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Macrocytic Anemia and Liver Inflammation Induced by Eucalyptus Oil

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ABSTRACT

The toxic effects of a toxicant result in histo-pathological and hematological variations that may lead to hepatic inflammation. Plant extracts and essential oils have long been used in medicines and food additives despite of their detrimental effects. Eucalyptus oil (EO) is commonly used in pharmaceutical industry for its high contents of menthol. The current study was planned to study the hepatotoxicity and hemotoxicity in Wistar rats. A dose dependent study was executed with T_1= 1.25ml/kg, T_2 = 2.5ml/kg and T_3 = 5ml/kg (kg of body weight) of oral administration of EO. The three experimental groups were compared with control. The animals were dissected and blood extracted by direct heart puncture. Liver was excised and formalin fixed for H&E staining.

A statistically significant increase was observed in MCV, percentage RDW and HCT that arguments for macrocytic anemia. With conserved morphology, the hepatic lobules have shown recruitment of inflammatory cells in perivascular areas with lower degree of hepatocytic necrosis.

Taken together these results we can conclude that EO can cause sterile inflammation to the liver with macrocytic anemia.

Keywords: Eucalyptus Oil, inflammation, Macrocytic anemia, Hematology, Liver histology

INTRODUCTION

Essential oil extracted from Eucalyptus, is commonly used in food and pharmacological industries (Batish *et al.*, 2008; Poke *et al.*, 2005) for its various biological properties such as its cognitive performance (Gobel *et al.*, 1994; Lis-Balchin, 1997; Serafino *et al.*, 2008) and antiseptic activity particularly against *Streptococcus mutans* (Takarada *et al.*, 2004).

Hematological variations provide direct indices for diagnosis of many disorders and malfunctioning physiological parameter e.g. hemolytic anemia in hepatitis (Agrawal *et al.*, 2011; KIVEL, 1961), and drug induced variations in blood structure (Lubran, 1989; Vandendries & Drews, 2006). Particularly *red blood cell distribution width* (RDW%) is routinely assessed as part of the complete blood count (CBC) to gather information on the variations in the size (volume more precisely) of circulating RBCs and therefore higher RDW% reflects greater variability in RBC size *(anisocytosis)*, which is usually caused by perturbation in erythrocyte maturation or degradation (Evans & Jehle, 1991). The RDW is used as supporting index to help diagnose different types of anemia. It has also been serving as a marker for colon cancer and celiac disease because of its responsiveness to subtle nutrient deficiency (Patel *et al.*, 2009; Sategna *et al.*, 2002; Spell *et al.*, 2004). Recent studies provide RDW% to be an efficient marker of cardiac disorders (Allen *et al.*, 2010; Felker *et al.*, 2007; Tonelli *et al.*, 2008). Mean corpuscular volume (MCV) is a ready and direct indication for various kinds of anemia and *macrocytosis* (increased MCV) (Seward *et al.*, 1990). Macrocytosis, a condition that refers to enlargement of RBC with nearly constant hemoglobin concentration, can be categorized in two types (Kaferle & Strzoda, 2009). Macrocytosis with *megaloblastic features* (with increased Reticulocytes, due to bone marrow dysfunction and folate or vitamin B_{12} deficiency) whereas the other types refers to *non megaloblastic features* (with normal Reticulocytes percentage, occurring by dint of iron deficit, pregnancy, alcoholic liver diseases, thyroid dysfunction, renal disorders, spleenotectomy and gammopathy (Horstman *et al.*, 2005; Mukhopadhyay *et al.*, 2007; Oosterhuis *et al.*, 2000)).

Acute inflammation is first strategy in case of any immunological assault to body including changes in various chemical mediators level and metabolic pathways (Gabay & Kushner, 1999; Malik *et al.*, 2010; Malik *et al.*, 2011; Sheikh *et al.*, 2007). The liver is most prominent organ in detoxifying unwanted agents; it is vulnerable for the acute inflammation and necrotic damage more than any other organ in body (Kuntz & Kuntz, 2006; Sheikh *et al.*, 2006) so liver is most hot topic of toxicological or pharmacological studies of various plant extracts.

The point of conflict is that despite of popularity in common uses of essential oils, they do have some side effects (Bakkali *et al.*, 2008). *Eucalyptus* oil is reported for met-hemoglobin formation and chronic liver failure (Craig, 1953; Eisen *et al.*, 2004; Webb & Pitt, 1993). The aim of current study was to particularly describe acute phase response induced by *Eucalyptus* oil in comparison to previously reported chronic or sub chronic investigations.

Author's Contribution: N.S., Developed concept and supervised the project; **M.S**., Designed and performed experiments; **N.Z**., Wrote manuscript. **Corresponding author; s_nadeem77@yahoo.com,*

MATERIALS AND METHODS

The Wistar rats *(Rattus norvegicus)* were segregated in three experimental groups (n=5) and one group as negative control (treated with 0.9% pyrogen free saline solution orally). The experimental groups were designated asT_1 (1.25ml/kg), T_2 (2.5ml/kg) and T_3 (5ml/kg of body weight). The animals were anesthetized, dissected and blood was collected by direct heart puncture in EDTA vials. CBC indices were obtained using automated hematology analyzer (Model MEK-6318; Power input 190V A; 220-240 Volts; Nihon Kohden Corp.). Livers were excised, washed with physiological saline solution and fixed in 10% formalin which was later used for H&E histological staining. The data were analyzed using one way ANOVA and the significance level was accepted where P<0.05 using Megastat Add-in for Microsoft Excel 2010.

RESULTS

Hematology

Statistically significant elevations were observed in MCV, RDW% and HCT as summarized in Table 1. Mean count values explain the increasing trend in the RBCs number. (R^2–value=0.959). While the p-value from the one factor ANOVA of RBCs count is 0.2791 which represent the non-significant differences among the groups (control and test groups) suggesting a lesser significant effects of EO doses over the RBCs number of test groups' animals (Table 1).

Comparison for MCV exhibited a trend in increase (R^2- value=0.8064). One factor analysis of variance proves the significant difference between the treatments (p= 0.0306). There was significant change in MCV level in control and experimental groups by applying one factor ANOVA and Tukey's post hoc test. Significant increase in values of T2 (2.5ml/kg *EO*) and T3 (5ml/kg *EO*) was observed as compared to control group (Table I).

Table I: Mean values with standard error of mean (S.E.M.) of complete blood count parameters in different treatments against control animals.

	CON	T1 (1.25ml/kg)	T2 (2.5ml/kg)	T3 (5ml/kg)	*P*-values
RBCs count (10^{12}/l)	8.49±0.26	8.85±0.35	9.2±0.4	9.35±0.05	0.27
Mean corpuscular volume (*fl)*	41.1±0.3	46.95±0.45	48.25± 1.75	48.95± 1.55	0.03
Red blood cell distribution width	14.85 ±0.25	17.45±0.55	17.3±0.2	17.1 ±0.3	0.02
Hematocrit	34.9 ±1.3	41.9±1.2	44.6±0.3	45.95±1.55	0.009
Platelets (10^9/l)	712 ± 5	943±0 133	687.5± 279.5	794±0 20	0.673
Mean platelets volume *(fl)*	7.25 ±0.05	65± 0	7.05± 0.35	6.55± 0.05	0.096
WBCs count (10^9/l)	8.25 ± 1.45	6.7± 0.8	6.5± 0.35	3.5± 1.1	0.111
Hemoglobin content *(g/dl)*	15.75± 0.35	16.3 ± 0.2	16.3± .85	17.4± 0.1	0.069
Mean corpuscular hemoglobin *(pg)*	17.3 ± 0.1	18.3 ± 0.5	17.6± 0.15	18.5± 0.15	0.947
Mean corpuscular hemoglobin concentration *(g/dl)*	42.2 ± 0.6	39 ± 0.6	36.7± 1.6	37.95± 2.05	0.102

A mild increasing trend in RDW% can be inferred from the graphical representation. RDW % increased from control in first dose T1, while, from T1 to T2 and T3, the index has not shown variation (R^2 values= 0.4837). Significant change in RDW% in control and experimental groups was found by one factor ANOVA (*p*=0.0178) and Tukey's post hoc test. There was significant increase in values in T1 (*p*=0.0064) T2 (*p*= 0.007) and T3 (*p*= 0.0106) when compared to control group (Table I).

Hematocrit percentage exhibited an overall increasing trend in the experimental groups. Percentages tended to vary between control and test groups (R^2 value=0.884). There resided a statistically significant increase in HCT in treated groups in comparison to control (*p*-Value=0.0094). Post-hoc analysis maximum difference (increase) was found between the control and treatment groups. There was significant increase in values of T1 (*p*=0.0140) T2 (*p*= 0.0044) and T3 (*p*= 0.0027) as compared to control groups (Table I).

Platelets percentages tended to vary least between control and test groups (R^2 value = 0.0001). PLT increased in the T1 then platelets

count decreased abruptly. No significant difference was observed between test groups (*p*-value= 0.6732) (Table I).

MPV tended to vary between control and test groups**Error! Reference source not found.** With the (R^2-value=0.2917) non-significant intergroup variations were observed for mean platelets volume (*p*-value =0.0961) (Table I).

WBCs count (10^9/l) showed marked decrease (R^2- value=0.8746) While the *p*-value = 0.1117 narrated non-significant intergroup variations for WBCs counts the experimental groups (Table I).

Hemoglobin contents were noticed to have an increasing trend in the test groups as compared to the control (R^2=0.8951). *p*-value = 0.0699 from one factor ANOVA suggests non-significant difference in HGB content between the groups (Table I).

MCH (mean corpuscular hemoglobin) level decreased in treated groups in comparison to control animals (R^2 = 0.4829) While *p*-value = 0.0947 from one factor ANOVA shows that there is non-significant variation among the groups (Table 1).

MCHCH (mean corpuscular hemoglobin concentration) exhibited minimum variations between control and experimental groups (R^2 = 0.6811). Whereas *p*-value=0.1025 from one factor ANOVA represented non-significant intergroup variations (Table 1).

Histopathology

Control: H&E stained sections of the control liver showed no infiltration of leucocytes to the neighboring surrounding sinusoid spaces. Hepatic lobules are not fragmented or distorted suggesting no disorientation in the microtubules architecture of liver tissue (Fig., 1a).

T1 (1.25ml/kg) test group: In the 1st test group with T1 the infiltration of leucocytes from the hepatic arteries to the sinusoids has been marked showing

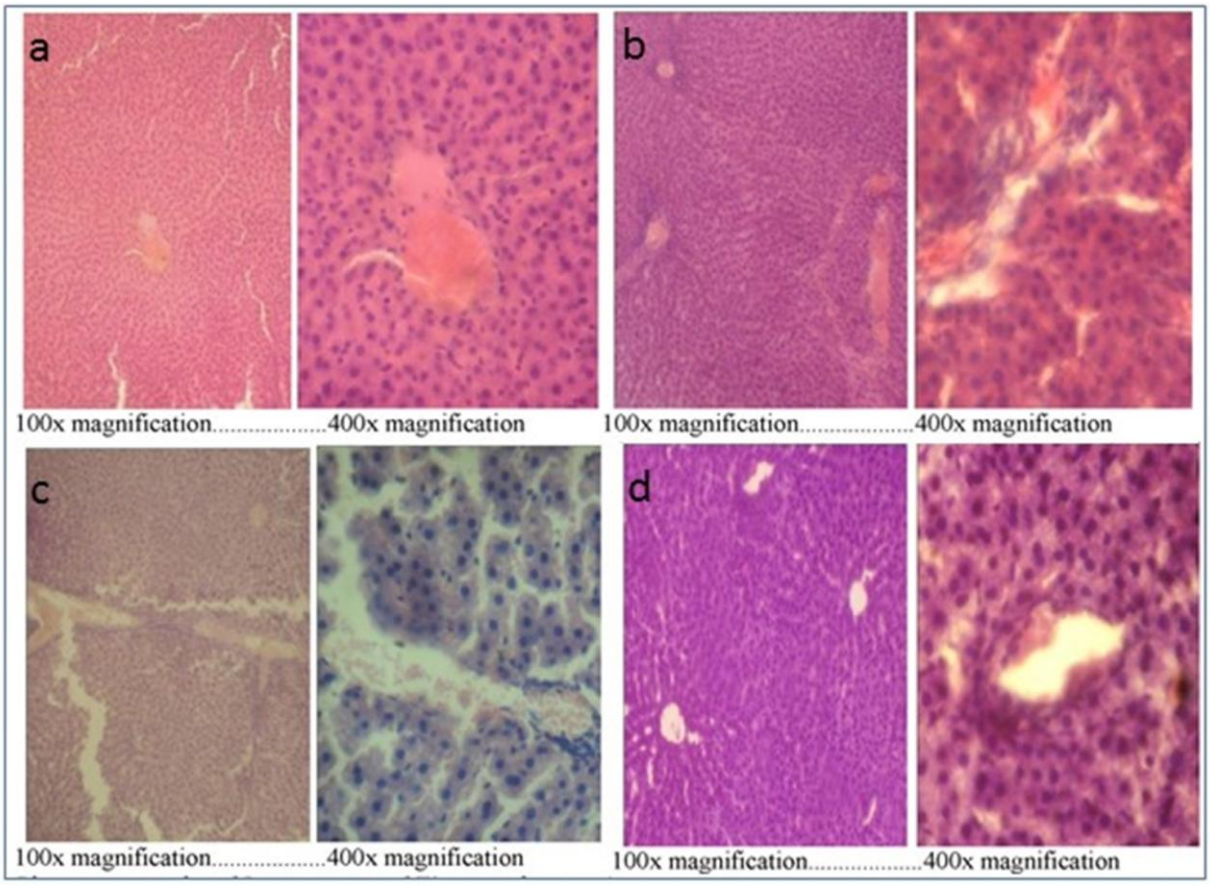

Fig., 1: Histological staining of the liver sections to demonstrat the tissue architecture integrity and effect of the eucalyptus oil. Normal hepatic lobular architecture is visible in control (a), with infiltration of leucocutes in T1 group (b), infiltration of leucocyte and mild degeneration with diffused necrosis in T2 (c) and high degree of the tissue damage along with recruitment of lymphocytes in T3 (d).

the immune response of the organisms. The portal vein and artery are not disrupted or distorted. Cellular degeneration is least observed in T1 animals (Fig., 1b).

T2 (2.5ml/kg) test group: In the 2nd test group with T2 the infiltration of leucocytes to sinusoids has been noticed representing that body has fired immune response. The portal vein and hepatic artery are evident with thinning of walls and damage. Lower level of cellular degeneration and diffused necrosis has been observed (Fig., 1c).

T3 (5ml/kg) test group: Livers of the animals with the 5 ml/kg EO administration revealed to have a maximum degree of the tissue damage by dint of inflammation and recruitment of lymphocytes to the surroundings and the vasculature of the hepatic tissues is deformed at its maximum relatively. Higher degree of coagulative necrosis and cellular degeneration has been observed in liver sections belonging T3 animals (Fig., 1d).

DISCUSSION

Taken together the variations in hematological parameters, it can be inferred that Eucalyptus oil high dose is able to result in macrocytosis with non-megaloblastic features (increased MCV, RDW% and HCT) which were in agreement with the studies reported by Breedveld *et al.* (1981) and acute inflammation in the liver: infiltration of lymphocytes to sites of hepatocellular damage has also been reported by Gruys *et al.* (2005).

Acute hemotoxicity can be correlated to the occurrence of acute inflammation in the living system, as there is variation in serum levels of many chemical mediators having distinct characteristic functions. Their variations result in variability of the physiology of the body as well (Ceciliani *et al.*, 2002; Gitlin & Colten, 1987; Gruys *et al.*, 2005; Liz *et al.*, 2010).

Increase in HCT level is evident in severe cases of dehydration (Tannheimer *et al.*, 2010), and dehydration in acute phase response, on the other hand is caused due to the down regulation of serum albumin level i.e. a negative acute phase protein, dropped systematically in serum to facilitate synthesis of other immune active positive acute phase proteins (Ceciliani *et al.*, 2002; Gitlin & Colten, 1987; Gruys *et al.*, 2005; Liz *et al.*, 2010; Ritchie *et al.*, 1999). Serum albumin level along with immunoglobulins is responsible for osmotic balance and fluidity of blood, a decrease in the serum albumin level makes the blood impotent to leech tissue water (Zetterstrom & Hedstrand, 1981).

Another possible reason of increase in Hematocrit and mild increase in RBCs can be, increase in the Erythropoeitn (EPO) as it is a positive acute phase protein and it is evident to be synthesized from liver cells during inflammation (Ramadori *et al.*, 2010).

Erythropoietin is the principal hormone involved in the regulation of erythrocyte differentiation and the maintenance of a physiological level of circulating erythrocyte mass. It performs its function by activating the EPO- receptors particularly in Erythroid lineage of stem cells upon stimulation; EPOR dimerizes and activate JAK2/STAT5 signaling cascade (STAT1 and STAT3 are rarely initiated) that serves to switching on the transcription of various proteins. Erythroid transcription factor (Eryf1) (GATA-binding factor 1) also gets activated by action of EPO on stem cells. Transcriptional activator which probably serves as a general switch factor for erythroid development works by binding to DNA sites with the consensus sequence of [AT]GATA[AG] within regulatory regions of globin genes and that of other genes up regulated in erythroid cells.

To our knowledge EPO also regulates the body iron by causing an up-regulation of NRAMP 2 (Natural resistance-associated macrophage protein 2), DMT-1 (Divalent metal transporter 1). These proteins are involved in the iron uptake into duodenal enterocytes; in iron transport from acidified endosomes into the cytoplasm of erythroid precursor cells and also play an important role in hepatic iron accumulation and tissue iron distribution (Aird *et al.*, 1994; Digicaylioglu & Lipton, 2001; Kapur & Zhang, 2001). All these biochemical changes may result in the increase of packed cell volume.

Macrocytosis is evident to occur under the effect of various toxicants and reason can be the oxidative stress and direct membrane damage to the cells in particular RBCs (Horstman *et al.*, 2005; Kaferle & Strzoda, 2009; Mukhopadhyay *et al.*, 2007; Tonnesen *et al.*, 1986).

Hepatic histopathological variations are most obvious to occur under the influence of toxicants due to its detoxification potential and changes in Acute phase proteins, and various other hormones (Sundaram & Shaikh, 2011).Hepatocellular damage has its roots in mitochondrial dysfunction leading to initial difused hepatocellular necrosis.

Mechanism for drug-induced liver injury is the formation of reactive metabolites, direct toxicity of liver cells or immune reactions resulting in mitochondrial membrane disruption. Organic toxicant itself can trigger mitochondrial membrane disruption or inhibits mitochondrial function through different mechanisms, such as it can seize coenzyme A or can inhibit mitochondrial β-oxidation enzymes, the transfer of electrons along the respiratory chain, or adenosine triphosphate (ATP) synthase.

Mitochondrial central dogma might also be impeded due to the organic molecules. A severe impairment of oxidative phosphorylation decreases hepatic ATP, leading to cell dysfunction or necrosis; inhibition of pyruvate catabolism, leading to lactic acidosis is also observed. Susceptibility to drug-mediated mitochondrial dysfunction can be increased by factors impairing the removal of the toxic parent compound or by the presence of other medical condition impairing mitochondrial function (Pessayre *et al.*, 2012). Conclusively it can be said that EO can cause macrocytic anemia and inflammation of liver in *Rattus norvegicus*.

REFERENCES

Agrawal, A.K., Haddad, F.G. & Matsunaga, A., 2011. Acute nonimmune hemolytic anemia without fulminant hepatitis in Wilson disease. *J. Pediatr. Hematol. Oncol.*, **33**(**4**): e163-e165.

Aird, W.C., Parvin, J.D., Sharp, P.A. & Rosenberg, R.D., 1994. The interaction of GATA-binding proteins and basal transcription factors with GATA box-containing core promoters. A model of tissue-specific gene expression. *J. Biol. Chem.*, **269**(**2**): 883-889.

Allen, L.A., Felker, G.M., Mehra, M.R., Chiong, J.R., Dunlap, S.H., Ghali, J.K., Lenihan, D.J., Oren, R.M., Wagoner, L.E., Schwartz, T.A. & Adams, K.F., JR., 2010. Validation and potential mechanisms of red cell distribution width as a prognostic marker in heart failure. *J. Card Fail.*, **16**(**3**): 230-238.

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M., 2008. Biological effects of essential oils--a review. *Food Chem. Toxicol.*, **46**(**2**): 446-475.

Batish, D.R., Singh, H.P., Kohli, R.K. & Kaur, S., 2008. Eucalyptus essential oil as a natural pesticide. *Forest Ecol. Manag.* **256**(**12**): 2166-2174.

Breedveld, F.C., Bieger, R. & Van-Wermeskerken, R.K., 1981. The clinical significance of macrocytosis. *Acta Med. Scand.*, **209**(**4**): 319-322.

Ceciliani, F., Giordano, A. & Spagnolo, V., 2002. The Systemic Reaction During Inflammation: The Acute-Phase Proteins. *Protein Pept. Lett.* **9**(**3**): 211-223.

Craig, J.O., 1953. Poisoning by the volatile oils in childhood. *Arch. Dis. Child*, **28**(**142**): 475-483.

Digicaylioglu, M. & Lipton, S.A., 2001. Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-kappaB signalling cascades. *Nature*, **412**(**6847**): 641-647.

Eisen, J.S., Koren, G., Juurlink, D.N. & NG, V.L., 2004. N-acetylcysteine for the treatment of clove oil-induced fulminant hepatic failure. *J. Toxicol. Clin. Toxicol.*, **42**(**1**): 89-92.

Evans, T.C. & Jehle, D., 1991. The red blood cell distribution width. *J. Emerg. Med.*, **9 (Suppl 1**): 71-74.

Felker, G.M., Allen, L.A., Pocock, S.J., Shaw, L.K., Mcmurray, J.J., Pfeffer, M.A., Swedberg, K., Wang, D., Yusuf, S., Michelson, E.L. & Granger, C.B., 2007. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. *J. Am. Coll. Cardiol.*, **50**(**1**): 40-47.

Gabay, C. & Kushner, I., 1999. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*, **340**: 448-454.

Gitlin, J.D. & Colten, H.R., 1987. Molecular biology of the acute phase plasma proteins. *Lymphokines*, **14**: 123-153.

Gobel, H., Schmidt, G. & Soyka, D., 1994. Effect of peppermint and eucalyptus oil preparations on neurophysiological and experimental algesimetric headache parameters. *Cephalalgia*, **14**(**3**): 228-234.

Gruys, E., Toussaint, M.J., Niewold, T.A. & Koopmans, S.J., 2005. Acute phase reaction and acute phase proteins. *J. Zhejiang. Univ Sci. B*, **6**(**11**): 1045-1056.

Horstman, A.L., Serck, S.L. & GO, R.S., 2005. Macrocytosis associated with monoclonal

gammopathy. *Eur. J. Haematol.*, **75**(**2**): 146-149.

Kaferle, J. & Strzoda, C.E., 2009. Evaluation of macrocytosis. *Am. Fam. Physician*, **79**(**3**): 203-208.

Kapur, R. & Zhang, L., 2001. A novel mechanism of cooperation between c-Kit and erythropoietin receptor. Stem cell factor induces the expression of Stat5 and erythropoietin receptor, resulting in efficient proliferation and survival by erythropoietin. *J. Biol. Chem.*, **276**(**2**): 1099-1106.

Kivel, R.M., 1961. Hematologic aspects of acute viral hepatitis. *Am. J. Dig. Dis.*, **6**: 1017-1031.

Kuntz, E. & Kuntz, H., 2006. *Hepatology: Principles and Practice*. Springer Medizin Verlag Heidelberg 2002, Germany: Verlag Heidelberg.

Lis-Balchin, M., 1997. Essential oils and 'aromatherapy': their modern role in healing. *J R. Soc Health*, **117**(**5**): 324-329.

Liz, M.A., Mar, F.M., Franquinho, F. & Sousa, M.M., 2010. Aboard transthyretin: From transport to cleavage. *IUBMB. Life*, **62**(**6**): 429-435.

Lubran, M.M., 1989. Hematologic side effects of drugs. *Ann. Clin. Lab Sci.*, **19**(**2**): 114-121.

Malik, I.A., Moriconi, F., Sheikh, N., Naz, N., Khan, S., Dudas, J., Mansuroglu, T., Hess, C.F., Rave-Frank, M., Christiansen, H. & Ramadori, G., 2010. Single-dose gamma-irradiation induces up-regulation of chemokine gene expression and recruitment of granulocytes into the portal area but not into other regions of rat hepatic tissue. *Am. J. Pathol.*, **176**(**4**): 1801-1815.

Malik, I.A., Naz, N., Sheikh, N., Khan, S., Moriconi, F., Blaschke, M. & Ramadori, G., 2011. Comparison of changes in gene expression of transferrin receptor-1 and other iron-regulatory proteins in rat liver and brain during acute-phase response. *Cell Tissue Res.*, **344**(**2**): 299-312.

Mukhopadhyay, A., Bhatla, N., Kriplani, A. & Saxena, R., 2007. Physiological macrocytosis of pregnancy. *Trop. Doct.*, **37**(**1**): 42-43.

Oosterhuis, W.P., Niessen, R.W., Bossuyt, P.M., Sanders, G.T. & Sturk, A., 2000. Diagnostic value of the mean corpuscular volume in the detection of vitamin B12 deficiency. *Scand. J. Clin. Lab. Invest.*, **60**(**1**): 9-18.

Patel, K.V., Ferrucci, L., Ershler, W.B., Longo, D.L. & Guralnik, J.M., 2009. Red blood cell distribution width and the risk of death in middle-aged and older adults. *Arch. Intern. Med.*, **169**(**5**): 515-523.

Pessayre, D., Fromenty, B., Berson, A., Robin, M.A., Letteron, P., Moreau, R. & Mansouri, A., 2012. Central role of mitochondria in drug-induced liver injury. *Drug Metab. Rev.*, **44**(**1**): 34-87.

Poke, F.S., Vaillancourt, R.E., Potts, B.M. & Reid, J.B., 2005. Genomic research in Eucalyptus. *Genetica*, **125**(**1**): 79-101.

Ramadori, P., Sheikh, N., Ahmad, G., Dudas, J. & Ramadori, G., 2010. Hepatic changes of erythropoietin gene expression in a rat model of acute-phase response. *Liver Int.*, **30**(**1**): 55-64.

Ritchie, R.F., Palomaki, G.E., Neveux, L.M., Navolotskaia, O., Ledue, T.B. & Craig, W.Y., 1999. Reference distributions for the negative acute-phase serum proteins, albumin, transferrin and transthyretin: a practical, simple and clinically relevant approach in a large cohort. *J. Clin. Lab Anal.*, **13**(**6**): 273-279.

Sategna, G.C., Scaglione, N. & Martini, S., 2002. Red cell distribution width as a marker of coeliac disease: a prospective study. *Eur. J. Gastroenterol. Hepatol.*, **14**(**2**): 177-181.

Serafino, A., Sinibaldi, V.P., Andreola, F., Zonfrillo, M., Mercuri, L., Federici, M., Rasi, G., Garaci, E. & Pierimarchi, P., 2008. Stimulatory effect of Eucalyptus essential oil on innate cell-mediated immune response. *BMC Immunol*, **9**: 17.

Seward, S.J., Safran, C., Marton, K.I. & Robinson, S.H., 1990. Does the mean corpuscular volume help physicians evaluate hospitalized patients with anemia? *J. Gen. Intern. Med.*, **5**(**3**): 187-191.

Sheikh, N., Batusic, D.S., Dudas, J., Tron, K., Neubauer, K., Saile, B. & Ramadori, G., 2006. Hepcidin and hemojuvelin gene expression in rat liver damage: in vivo and in vitro studies. *Am. J. Physiol.-Gast-L.*, **291**(**3**): G482-G490.

Sheikh, N., Dudas, J. & Ramadori, G., 2007. Changes of gene expression of iron

regulatory proteins during turpentine oil-induced acute-phase response in the rat. *Lab Invest*, **87**(**7**): 713-725.

Spell, D.W., Jones, D.V., JR., Harper, W.F. & David, B.J., 2004. The value of a complete blood count in predicting cancer of the colon. *Cancer Detect. Prev.*, **28**(**1**): 37-42.

Sundaram, V. & Shaikh, O.S., 2011. Acute liver failure: current practice and recent advances. *Gastroenterol. Clin. North Am.*, **40**(**3**): 523-539.

Takarada, K., Kimizuka, R., Takahashi, N., Honma, K., Okuda, K. & Kato, T., 2004. A comparison of the antibacterial efficacies of essential oils against oral pathogens. *Oral Microbiol. Immunol.*, **19**(**1**): 61-64.

Tannheimer, M., Fusch, C., Boning, D., Thomas, A., Engelhardt, M. & Schmidt, R., 2010. Changes of hematocrit and hemoglobin concentration in the cold Himalayan environment in dependence on total body fluid. *Sleep Breath.*, **14**(**3**): 193-199.

Tonelli, M., Sacks, F., Arnold, M., Moye, L., Davis, B. & Pfeffer, M., 2008. Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease. *Circulation*, **117**(**2**): 163-168.

Tonnesen, H., Hejberg, L., Frobenius, S. & Andersen, J.R., 1986. Erythrocyte mean cell volume--correlation to drinking pattern in heavy alcoholics. *Acta Med. Scand.*, **219**(**5**): 515-518.

Vandendries, E.R. & Drews, R.E., 2006. Drug-associated disease: hematologic dysfunction. *Crit Care Clin.*, **22**(**2**): 347-55, viii.

Webb, N.J.A. & Pitt, W.R., 1993. Eucalyptus oil poisoning in childhood: 41 cases in south-east Queensland. *J. Paediatr. Child Health*, **29**(**5**): 368-371.

Zetterstrom, H. & Hedstrand, U., 1981. Albumin treatment following major surgery. I. Effects on plasma oncotic pressure, renal function and peripheral oedema. *Acta Anaesthesiol. Scand.*, **25**(**2**): 125-132.

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