The Effect of *Echinacea purpurea* and Clove (*Syzygium aromaticum*) Extracts on Raising the Immune Response in Rats with Immune Deficiency Diseases

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Abstract

The present study aimed to investigate the effect of *Echinacea purpurea* and clove (*Syzygium aromaticum*) aqueous extract on raising the immune response in rats with immune deficiency diseases. Forty-eight male albino rats were randomly divided into 8 equal groups (n=6). Group 1 was negative group, whereas the other 7 groups injected by cisplatin. Group 2 kept as the immunotoxic control group (+ve group). Groups 3 and 4 received 1 and 2 ml of *Echinacea* extract, whereas 1 and 2 ml of clove extract were given to groups 5 and 6, respectively. Groups 7 and 8 received 1 and 2 ml of *Echinacea* and clove extract mixture, respectively, for 8 weeks. Results of body weight, spleen weight, serum immunogolobulin G, immunogolobulin M (IgM), total protein, albumin, globulin, neutrophil, lymphocyte, monocytes and eosinophil significantly decreased, whereas leukocyte significantly increased by cisplatin in positive control group as compared to the negative control group. On the other hand, administration of *Echinacea* and clove extracts and their combination attenuated these adverse effects and markedly ameliorated histopathological and biochemical alterations that caused by cisplatin administration. In conclusion, *Echinacea* and clove extracts stimulate the immune system of rats with cisplatin-induced suppressed immunity. This study recommended increasing the consumption of the *Echinacea* and clove in the diet, this may be raising the immune response in patient with immune disorders.

Keywords: *Echinacea purpurea*, Clove, Cisplatin, Immune System, Rats.

INTRODUCTION

The immune system plays the most effective role in preventing invasion of pathogens through immune response in order to maintain the physiological balance. However, several factors may affect or even destroy the immune response, and cause immune disorder further, such as malnutrition, application chemotherapy and stressors **(Huang *et al*., 2016)**. The damaged immune system can lead to inflammatory diseases, autoimmune diseases, and even cancer **(McComb *et al*., 2019)**. Immunotoxicity could be the result of direct or indirect action of a chemical on the immune system, causing a suppression or activation of the immune response. Compromised immune response can result in suppression of host resistance to infectious agents as well as tumor cells **(Zandvoort *et al*., 2001)**.

Cisplatin (also known as cisplatinum or cisdiamminedichloroplatinum) is a platinum-containing compound which inhibits synthesis of RNA, DNA and protein in cells. Cisplatin is one of the most effective anticancer drugs used for the treatment of various oncologic diseases **(Baek *et al*., 2003)**. Despite its effective anticancer efficacy, cisplatin exerts many undesirable adverse effects, including myelosuppression, hepatotoxicity, nephrotoxicity and immunotoxicity **(Hasaaan *et al*., 2010)**. The toxic effects of cisplatin are attributed to several factors, such as peroxidation of the cell membrane, DNA damage, mitochondrial dysfunction, inhibition of protein synthesis, and ability to affect host immune response **(Jordan and Carmo-Fonseca, 2000)**.

Herbs are traditionally used in many therapeutic practices, if not as the main, then as the accompanying therapy in combination with medications, aimed at boosting immunity for prevention. Phytotherapy has repeatedly proven its effectiveness, including its ability to cope with infectious diseases **(Zhang *et al*., 2020)**. *Echinacea purpurea* (family: Asteraceae) is an important medicinal plant with various pharmacological properties. E. purpurea was found to exhibit significant antioxidant, anti-inflammatory, and immunoregulatory activities **(Lee *at al*., 2009)**. Several active constituents, such as flavonoids, caffeic acid derivatives (alkamides), essential oils, and poly acetylenes, were isolated from this plant. Among various phytocomponents, alkamides were found to possess immunoregulatory effects **(Matthias *et al*., 2008)**. The caffeic acid derivatives, alkamides and polysaccharide fractions were found to show inhibition against in vitro Cu(II)□catalyzed oxidation of human low□density lipoprotein (LDL) that proves the presence of antioxidant property **(Dalby□Brown *et al*., 2005)**.

Clove, *Syzygium aromaticum*, is an aromatic medical plant of the family Myrtaceae. It is commonly applied as a natural additive in the food industry, antiseptic against infectious diseases, and local anesthetic in dentistry **(Cortés-Rojas *et al*., 2014)**. In addition to its antimicrobial, anti-fungal, and anti-viral properties, clove possesses anti-inflammatory properties **(Chaieb *et al*., 2007)**. Eugenol, eugenyl acetate, carvacrol, tanene, and thymol were detected as major constituents of the clove **(Amelia *et al*., 2017)**. It has been reported that constituents of clove impart anti-oxidant activities and inhibit lipid peroxidation **(Dibazar *et al*., 2015)**. Effects by clove or main constituents (like eugenol) on specific immune system components/mechanisms have only recently begun to be examined in detail **(Yogalakshmi *et al*., 2010; Bachiega *et al*., 2012; Grespan *et al*., 2012)**.

Therefore, this study was conducted to study the effect of aqueous extracts of *Echinacea purpurea* and Clove (*Syzygium aromaticum*) on raising the immune response in rats with immune deficiency diseases.

MATERIALS AND METHODS

Materials

Echinacea purpurea and *Syzygium aromaticum* were obtained from Agriculture Research Center, Egypt. Cisplatin, casein, cellulose, choline chloride, D-L methionine, vitamin and mineral constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soy oil, and sucrose were obtained from the Egyptian local market. Forty-eight adult male rats **(**Sprague Dawley strain), weighing about 180±10 g b.wt. were obtained from the Laboratory Animal Colony, Agricultural Research Center, Giza, Egypt.

Methods

1. **Preparation of *Echinacea* and Clove Aqueous Extract:**

Twenty-five gm of dried Echinacea was submerged in 100 ml of distilled water and allowed to soak overnight, then filtered to obtain a liquid extract. A known concentration of *Echinacea* aqueous extract was given orally by stomach tube.

The Clove flower buds were dried in the sun and ground to fine powder with the aid of an electric blender. Thereafter, 25 g of the milled clove powder was soaked in 100 ml distilled water, then filtered to prepare an aqueous extract **(Dibazar *et al*., 2015)**. A known concentration of Clove aqueous extract was given orally by stomach tube.

2. **Induction of Immune Deficiency Diseases:**

Cisplatin-induced immune deficiency diseases in rats. Intraperitoneal injection of male albino rats with cisplatin (3.5 mg/kg) once every 3 days for consecutive 2 weeks **(Wang *et al*., 2013)**.

3. **Diet Preparation and Experimental Design:**

The basal diet was prepared according to AIN-93M diet **(Reeves *et al*., 1993)**. Forty-eight male albino rats were randomly divided into 8 equal groups (n=6). Group 1 was negative control group, whereas the other 7 groups injected by cisplatin. Group 2 kept as the immunotoxic control group (+ve group). Groups 3 and 4 received 1 and 2 ml of *Echinacea* extract, whereas 1 and 2 ml of clove extract were given to groups 5 and 6, respectively. Groups 7 and 8 received 1 and 2 ml of *Echinacea* and clove extract mixture (1:1), respectively, for 8 weeks. During the experiment period, the quantities of diet, which were consumed and/or waste, were recorded every day. In addition, rat's weight was recorded weekly to determine body weight gain and feed efficiency ratio according to **Chapman *et al*., (1959)**.

4. **Biochemical Analysis:**

At the end of the experimental period (8 weeks), rats were fasted overnight before scarifying and blood samples were collected from each rat and centrifuged at 3000 rpm for 15 min to obtain the serum for biochemical analysis. Levels of leukocytes, neutrophil, lymphocyte, monocytes, eosinophil and basophil were estimated according to **Ochei and Kolharktar, (2008)**. Immunoglobulin M (IgM) and immunoglobulin G (IgG) were measured according to **Ziva and Pannall, (1984)**. Concentration of total protein was determined according to **Burtis and Ashwood (1999)**, albumin and globulin were determined according to **Young, (1995)**.

5. **Histopathological Examination:**

Specimens from the spleen were placed in 10% neutral buffered formalin for histopathological examination according to **(Bancroft and Stevens, 1996)**. Histopathological examination was done in Veterinary medicine, Cairo University, Egypt.

6. STATISTICAL ANALYSIS:

Results were expressed as the mean standard error ± SE. Data were statistically analyzed for variance "ANOVA" test at $P \leq (0.05)$ using SPSS statistical software, version 20 was used for these calculations according to **Armitage and Berry, (1987)**.

RESULTS AND DISCUSSION

The results in **Table 1** showed that intraperitoneal injection of cisplatin to rats caused a decrease in feed intake and a significant ($P<0.05$) reduction in body weight gain (BWG%), feed efficiency ratio (FER) and spleen relative weight % when compared to the negative control group. Oral administration of *Echinacea* and Clove extracts and their mixture to rats inflicted with immune deficiency diseases (IDD) caused increasing in feed intake and a significant ($P<0.05$) increases in FER, body weight and spleen weight as compared to the positive control group.

Lin *et al*., (2018) demonstrated that cisplatin administration resulted in significantly decrease in

feed intake, body weight and feed efficiency, as found in the present study. Immunotoxicity may parallel alterations in the weight of lymphoid organs (spleen) **(Pearse *et al*., 2009)**. The results in the present study were in the same line with **EL-Sherbiny *et al*., (2021)** that the administration of *Echinacea* extract stimulated the increase in weight of spleen as well as body weight in rats with immune deficiency. Furthermore, **Ali, (2008)** reported that *Echinacea* has a positive effect on body weight gain after 4 weeks of treatment. Moreover, the obtained results are agreed with **Agbaje *et al*., (2009)** who showed an improvement in body weight by clove in rats.

Results presented in **Table 2**, revealed that rats inflicted with immune deficiency diseases by cisplatin had significant ($P<0.05$) reduction in serum immunogolobulin G (IgG) and immunogolobulin M (IgM) antibodies levels when compared with the negative control (-ve) group. Administration with *Echinacea* and Clove extracts and their mixture to IDD rats resulted in significant ($P<0.05$) increases in serum IgG and IgM as compared to the positive control group. It was also observed that rats administrated with 2 ml mixture of *Echinacea* and Clove extracts recorded the best results for increasing IgG and IgM when compared to the negative control group.

Results in **Table 2,** were confirmed by **Nassef *et al*., (2018)** reported that cisplatin injection to rats caused a significant reduction is serum immunoglobulin (IgG, IgM). **Rehman *et al*., (1999) Mahmoud *et al*., (2022)**, showed that *Echinacea* administration increased IgG and IgM production in rats with immune deficiency. The effects of immune activation by *Echinacea* were investigated by measuring total immunoglobulin (IgG, IgM). **Mishima *et al*., (2004)** investigated the effects of immune activation by *Echinacea* by measuring T lymphocyte subsets in the peripheral blood of mice following whole-body irradiation and reported that *Echinacea* activates macrophages to stimulate IFN-gamma production in association with the secondary activation of T lymphocytes, resulting in decreases of IgG and IgM production. Also, the improvement of IgG and IgM may be due to that clove act as additional bonds with immunoglobulin molecules at the Fc receptors, which stimulated the immune response **(Ahmed *et al*., 2013)**.

Results illustrated in **Table 3**, showed that rats injected intraperitoneally with cisplatin had significant decreases in the serum levels of total protein, albumin and globulin when compared with the negative control group. Oral administration of *Echinacea* and clove extract (2 ml) and their mixture to IDD rats significantly normalized ($P < 0.05$) the levels of total protein, albumin and globulin in the serum. These findings were confirmed by **Parameshappa *et al.*, (2012) and Khalaf *et al*., (2019)**, who observed significant reduction in total protein, globulin and albumin concentration in rats administrated with cisplatin. Results also, in agreement with **Sadigh-Eteghad *et al*., (2011)**, who showed that treatment with *Echinacea* (500 mg /4 weeks) ameliorates the alteration in total protein and albumin. Concerning results of clove administration, were in agreement with **Abozid and El-Sayed (2013)**, who found that rats treated with clove extract increased plasma total protein and albumin compared with +ve group due to the polyphenolic compound and flavonoids that present in clove extract **(Gulcin *et al*., 2004)**.

Results in **Table 4**, showed that the positive control group had a significant increase ($P < 0.05$) in leukocytes and a significant decrease ($P < 0.05$) in levels of neutrophil, lymphocyte, monocytes and eosinophil as compared to the negative control group. On the other hand, rats that administrated with different levels of *Echinacea* and Clove extracts and their mixture had significant reduction in leukocytes and a significant elevation in neutrophil, lymphocyte, monocytes and eosinophil as compared to the positive control (+ve) group. The highest improvement was recorded in group that treated with the high level (2 ml 1E:1C) of combination of *Echinacea* and Clove extract.

In complementary of the present results, **Khalaf *et al*., (2019)** found that administration of cisplatin to mature rats resulted in marked immunotoxic effects represented by leukopenia, lymphocytopenia and neutrophilia. On the other hand, **Markovic *et al*., (2011)** reported that cisplatin increased the number of leukocytes due to the consequence of infection and inflammation. On the other hand, administration with *Echinacea* extract significantly reduced leucopenia induced by cisplatin which indicates that the extract could stimulate the haemopoetic system. This may be attributed to the contents of *Echinacea* as cichoricacid and echinacocide that stimulate bone marrow and the reformation of hematopoietic stem cells **(Goel *et al*., 2002)**. **Mishima *et al*., (2004)** reported that administration of *Echinacea* 360 mg/kg/day for 3weeks increases the number of leukocytes; this elevation is due to ability of polysaccharides and echinacocide to increase the number of leukocytes. **Ezz, (2011)** showed that *Echinacea* is involved in the modulation of immune response. Various phytoconstituents present in *Echinacea*, such as caffeic acid derivatives, alkamides, flavonoids, essential oils and polyacetylenes, are known to activate the non-specific cellular and humoral by increasing the production and activation of leukocytes, lymphocytes, monocytes and cytokines **(Kim *et al*., 2002)**. These components also modulate the immune response by macrophage phagocytosis, pro-inflammatory cytokine production, activation of NK cell activity, enhancement of B cell response, increased T cell proliferation and elevated production of T cell cytokines **(Chen *et al*., 2005; Thygesen *et***

***al*., 2007 and Khalaf *et al*., (2019)**. Hence, it is plausible that *Echinacea* extract may confer immunoprotection through multifactorial immunomodulatory effects which could be achieved through chemical synergy of various bioactive constituents. On the other hand, **Dibazar *et al*., (2015) and Kmiec *et al*., (2017)**, revealed that clove extract containing eugenol, β-caryophyllene, caryophyllene oxide and α-humulene can increase the proliferation of activity of lymphocytes, lymphoblasts and reactive oxygen intennediate secretion of macrophages. Infected mice will increase IFN-γ levels because there are immunogens that activate the immune system.

Histopathological Examination

Microscopically, spleen of rats from group 1 revealed the normal histological structure of white pulp **(Photo 1)**. On the other hand, spleen of rats from group 2 showed lymphocytic necrosis and depletion **(Photo 2)**. Some examined sections from group 3 revealed intravascular permeation of inflammatory cells **(Photo 3)**. Moreover, spleen of rats from group 4 showed no histopathological alterations except extramedullary megakaryocytes in some examined sections **(Photo 4)**. Furthermore, spleen of rats from group 5 exhibited no histopathological alterations **(Photo 5)**. Moreover, slight lymphocytic necrosis and depletions in some follicles was the only histopathological lesion observed in spleen of rats from group 6 **(Photo 6)**. Additionally, spleen of rats from group 7 revealed no histopathological alterations **(Photo 7)**. Furthermore, spleen of rats from group 8 exhibited no histopathological alterations except slight lymphocytic necrosis and depletion in some sections **(Photo 8)**.

Immunotoxicity may parallel alterations in the normal microscopic architecture of lymphoid organs. However, splenic immunosuppression may attribute to the decreased lymphatic cells numbers in the spleen and other immune organs **(Pearse *et al*., 2009 and Monfared *et al*., 2014)**. **Menezes *et al*., (2005)**, indicated that all extensive injuries were repaired with collagen fibers which may lead to the fibrosis as observed here. **Crăciun and Paşca, (2014)**, reported that cisplatin had a significant toxic effect in both central (thymus) and peripheral (spleen) lymphoid organs. On the other hand, **Susan, (2006)** reported that the *Echinacea* treatment markedly ameliorated the altered histopathological features in the spleen tissues. This observed effect may be due to the antioxidant properties of various constituents (e.g., polysaccharides and flavonoids) present in the *Echinacea* extract. As well as, *Echinacea* is a potent scavenger of a variety of free radicals and a restoration agent in the production of immune cells which were decreased in positive control group. This observed effect may be due to the antioxidant properties of various constituents (e.g., polysaccharides and flavonoids) present in the *Echinacea* extract. Furthermore, **Dwivedi *et al*., (2011)**, reported that the eugenol present in clove extract is an effective cytotoxic agent for different type of cancer cells and it is endowed with apoptotic inducing capability.

Conclusion

The findings of this study demonstrated that Echinacea and clove extract is a promising immunomodulatory agent with a potent therapeutic value in stimulating the suppressed immune response.

Table 1: Effect of Echinacea and Clove Extracts on Feed Intake (FI), Efficiency Ratio (FER), Body Weight Gain (BWG), and Spleen Relative Weight (SRW) of Rats with Immune Deficiency Diseases

Parameters / Groups	FI (g/d/rat)	FER	BWG%	SRW%
1- Control (-ve)	25	0.039±0.001^{a}	28.54±1.04^{a}	0.88±0.05a
2- Control (+ve)	16	0.029±0.001^{c}	14.06±0.93^{c}	0.42±0.08f
3- 1ml *Echinacea*	18	0.036±0.001^{ab}	19.03±0.91b	0.52±0.08e
4- 2ml *Echinacea*	21	0.035±0.002^{ab}	21.76±2.22^{b}	0.61±0.11d
5- 1ml Clove	18	0.036±0.003^{ab}	19.45±1.20^{b}	0.52±0.09e
6- 2ml Clove	20	0.037±0.002^{ab}	22.07±1.74^{b}	0.64±0.08d
7- 1ml E:C	22	0.036±0.001^{ab}	23.05±0.77^{b}	0.71±0.15c
8- 2ml E:C	22	0.036±0.002^{a}	23.59±1.93^{b}	0.77±0.16b

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
***E:C=** Echinacea: Clove.

Table 2: Effect of Echinacea and Clove Extracts on Serum Immunogolobulin G (IgG) and Immunogolobulin M (IgM) of Rats with Immune Deficiency Diseases

Parameters / Groups	IgG	IgM
	(g /L)	
1- Control (-ve)	12.00±0.57[a]	252.33±3.56[a]
2- Control (+ve)	5.46±0.88e	127.34±2.04[e]
3- 1ml *Echinacea*	6.90±0.62d	219.66±1.76[cd]
4- 2ml *Echinacea*	8.32±0.18c	231.66±3.35[bc]
5- 1ml Clove	6.89±0.16d	212.33±2.84[d]
6- 2ml Clove	8.46±0.32[c]	233.66±4.25[bc]
7- 1ml E:C	9.80±0.21b	231.00±2.40[bc]
8- 2ml E:C	10.72±0.15b	235.52±1.15b

*Mean values are expressed as means ± SE; *Mean values at the same column with the same superscript letters are not statistically significant at P<0.05; ***E:C=** Echinacea: Clove.

Table 3: Effect of Echinacea and Clove Extracts on Serum Total Protein, Albumin and Globulin of Rats with Immune Deficiency Diseases

Parameters / Groups	Total Protein mg/dl	Albumin mg/dl	Globulin mg/dl
1- Control (-ve)	7.82±0.75a	4.85±0.61a	3.57±0.07a
2- Control (+ve)	3.87±0.89d	2.15±0.16[d]	1.66±0.12[d]
3- 1ml *Echinacea*	4.55±0.42cd	2.36±0.26[cd]	1.89±0.12d
4- 2ml *Echinacea*	5.10±0.27bc	3.24±0.15b	2.37±0.22c
5- 1ml Clove	4.42±0.20cd	2.38±0.29cd	1. 83±0.21d
6- 2ml Clove	5.24±0.55bc	3.13±0.16bc	2.82±0.60b
7- 1ml E:C	5.48±0.25b	3.32±0.83b	3.04±0.17b
8- 2ml E:C	6.00±0.12b	3.38±0.63b	3.17±0.14b

*Mean values are expressed as means ± SE; *Mean values at the same column with the same superscript letters are not statistically significant at P<0.05; ***E:C=** Echinacea: Clove.

Table 4: Effect of Echinacea and Clove Extracts on Leukocytes, Neutrophil, Lymphocyte, Monocytes, Eosinophil, and Basophil Count of Rats with Immune Deficiency Diseases

Parameters / Groups	Leukocytes (x 10^3/ul)	Neutrophil (x 10^3/ul)	Lymphocyte (x 10^3/ul)	Monocytes (x 10^3/ul)	Eosinophil (x 10^3/ul)	Basophil (x 10^3/ul)
1- Control (-ve)	5.30±0.57e	5.41±0.12[a]	4.83±0.19[a]	1.84±0.07[a]	1.37±0.02a	1.50±0.05a
2- Control (+ve)	12.03±0.24[a]	0.79±0.02[e]	1.01±0.05f	0.35±0.02[d]	0.30±0.03f	0.19±0.01e
3- 1ml *Echinacea*	11.22±0.18b	1.52±0.19[d]	1.87±0.05e	0.49±0.03[cd]	0.55±0.02de	0.33±0.01de
4- 2ml *Echinacea*	8.40±0.15[c]	2.83±0.09[c]	2.80±0.09d	0.56±0.01[c]	0.71±0.01cd	0.47±0.03d
5- 1ml Clove	10.88±0.34[b]	1.73±0.09[d]	1.69±0.09e	0.46±0.01[cd]	0.49±0.01e	0.29±0.01de
6- 2ml Clove	7.99±0.26[c]	2.71±0.14[c]	2.91±0.03cd	0.63±0.03[c]	0.80±0.01bc	0.51±0.01d
7- 1ml E:C	6.73±0.15[d]	3.03±.04[c]	3.10±0.06c	0.58±0.04[c]	0.79±0.04bc	0.88±0.01c
8- 2ml E:C	6.33±0.12[d]	4.02±0.10[b]	4.18±0.10b	0.94±0.03[b]	0.94±0.01b	1.16±0.09b

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
***E:C=** Echinacea: Clove.

HISTOPATHOLOGICAL EXAMINATION

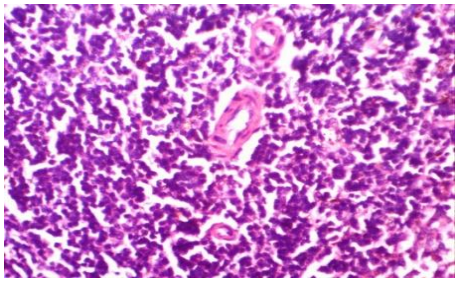

Photo 1: Spleen of rat from group 1 showing the normal histological structure of white pulp (H & E X 400).

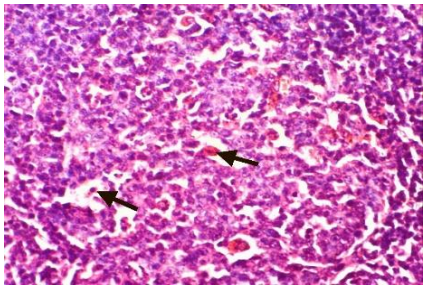

Photo 2: Spleen of rat from group 2 showing lymphocytic necrosis and depletion (H & E X 400).

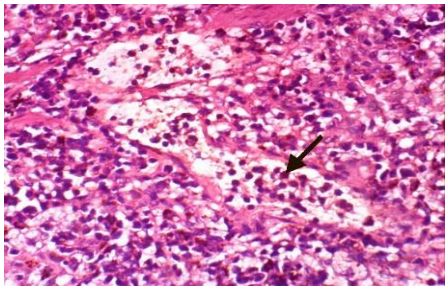

Photo 3: Spleen of rat from group 3 showing intravascular permeation of inflammatory cells (H & E X 400).

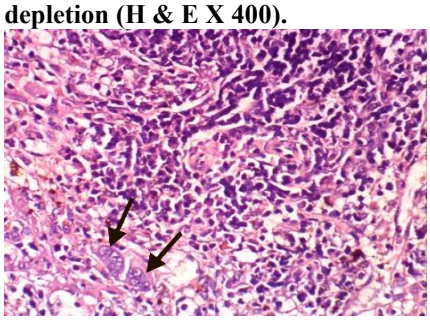

Photo 4: Spleen of rat from group 4 showing extramedullary megakaryocytes (H & E X 400).

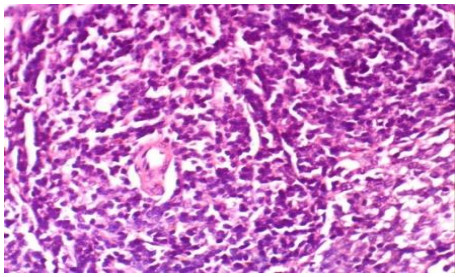

Photo 5: Spleen of rat from group 5 showing no histopathological alterations (H & E X 400).

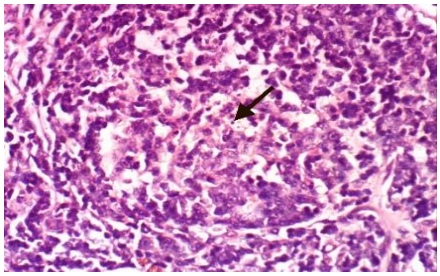

Photo 6: Spleen of rat from group 6 showing slight lymphocytic necrosis and depletion (H & E X 400).

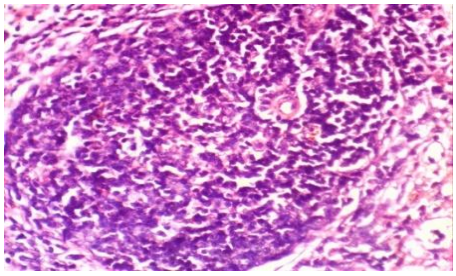

Photo 7: Spleen of rat from group 7 showing no histopathological alterations (H & E X 400).

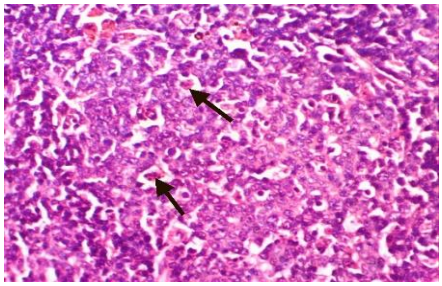

Photo 8: Spleen of rat from group 8 showing slight lymphocytic necrosis and depletion (H & E X 400).

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