, Platelet crit, Mean platelet volume, Platelet distribution width, Hematocrit, Serum glutamic pyruvic transaminase, Cholesterol, Low-density lipoprotein, triglycerides, Serum glutamic oxaloacetic transaminase, high-density lipoprotein, Urea and Total Billirubin by using hematological techniques. We have noted that the number of WBCs and RBCs decrease sharply while platelet cells increases gradually. We also have revealed the shape changes like WBCs show disintegration around RBCs and clumping of Platelet cells. This work will be extended towards the three dimensional imaging using Optical Coherence Tomography (OCT) for monitoring the level of Ethanol in blood via vasculature maps both for the normal and breast cancer.

Keywords: Estrogen, mammograms, oestradiol level, carcinogens, biopsy.

INTRODUCTION

Ethyl alcohol (ethanol) is one of the flame able, colorless, volatile and a drinking liquid member of alcohol family with chemical formula C_2H_5OH and structural formula CH_3CH_2OH (Brust *et al.*, 2010) It is useful as solvent, antiseptic, antitussive and antidote to methanol. (McDonnell 1999, Calesnick, 1971) but it also has adverse effects like loss of balance, gastrointestinal diseases, allergy, birth defects and cancer (Morch *et al.*, 2007). There are many types of cancer like blood cancer, lungs cancer, liver cancer and breast cancer etc. Our research work is only about blood cancer. The cancer which is developed from breast cells in which inner lining of licks or milk ducts have uncontrollable multiplication of cells is called ductal carcinoma. There is clear scientific evidence that carcinogens are the greater sources of breast cancer and alcohol is one of them (Flatt *et al.*, 2010). Statistical data from literature shows that rate of breast cancer depends upon the average quantity of alcohol taken by a woman. More than 60% breast cancer in women is due to alcohol drinking. There is 55% chances enhancement of breast cancer if a woman drinks four or five drinks daily and high drinking of alcohol doubles the chances of breast cancer (Barnett *et al.*, 2008). Alcohol consumption, genetics, dense breast tissues, getting older, lumps in breast, longer exposure to estrogen, overweight, over height, radiation exposure and hormone replacement therapy (HRT) etc are the main causes of breast cancer. There are different techniques to

diagnose breast cancer at nearly stages like medical history and exam, mammograms, imaging tests, breast ultrasound, ductogram and biopsy. Low use of alcohol is beneficial against breast cancer for old women or even moderate consumption of alcohol is anti breast cancer for young women (Singletary, 2001, Stevens, 2001). The mechanism due to which alcohol consumption is a risk factor for breast cancer is very complicated and varies with daily average drinking quantity of alcohol. Here are few of the mechanisms which may cause the breast cancer (I) high alcohol consumption increases the level of estrogen in female and androgen in male (very rare cases), (II) it may influence or increase the risk factor of breast cancer by enhancing mammary glands, (III) It may also highly damages the DNA of mammary glands (IV) It also increases the metastatic potential which is a risk factor for breast cancer (Kabat *et al.*, 2011). Alcohol drinking has heritage effects i.e. it not only affects the drinking pregnant mother but also the new born daughter has life time elevated threat of breast cancer by elevated oestradiol level (type of estrogen hormone) mechanism (Mcfarland *et al.*, 1963). Alcohol consumption is not a risk factor for all types of cancer but is in women type of breast cancer is associated with alcohol usage, estrogen positive breast cancer increases with alcohol consumption and the risk of triple-negative cancer I is less in those who drank alcohol as compared to who do not drank alcohol (Lindenbaum *et al.*, 1987). Thus the rate of risk of breast cancer in women with consumption of alcohol is higher as compared to those who do not use alcohol.

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MATERIALS AND METHODS

We have used blood smear preparation which is a hematological technique to analyze the blood components and parameters. We took fresh blood of breast cancer patient and poured in five different anticoagulant tubes with 0.3% sodium citrate. Each tube contains 2ml of blood and then we add five different concentrations of ethanol i.e. (0 μ l, 10 μ l, 20 μ l, 30 μ l and 40 μ l) in each tube. Here 0 μ l refers no external addition of alcohol i.e. pure blood. Blood smear of each sample was prepared by using ethanol as fixing agent and field stain (A, B) for staining. Slide for each sample were then examined after putting one drop of emersion oil under microscope model (Olympus CX41) at 100X. The images of each sample set were captured with digital camera model (Canon EOS 600D, Japan) and were saved. Then we put all samples on roller mixer to mix the ethanol in whole blood and perform complete blood count (CBC) with the help of hematological analyzer and noted the blood cells count and blood parameters. We put these samples into centrifuge machines to get platelet rich plasma (PRP) of each sample at 800rpm for 4 minutes. Then PRP was examined under microscope at 40X (dark field microscopy) to analyze the morphology of platelet cells. Then PRP of each sample was put in Selectra junior and noted the blood parameters. Whole experiment was conducted at room temperature

Ethical approval

It is stated that all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

RESULTS

Sample with 0 μ l ethyl alcohol shows that there are broken and abnormal WBCs are present in leukemic patient as shown in fig. 1(a). With increasing ethyl alcohol concentration white blood cells absorb ethyl alcohol undergo shape changes from round to oval shape and starts to disintegrate and parts of WBCs spreads all around RBCs as shown in fig. 1(b, c, d). At further higher concentration of ethyl alcohol along with disintegration and shape changes of leukocytes, they start accumulating. Upon high intake of ethyl alcohol accumulation of leukocytes increases but distortion decreases as shown in fig. 1(e). For platelet cells we see that in breast cancer sample with no alcohol clumping of PLTs is already present as shown in fig. 2(a). Increasing ethyl alcohol concentration causes to increase the aggregation of platelets in leukemia because ethyl alcohol increases the cancer growth. Ethyl alcohol concentration is direct in proportion with rate of cancer growth, thus increase the leukemia by malignant WBCs which indicates an increased rate of platelet cells aggregation as shown in fig. 2(b, c, d, e).

DISCUSSION

We have tried to the depth and detailed knowledge about the changes in blood components and parameters of breast cancer patient prior and after mixing five different concentrations (0 μ l, 10 μ l, 20 μ l, 30 μ l and 40 μ l) of ethyl alcohol. Alcoholism is the worldwide socioeconomic factor/problem which almost disturbs every organ/ system and thus is the cause of metabolic and pathological changes in biological bodies. Immune/defensive system is run by WBCs. WBCs such as neutrophils which give response to bacterial infection and their count usually

Table 1: Blood cells and parameter under aforementioned concentrations of alcohol

Sr. No.	Ethanol Concentration (μ L)	No. of WBCs ($10^3/\mu$ L)	No. of platelet cells ($10^3/\mu$ L)	No. of RBCs ($10^6/\mu$ L)	HGB g/dL	RDW %	PDW %	WBC %	PCT %	MCV fL	MPV fL
1	0	3.9	306	3.49	9.0	17.9	19.1	32.1	0.14	92.0	4.5
2	10	3.7	306	3.28	8.7	18.0	20.6	30.1	0.15	91.8	4.9
3	20	3.3	309	3.19	8.3	18.8	18.2	29.3	0.16	91.8	5.1
4	30	3.1	305	3.04	7.9	18.3	19.7	27.7	0.19	91.1	6.1
5	40	2.9	328	2.89	7.6	18.5	17.8	26.2	0.23	90.7	7.0

Table 2: Blood parameters under afore mentioned five different concentrations of alcohol.

Sr. No.	Ethanol Concentration (μ L)	SGPT (μ L)	SGOT (μ L)	CHO (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	TG (mg/dl)	Total Billirubin (mg/dl)
1	0	16	21	185	119	39.2	25	0.78	125	0.33
2	10	17	28	182	113	35.3	23	0.77	129	0.58
3	20	20	54	178	106	45	21	0.75	120	1.87
4	30	14	61	164	97	37.3	20	0.68	136	1.99
5	40	19	70	164	85.3	28.1	20	0.68	143	2.59

increases but under high consumption of alcohol neutrophils count decreases i.e. neutropenia as shown in fig. 3 due to less production at bone marrow. Moreover leukotriene is a hormonal material that controls adhesive function of neutrophil is also decreased (Homaidan *et al.*, 1984). Blood coagulation/clotting system is controlled by platelet cells and different proteins through haemostatic plug formation. A stretched mesh is formed around the cut or injured part by stringy protein called fibrin. Under hyper condition of alcohol disorders of platelet cells are caused like prolongation of bleeding, less secretion of fibrin protein and impaired aggregation of platelet cells (Bilban *et al.*, 1999). Tumor cells produce and release many cytokines that increases the accumulation of leukocytes and PLTs. Platelet cells count varies in individuals and quantity of drinking. In this research work platelet cells count is increased which also indicates that dengue patients can be treated by high alcohol

consumption. Transportation of oxygen is carried out by RBCs which have iron made component called hemoglobin. Under excessive usage of alcohol iron is converted into ferritin that has cells called ringed sideroblasts which envelope the nucleus and RBCs remain immature. Thus cells count of RBCs decreases significantly as shown in fig. 3 and cause anemia along with macrocytosis, oddly shape and enlargement of RBCs which results destruction/lyses (Cohen *et al.*, 1979). HGB is a red pigment present in blood which plays a vital role to carry oxygen to different parts of body and gives red color to blood. HGB was 9 g/dL and its value reduces by increasing concentration of alcohol and becomes 7.6 g/dL as shown in fig. 4, which indicates anemia due to less count of RBCs (Taskinen *et al.*, 1987). HCT or PCV (packed cell volume) or EVF (erythrocytes volume fraction) is the volume percentage of RBCs. Low HCT results anemia and leukemia. Its abnormal value is life

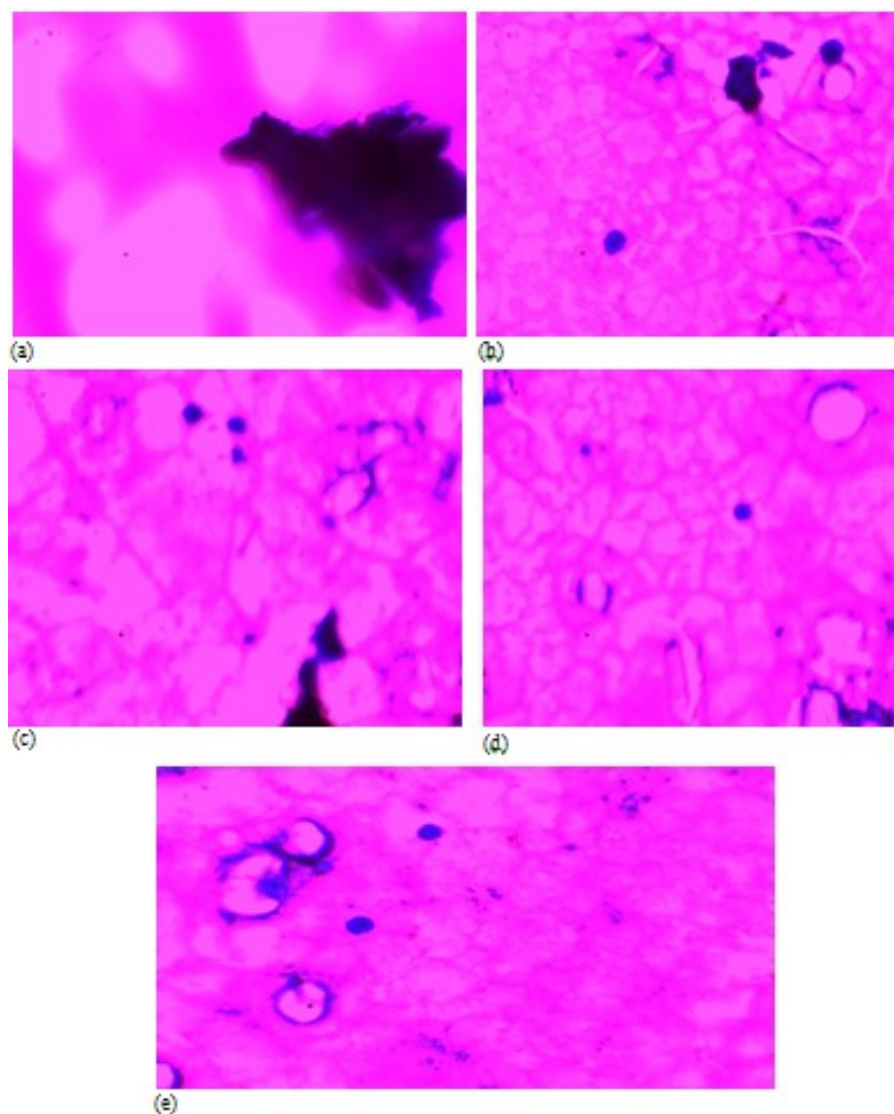


Fig. 1 (a, b, c, d, e): Showing shape changes from round to ellipse and finally burst of WBCs of each phantom at 100X under transmission mode microscopy.

threatening, a low HCT or EVF value is noticed against increasing concentration of alcohol as shown in fig. 4 this indicates symptoms of cancer. MCV is the measure of average volume of RBCs and is helpful in classification of type of anemia. It decreases as shown in fig. 4 with increasing concentration of alcohol thus showing trend from macrocytic anemia to normocytic anemia. RDW is the measure of range of variations of RBCs. RDW shows an increasing trend with increasing concentration of alcohol as shown in fig. 4 at higher concentration. High value of RDW also causes anemia (Bilban *et al.*, 1999). MPV is the measure of average size of platelet cells and is useful to predict the destruction or production of platelet cells. In this case it increases with increasing concentration of alcohol as shown in fig. 4. High MPV results destruction of platelet cells. PDW is used to express the variations in size of platelet cells. PDW decreases with increasing concentration of alcohol as shown in fig. 4. MPV and PDW are related with each other and are generally direct in relation but here is opposite condition. PCT is a source to measure quantitative disorders / abnormalities of platelet cells. It increases with increasing concentration of alcohol as shown in fig. 4. HGB, RDW, HCT and MCV are the parameters related to RBCs while MPV, PDW and PCT are related with platelet cells.

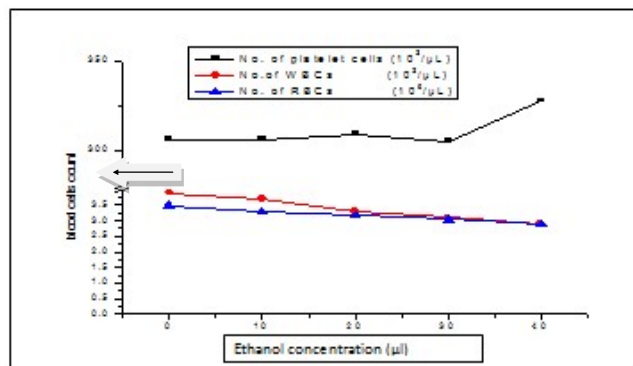


Fig. 3: Effects of different concentrations of Ethanol on blood cells count hematological analyzer.

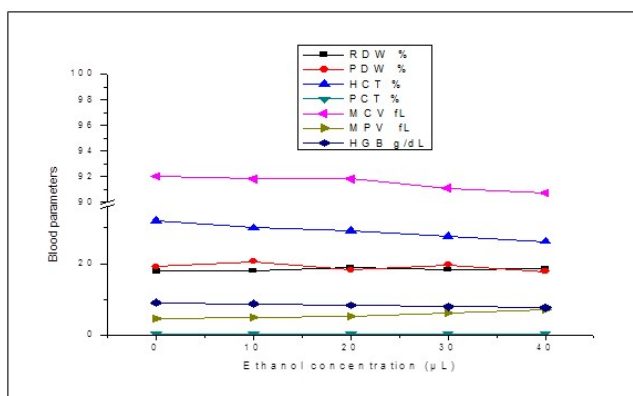


Fig. 4: Blood parameters under different concentrations of Ethanol by hematological analyzer.

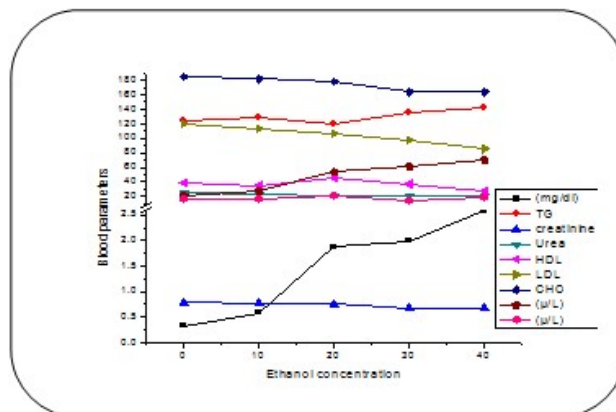


Fig. 5: Blood parameters under different concentration of Ethanol by Selectra junior.

SGPT and SGOT are both liver and heart cells enzymes and their level is increased when liver or heart damages then SGPT is released in blood. SGOT sometime increase in heart patients and increase with age factor. SGPT and SGOT both increases with increasing concentration of alcohol as shown in fig. 5. This indicates that high consumption of alcohol in breast cancer patients increases the level of SGPT and SGOT and thus damages not only heart but also liver (Cohen *et al.*, 1979). Cholesterol is a waxy liquid in our body which is either produced by food that we take or by liver. Its higher level in blood can make harder for heart to transfer blood due to clot formation in capillaries. LDL is a type of cholesterol also called bad cholesterol which also blocks the flow of blood its level is also lowered by high alcohol usage. HDL is good cholesterol which removes the blockage of blood vessels and reduces the risk of heart attack. Its level is decreased under increasing concentration of alcohol. HDL and LDL levels in blood are lowered as the concentration of alcohol goes on increasing as shown in fig. 5. This indicates that by high drinking of alcohol HDL and LDL levels in blood are lowered and thus increases the risk of heart attack. For the patient Cholesterol level is increased under low beverage (Taskinen *et al.*, 1987). Kidney and liver are associated with each other and the disorders in one can create the problem to other at primary level. Urea level is decreased under increasing concentration of alcohol as shown in fig. 5 due to many structural and functional changes in kidneys like abnormal thickened of glomerulus membrane, enlargement, swelling and altered of kidneys cells. This lowering level of urea indicates failure of liver, which is also proved by elevated level of SGPT and SGOT (CHAIKOFF, 1948, VAN THIEL, 1977). Creatinine level in blood is increased for non cancerous persons under low drinking but in our case Creatinine level continuously goes on decreasing as shown in fig. 5. This lowering level of Creatinine in blood indicates less muscular mass or aging which is either due to high alcohol consumption or malnutrition (Cockcroft *et al.*, 1976). Triglycerides are the fats whose high level is a risk

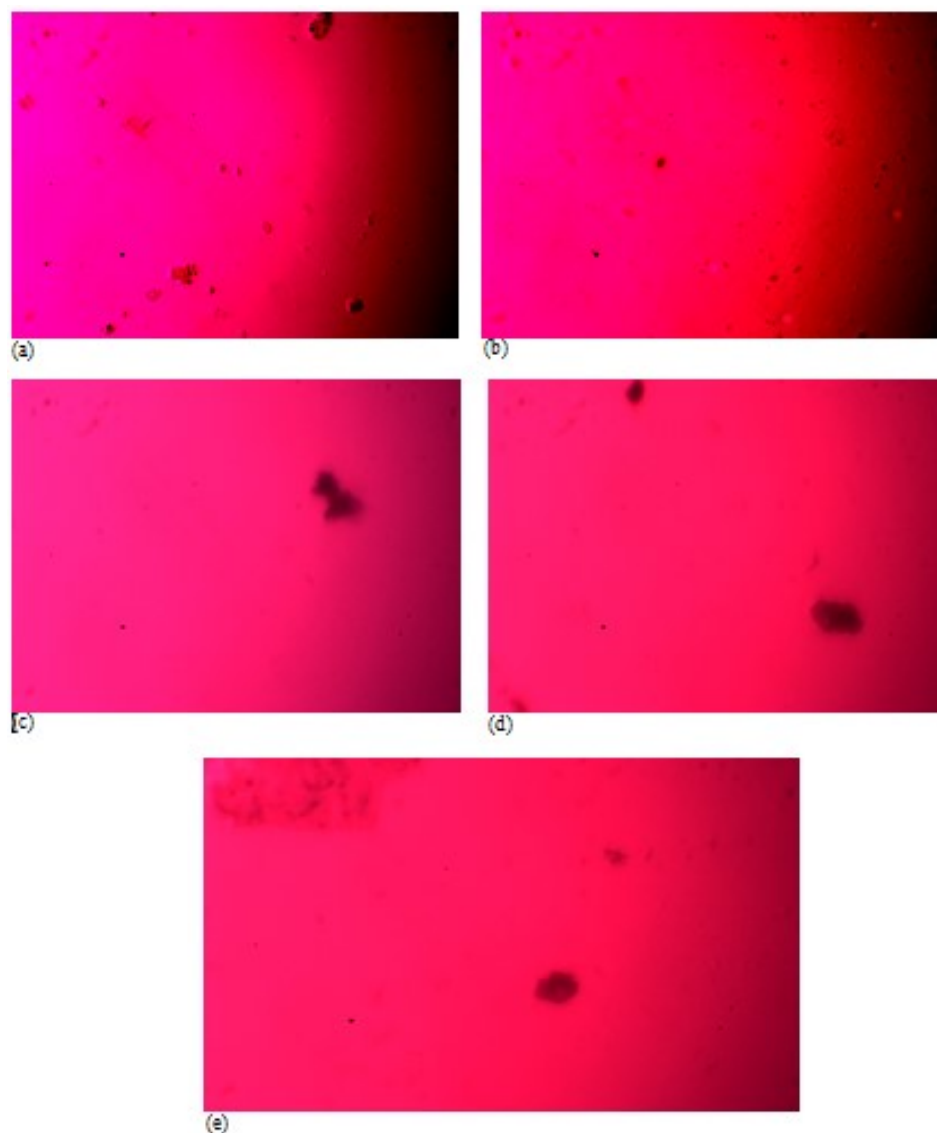


Fig. 2 (a, b, c, d, e): Showing clumping (rouleaux formation) of platelet cells. Mean Platelet Volume increases gradually with increasing concentration of Ethanol

factor for heart. TG goes on increasing with increasing concentration of alcohol as shown in fig. 5. It is noted that 30 gram of alcohol after meal can raise the TG level up to 15.3% after one hour. This increasing value of triglycerides indicates failure or risk of heart failure (Veenstra *et al.*, 1990]. Total Billirubin is a yellow pigment; its high level indicates whitening of eyes or yellowing of body skin. Total Billirubin is produced by the action of biliverdin reductase enzyme its level is increased under high concentration of alcohol as shown in fig. 5. Low count of RBCs indicates high value of Total Billirubin due to broken of RBCs. Thus high alcohol consumption elevates Total Billirubin level which in turns causes liver diseases, blockage of bile ducts and anemia (Veenstra *et al.*, 2010).

CONCLUSION

Alcohol consumption in female is very alarming for health. Blood cells are highly affected from ethanol. WBCs are disintegrated, RBCs remain immature and Platelet cells accumulate in clusters. Their count rate/ μL changes from normal level to abnormal, specially platelet cells count goes on increasing at higher concentration which is beneficial in case of dengue patients. Their shape also goes changing from normal to abnormal shape under high consumption of alcohol. Blood parameters are also affected from alcohol consumption; especially the level of LDL (mg/dl), HDL (mg/dl) and Urea (mg/dl) are highly disturbed. LDL and HDL are very vital blood parameters to remove the blockage in blood vessels while abnormal

Urea level can disturb the proper functioning of kidney. Thus it can be said that high consumption of alcohol leads to breast cancer with destruction of immune system which leads to death.

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