

## Toxicology Study the Acute effects of Diethanolamine in Mice Blood and Liver (Oral study)

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**Abstract:** The most important goals of this study is screening effects of Diethanolamine (DEA) which is consider one of the most important ingredients in cosmetic products in some tissues of female albino mice (blood and liver) as laboratory models;(DEA) which using for neutralizing the pH through the industry of cosmetic product or adding to make the product fatty or more sudsy; and as a preservative in vegetables and meat, also in some Pharmaceutical preparations as one of the ingredients of medicine for patients of Pulmonary Arterial Hypertension (PHA); and in industry of intravenous solutions and preservative solution for contact lenses. Thirty mice at the average age of (40 days) and weight (21- 24 gm); were divided into 6 groups (control, 603, 1250, 2500, 5000, 10000 ppm), each group includes five mice were treated for two weeks; weight gain except the higher concentrations, ruffled fur, pale foot pads and abortions were recorded. Water consumption was increased in mice groups which treated with low concentrations and decreased in higher concentrations resulting in severe dehydration which cause death. The hematological results showed decreasing in Hemoglobin, Packed Cell Volume and platelets count; but the White Blood Cells were recorded increasing in their count with the increasing in concentrations and exposure period. Monocytes, Neutrophils and Lymphocytes were increased also because the decreasing immunity for mice. The histopathological sections showed the increasing in liver weight and showed hepatocytes degeneration, congestion of the central vein, sinusoid dilation, necrosis and lysis of hepatocytes, sever fatty changes, nuclear pyknosis and karyorrhexis and infiltration of inflammatory cells.

**Keywords:** *Diethanolamine, acute effect, toxicity.*

### Introduction

Diethanolamine (DEA) is a general ingredient of care products (Carcunescu *et al.*, 2011); an organic compound resulting from ethylene oxide and ammonia reacting, it isn't occurring naturally; it's one of the Ethanolamine's family which consist of monoethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA) are used in spread industries (Knaaket *et al.*, 1997); This family is using in non- colouring agents and rinse- off products with concentrations  $\leq 30\%$ ; while in leave- on products about  $\leq 10\%$  and in baby products 0.3- 3% but in lipsticks about 3- 10% (CIR, 2011).

Diethanolamine is used for the preparations of amides and amides salts of DEA which are forming cosmetics, detergents shampoos and hair conditioners. It's a contaminant; having double functional groups (amino and hydroxyl) which make them play as intermediates of surfactants in soaps and other pharmaceuticals applications (Knaak *et al.*, 1997), the chemical structure of DEA is: (OH - CH<sub>2</sub> - CH<sub>2</sub>- NH- CH<sub>2</sub> - CH<sub>2</sub>- OH) and Chemical formula (C<sub>4</sub>H<sub>11</sub>NO<sub>2</sub>) with molecular weight is 105.14 (IARC, 2012). Diethanolamine has several uses in cosmetics is using to make the cosmetic product more creamy or sudsy and using as pH adjuster to naturalize the other ingredients with high acidity (David, 2010). In pharmacology the medicine of UT- 15C SR (United Therapeutics- Sustained Release treprostini diethanolamine) an oral tablet to treat patients of PHA (Pulmonary Arterial Hypertension), global spread disease with nearly 500,000- 100,000 UT- 15C (oral Treprostini: UT-15C SR) has two forms salt from of Remodulin (Treprostini) injection and Tyvaso (Treprostini) inhalation solution. In United States This drug is not altered but approve that it has bioactivity form which is (DEA) which existing in blood stream. Troprostini sodium (in the form of salt) has its potential effects on the vascular function, proliferation of pulmonary artery smooth muscle cells and aggregation of platelets and mechanism action on the cardiovascular system, 1.5% of DEA solvents are using as intravenously drugs (FDA, 2013). Diethanolamine stimulates deficiency of hepatic chlorine in mice (Lehman-

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Mckeeman *et al.*, 2002) possibly because of the inhibition of choline uptake (Lehman-Mckeeman *et al.*, 2000). When mice exposed to higher tolerated doses of DEA; this was resulting choline Levels reduction in the liver (Lehman-Mckeeman *et al.*, 2002) and induced of morphological transformation of SHE cells (Syrian Hamster Embryo Cells) was banned by additional concentrations of DEA (Lehman-Mckeeman *et al.*, 2000) than DEA prevented choline uptake inhibition and by the excess of choline to the culture media prevented the cells of Chinese hamster cells (Lehman-Mckeeman *et al.*, 2000; Lehman-Mckeeman & Gamsky, 1999), in this stage DNA methylation was altered, hepatocytes grown in environment with choline deficiency and DEA presence (Bachman *et al.*, 2006); in primary culture of a mouse or rat hepatocytes which incubating by DEA prevented by choline excess (Kamendulis & Klauning, 2005) but without sodium nitrate (Stott *et al.*, 2000). NTP in (1999) put the clear evidence of DEA carcinogenicity in male and female mice by growing of liver neoplasms in both sexes and renal tubule increasing cytoplasmic alternation, syncytial alternation, renal tubule hyperplasia and thyroid gland follicular cell hyperplasia, skin hyper keratosis inmales under 2- years dermal injection studies. While Shine *et al.* in (2016) by detection of Mono-, Di-, Triethanolamine in shampoos, creams and lotions found that DEA isn't harmful but might react with other cosmetic formula ingredients resulting in very potential carcinogen called nitrosodiethanolamine (NDEA) because of long storage period of cosmetic product; to improve this possibility of DEA carcinogenicity and because the great risk from oral DEA dose can be most dangerous than dermal administration because the liver receives the toxicant directly through the portal system (Leung *et al.*, 2005). This study came for assess the acute effects of DEA on female mice farther than male because of the broad DEA uses in cosmetics industries.

## Method and Materials

Diethanolamine (DEA) (31590) was obtained from SIGMA ALDIRCH Company. The analytical data indicated that the purity was 99.5%. No impurities greater than 0.5% relative to the Diethanolamine peak were observed by gas chromatography. The chemical was stored at room temperature for 20 days than refrigerated and protected from light (NTP, 1992). Oral administration of DEA was given in drinking water, DEA doses were prepared with deionised water; the pH was adjusted to  $7.4 \pm 0.2$  with (1N) hydrochloric acid. These dose solutions were stored no longer than 20 days at room temperature in polypropylene carboys than refrigerated, protected from light (NTP, 1992). To study the acute effects of DEA; thirty Healthy albino female mice at the age of 40 days and average weight ( $24 \pm 5$ g) were divided randomly into six groups of five animals each (5 animals/cage) in polypropylene cages at the temperature of  $25 \pm 5$  °C and  $12 \pm 2$  hours light/day Diet was given *ad libitum*, five female of each cage received drinking water solutions containing diethanolamine at concentrations of (control, 630, 1250, 2500, 5000, and 10000 ppm) *ad libitum* daily for 14 days. Animals were treated by using cylinder plastic bottles and have a metal nosel and all mice were weighed in the first day and eighth day from the experiment. According to the protocol of 5 days/week water consumption was determined once daily. Protocol-required tissues examined microscopically in all control and treated animals; the target organ was liver in two weeks study; they were isolated and washed with normal saline twice and kept immediately in 10 % formalin for the microscopic study of the expected histological changes. Blood was collected from the animals which survived till the end of the study by heart puncture and placed into anticoagulant tubes for blood analysis (NTP, 1992).

## Results and Discussion

All females in both control and treated groups survived to the end of the acute toxicity experiment of 2 weeks except for two females from group receiving dose. Early deaths one of the most important sings in animals with high doses (5000 and 10000 ppm) the other toxic signs in other treated groups except the control: weight gain except the two high doses; thin appearance, icterus (or yellow skin), abnormal condition, hypoactivity, ruffled fur especially in animals receiving (2500 ppm) as in (fig. 1) . In severe cases animals suffering from being incapable of standing, eating or drinking, clear dehydration with recessed eyes as in (Figure 2). Weight gain have been found in control and treated female mice groups except those which received high doses (5000 and 10000 ppm) weight was reduced; the lowest mean value were recorded in control in the first day of acute toxicity experiment ( $21.73 \pm 1.92$ ) and ( $24.81 \pm 0.95$ ) in females receiving (10000 ppm) while in the 8th day were recorded ( $23.49 \pm 1.04$ ) and ( $20.58 \pm 2.69$ ) respectively; as it shown in Table 1; there is significant differences

( $P < 0.05$ ) after one week of oral administration of DEA in mice body weight in all treated groups. Low water consumption can affect body weight (NTP, 1992); in case of water deficiency, causes decreasing in food intake; that means when a mouse consumes any quantity of food depend on water availability (Hamilton & Flaherty, 1973; Rowland, 2007).

**Table 1.**Effect of DEA concentrations on mice weight

Concentration	Mean $\pm$ SE (gm)	
	First day	8 <sup>th</sup> day
<b>Control</b>	21.73 $\pm$ 1.92	23.49 $\pm$ 1.04
<b>630</b>	24.21 $\pm$ 1.22	25.16 $\pm$ 0.78
<b>1250</b>	22.97 $\pm$ 0.40	24.32 $\pm$ 1.70
<b>2500</b>	22.80 $\pm$ 1.82	22.78 $\pm$ 1.54
<b>5000</b>	25.97 $\pm$ 0.39	21.98 $\pm$ 0.58
<b>10000</b>	24.81 $\pm$ 0.95	20.58 $\pm$ 2.69
<b>LSD value</b>	3.721 *	4.067 *
<b>P-value</b>	0.0407	0.0512
<b>* (P&lt;0.05).</b>		



**Figure 1.** Mice with ruffled fur resulting from oral administration with (2500 ppm DEA)



**Figure 2.** Mice with dehydration and recessed eyes resulting from oral administration with (10000 ppm DEA)

According to the results of Table 2 that there was highly significant differences ( $P < 0.01$ ) between control and all treated groups with DEA in acute toxicity experiment due to LSD value (14.327); control recorded the highest mean value was (41.30  $\pm$  6.79) while the lowest mean value were recorded (14.90  $\pm$  3.41) in treated group with (10000 ppm); whenever the concentration of DEA in drinking water increased, the less consumed by mice. The small size of mice, variations in body weight, appearance, and physiology make them different in facing on dehydration but mice have endogenous nycthermal rhythms which lead them to adaptation (Rowland, 2007); this type of mice usually die quickly, dehydration like weight loss a indicators of death (Foltz *et al.*, 1999). Water consumption was reduced in high doses remarkably because of the minimized palatability of (5000 and 10000 ppm) in the drinking water; this leads to refrain the animal from drinking water and gets severe dehydration (NTP, 1992).

**Table 2.**Effect of difference DEA concentration in Water consumption and Liver weight

Concentration	Mean $\pm$ SE (ml)	
	Water consumption	Liver weight
<b>Control</b>	41.30 $\pm$ 6.79	1.210 $\pm$ 0.106
<b>630</b>	24.10 $\pm$ 4.84	1.466 $\pm$ 0.048
<b>1250</b>	20.70 $\pm$ 3.38	1.526 $\pm$ 0.104
<b>2500</b>	30.50 $\pm$ 6.62	1.726 $\pm$ 0.013
<b>5000</b>	19.10 $\pm$ 4.07	3.000 $\pm$ 0.346
<b>10000</b>	14.90 $\pm$ 3.41	3.153 $\pm$ 0.204
<b>LSD value</b>	14.327 **	0.544 **
<b>P-value</b>	0.0077	0.0001
<b>** (P&lt;0.01).</b>		

Doctor *et al.* in (2016) found that liver and kidney are target organs for accumulated DEA in higher concentrations; liver weight showed increasing through DEA oral administration; the lowest mean value was recorded in control group ( $1.210 \pm 0.106$ ) while the highest mean value ( $3.153 \pm 0.204$ ) in treated group with (10000 ppm) as it shows in table (2); which shows highly significant differences ( $P < 0.01$ ) due to LSD value (0.544); As a result of oral administration; 27% from DEA deposit in liver and 5% in kidney than ( $< 1.0\%$ ) in blood, brain, spleen and heart (Arton *et al.*, 1949). DEA has special affinity for liver and kidney (Mathews *et al.*, 1995). The changes in hematological alterations can serve as early indicators for toxic effects on tissue (Paprikar and Sharma, 2003). Doctor *et al.* in (2016) DEA is a strange compound to the body which is an important example of for toxins are metabolized and detoxified, oral DEA administration for 30 days increased lipid peroxidation in liver because of reduction of (glutathione peroxidase, super oxide dismutase and catalase) enzymes.

The blood considers the most important tissue in which way all metabolic processes changes are reflected, for this reason, the dependable indicator on toxic effects of drugs, chemicals and disease are the abnormal alteration in blood parameters (Lodia & Kansala, 2012). The Table 3 refers to mean  $\pm$  SE of hemoglobin, Packed Cell Volume (PCV) and platelets counts measured in mice after being exposed to different concentrations of DEA for two weeks only and control mice without DEA treatment:

**Table 3.** Effect of difference DEA concentrations in Hemoglobin, Packed Cell Volume (PCV) and Platelets count

Concentration	Mean $\pm$ SE		
	Hb g/dl	PCV%	Platelets count $\times 10^3$ (mm <sup>3</sup> )
<b>Control</b>	12.30 $\pm$ 0.49	38.00 $\pm$ 1.15	335.00 $\pm$ 129.71
<b>630</b>	11.98 $\pm$ 1.93	37.60 $\pm$ 5.85	212.00 $\pm$ 90.74
<b>1250</b>	11.50 $\pm$ 1.49	35.40 $\pm$ 4.53	159.00 $\pm$ 12.18
<b>2500</b>	10.00 $\pm$ 1.28	31.40 $\pm$ 3.51	107.40 $\pm$ 57.12
<b>5000</b>	8.38 $\pm$ 0.64	25.80 $\pm$ 1.98	97.00 $\pm$ 17.50
<b>10000</b>	8.18 $\pm$ 1.06	25.60 $\pm$ 2.92	71.67 $\pm$ 14.24
<b>LSD value</b>	4.008 *	11.797 *	223.86 *
<b>P-value</b>	0.0497	0.0415	0.0302
* ( $P < 0.05$ ).			

For hemoglobin content in mice blood, the highest value recorded ( $12.30 \pm 0.49$ ) in control group while lowest mean value ( $8.18 \pm 1.06$ ) in treated group with 10000 ppm; the table shows significant differences ( $P < 0.05$ ) between all DEA treated groups with control group due to LSD 4.008 (table 3). Packed Cell Volume also recorded highest value in control group ( $38 \pm 1.15$ ); but treated group with 10000 ppm was recorded the lowest value ( $25.60 \pm 2.92$ ) and there are significant differences ( $P < 0.05$ ) between values of PCV for all treated groups with control due to LSD 11.797 (table 3). Due to LSD (223.86); there is significant differences ( $P < 0.05$ ) between control and treated groups in count of platelets which recorded highest mean value in control group ( $335.00 \pm 129.71$ ) while lowest value ( $71.67 \pm 14.24$ ) in treated group with 10000 ppm (Table 3). All blood parameters are dependent on DEA concentrations; decreased with the highest concentrations but statically the different effects of DEA concentrations in blood parameters are very clear. It indicates anemia when its values under than normal values and it uses as physiological indicator of animal blood condition (Kemal, 2014). National Toxicity Program (1992) found that DEA caused microcytic and normochromic anemia but the bone marrow is hypocellular; lead to ineffective hematopoiesis and stem cells damage with iron deficiencies, chronic disease, and thalassemia. Sneha (2013) recorded in their study morphological alterations and hemolysis caused by the toxicant DEA with alteration in membrane and oxidative damage. Hemoglobin considers an oxygen carrier to the tissues (the red blood cell's function) the estimations of hemoglobin and PCV are using more than red blood cells count for accuracy (Norman, 2009); for more functional assessment PCV which is known as (hematocrit); its useful test to any hematological work (Bull And Hay, 2001).

Phillipson and Kubes found (2011) the Blood platelets are an important player in blood sepsis; and these cells have a basic role in keeping hemostasis; take part in immunity which make injury stays indefinite, mice showed survivals of 51% at 48 hours when their platelets have deficiency in comparison with control (Fujimi *et al.*, 2016); the higher mortality is association with platelets

deficiency after trauma and sepsis (Takashima, 1997; George *et al.*, 2001) because the platelets can sense and respond to their microenvironment's changes involving dangerous signals in respond to injury (Fujimiet *al.*, 2016). While white Blood Cells alteration in acute toxicity of DEA was illustrated in Table 4 after DEA exposure for two weeks, WBCs recorded lowest mean value in control group (4520± 717.9) but the highest mean value in treated group with 10000 ppm (9600.00± 3857.46) and the table shows significant differences (P< 0.05) between control and all treated groups due to LSD value 3038.20.

**Table 4.**Effect of difference DEA concentrations in WBC, Monocytes, Neutrophil and Lymphocytes counts

Concentration	Mean ± SE			
	WBC count	Monocyte count	Neutrophils count	Lymphocyte count
Control	4520.00 ±717.91	6.80 ±0.86	11.40 ±1.86	59.00 ±7.73
630	5080.00 ±646.84	7.40 ±0.67	15.00 ±2.89	63.00 ±2.79
1250	5240.00 ±1014.20	7.60 ±0.74	24.60 ±3.26	66.40 ±3.11
2500	5500.00 ±773.30	9.00 ±0.71	25.40 ±5.28	67.00 ±5.38
5000	6880.00 ±1536.03	9.20 ±1.06	29.60 ±3.38	70.00 ±2.88
10000	9600.00 ±3857.46	15.00 ±0.02	36.20 ±5.74	71.40 ±8.02
LSD value	3038.20 *	2.430 **	12.435 **	17.340 NS
P-value	0.0247	0.0001	0.0040	0.6847

\* (P<0.05), \*\* (P<0.01), NS: Non-significant.

The White Blood cells named (leukocytes) which defending the body from the occupying organisms as viruses and bacteria, their number can be increased when the animal's body is weakened and being stressed by metabolic toxin (resulting in acute failure of kidney with built up waste products) (Foster and Smith Education Staff, 2016). The normal WBC indicates several remarkable physiological functions; their basic function providing immunity to the body, if they increase donate on inflammation (Kemal, 2014). If the existing cells in the case of reduction; the body increases the white blood cells for compensation, but the high toxicity will dominate and the cell numbers will reduced (Mahdieh *et al.*, 2015).

About Monocytes; Table 4 shows highest mean value for monocytes (15.00±0.02) in group treated with 10000 ppm in acute toxicity experiment, while the lowest mean value also recorded in control group (6.80 ±0.86) with highly significant differences (P<0.05) between control and all treated groups due to LSD value 2.430.

The Monocytes are leukocytes which act as a key role in inflammation and hemostasis; but these cells have global plasticity and heterogeneity which maintain human health (Bio- Rad Laboratories, 2012), these cells have a strong secretory activities (Kemal, 2014); which represent 4% of the mice white blood cells and 10% in humans (Van Furth & Sluiter, 1986) and can change their functional phenotype in responding to environmental stimulation, monitor and sense to environmental changes (Yang *et al.*, 2014).

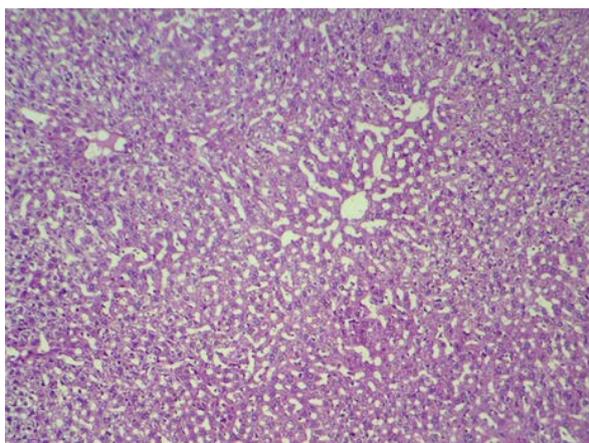
Monocytosis which means increasing in monocytes production is an indicator of different inflammatory diseases such as autoimmune disease, gastrointestinal disorders and occurs in case with cancer and chronic conditions (Dutta & Nahrendorf, 2014)., but the Neutrophils count according to Table 4 was found that after exposed mice to two weeks from DEA the highest mean value for neutrophils count (36.20±5.74) which recorded in treated group with 10000 ppm while control group was recorded lowest mean value (11.40 ±1.86) with highly significant differences (P< 0.01) between the last and other treated groups due to LSD value 12.435.

Neutrophils are granular leukocytes which the most density and very short- lived; play as first defenders towards infections (Nathan, 2006). These cells were found to be included in physiological and pathological process behind immune system (Mócsai, 2013). Their function is engulfing the causes of disease as bacteria and other small particles (Foster and Smith Education Staff, 2016), so stable count of neutrophils resulting in highly dynamic feedback system (Vietinghoff and Ley, 2008). When total number of neutrophil counts increased, it's a sign of some extreme condition or bacterial infection; it will be a sever reaction, caused the body is releasing more mature neutrophils to the blood circulation to protect itself from the infection (Foster &Smith, 2016). Decreased or increased counts of neutrophils, even within normal range in relationship with all- cause mortality (Vietinghoff & Ley,

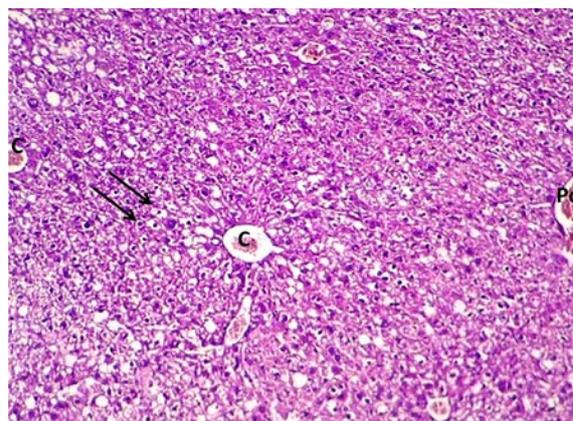
2008). Neutrophils act as regulation role in vascular inflammation depending on the situation; these cells are enlisted by different mechanisms; in case of systemic infection the neutrophils are immobilized in the lungs and liver (Phillipson & Kubes, 2011).

Table 4 shows the lowest and highest mean value of Lymphocytes ( $59.00 \pm 7.73$ ) and ( $71.40 \pm 8.02$ ) in control and mice treated with 10000 ppm respectively after two weeks from DEA treatment; there are no significant differences between control and other treated groups. These cells are a granulocytes which are defending the body in other ways not engulfing foreign organisms and particles but destroying the foreign materials and particles invading organisms, too (Foster and Smith Education Staff, 2016). They are considering major ingredients of immune system defense toward viruses, bacteria and Protista (Kemal, 2014); the lymphocytes are base for adaptive immune system in the body (Weir *et al.*, 2012). In response to chemical, physical and microbial damage inflammation takes place; acute inflammation can be described histopathologically via polymorphonuclear leukocytes permeation, in case not advanced to chronic inflammation (Kumar *et al.*, 2005); while an increasing in lymphocytes number can be noted in prolonged illness (Foster & Smith, 2016), excessed recruitment of leukocytes from blood into damage tissue consider a major character of inflammation (Sigmundsdottir & Butcher, 2008). During inflammation, T- cell (one of lymphocytes kinds) move toward the affected tissue (Brown *et al.*, 2010). Sigmundsdottir and Butcher found in (2008) that lymphocytes responded to tissue- specific and antigen- signals with functional specialization (as: special isotype or cytokine responses); so the characterization of chronic inflammatory diseases can be via chronic permeation of lymphocytes in extra lymphoid tissues (Kumar *et al.*, 2005).

The target organ liver had shown severe histological alterations; Figure 3 shows the normal liver structure of parenchyma with normal appearance tissue and liver extracellular matrix hepatocytes are arranged in cords located between the sinusoidal capillaries and orientated radially to the terminal venula, sheet of hepatocytes have polygonal shapes, most mononucleated cells.

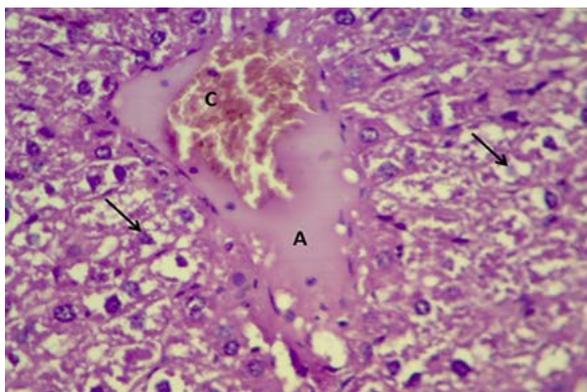


**Figure 3.** Cross section in liver showing normal structure in control mice treated with distal water for two weeks shows the central vein surrounded by the hepatic cords (hepatocytes) with sinusoids (H&E) (400X)

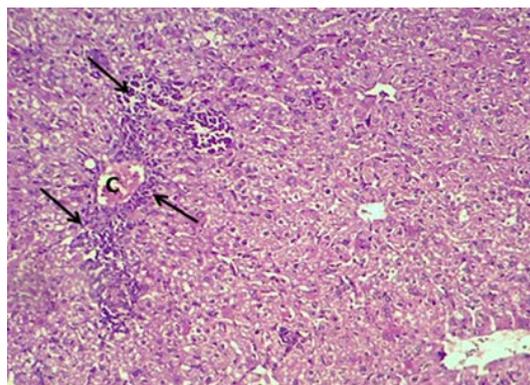


**Figure 4.** Section of liver for two weeks in mice treated with (630 ppm) showed central venous congestion (C) and portal venous congestion (pc) with mild hydropic degeneration (arrows). (H&E) (100X)

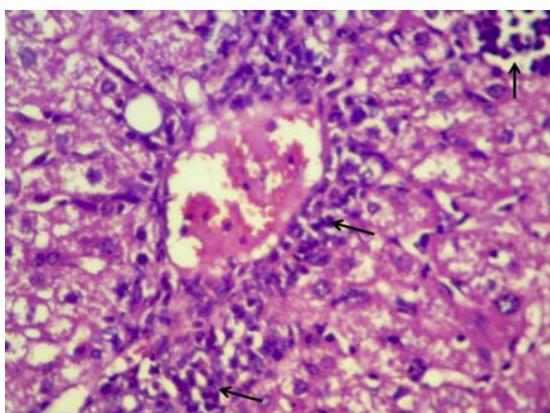
After 2 weeks from exposure to 630 ppm DEA; Figures 4 and 5 show the mild central venous and portal venous congestions with marked secondary amyloidosis at the portal area. There were mild hydropic degeneration with distortion of hepatocytes cords surrounded central vein which showed cytoplasmic vacuolation, nuclear pyknosis and karyorrhexis. While Figures 6 and 7 show alterations of hepatic tissue in mice treated with (1250 ppm); mild central venous congestion with little of necrotic foci composed of necrotic and lysis hepatocytes surrounded by infiltrated mononuclear leukocytes around central vein. The sections of mice liver treated with 2500 ppm showed focal fatty degeneration of hepatocytes with marked portal venous dilation and congestion. Mild necrosis of hepatocytes surrounded central vein with marked collapse of hepatic sinusoids (Figures 8 & 9).



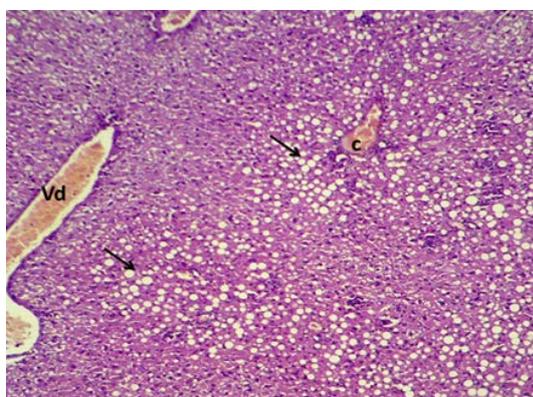
**Figure 5.** Section of liver for two weeks in mice treated (630 ppm) showed central venous congestion (C) and marked amyloidosis as acidophilic portentous fluids (A) with mild hydropic degeneration and nuclear pyknosis with karyorrhexis (arrows) (H&E) (400X)



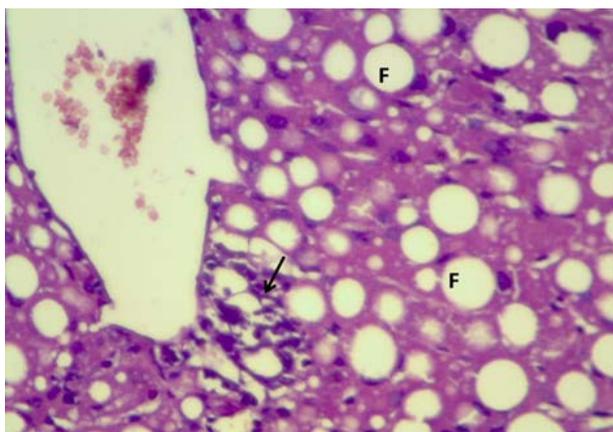
**Figure 6.** Section of liver for two weeks in mice treated with (1250 ppm) shows infiltration of mononuclear leukocytes surrounded necrotic hepatocytes and central vein (arrows) (H&E) (100X)



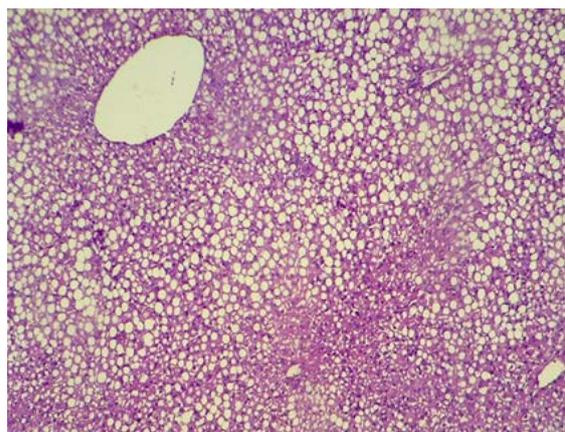
**Figure 7.** Magnified section of liver for 2 weeks in mice treated with (1250 ppm) shows central venous amyloid deposit with marked infiltration of mononuclear leukocytes, (arrows) (H&E) (400X)



**Figure 8.** Section of liver for 2 weeks in mice treated with (2500 ppm) shows: generalized fatty degeneration of hepatocytes (arrows) with marked portal venous dilation (Vd) with congestion of ventral vein (C) (H&E) (400sX)



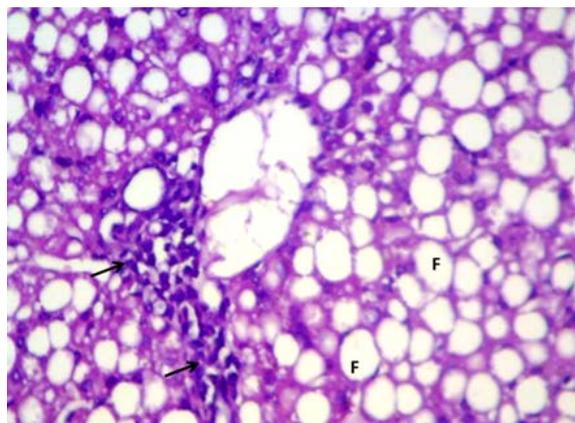
**Figure 9.** Section of liver for 2 weeks in mice treated with (2500 ppm) shows hepatocytes suffering from fatty changes (F) and necrotic focus surrounded with inflammatory cells (arrow) (H&E) (400X)



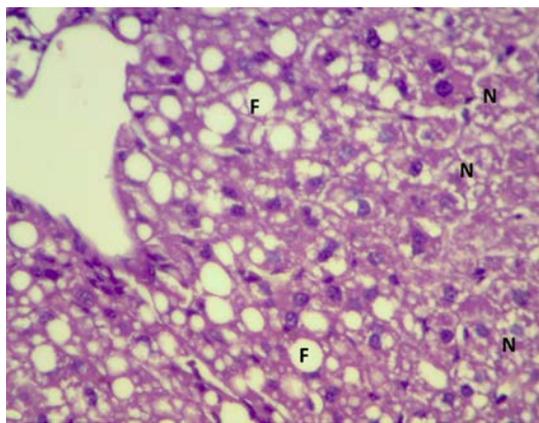
**Figure 10.** Section of liver for 2 weeks in mice treated with (5000 ppm) shows severe and generalized fatty changes. (H&E) (400X).

At the concentration of 5000 ppm; the fatty changes were more severe and generalized with necrosis of hepatocytes surrounded with mononuclear leukocytes. Most of hepatic sinusoids were disappeared and

some section showed finding of necrosis surrounded by infiltration of mononuclear leukocytes (fig. 10) and (11)



**Figure 11.** Magnified section of liver for 2 weeks in mice treated with (5000 ppm) shows: showed sever fatty changes (F) with necrosis of hepatocytes surrounded with mononuclear leukocytes. (H&E) (400X)



**Figure 12.** Section of liver for 2 weeks in mice treated with (10000 ppm) Shows fatty changes (F) and necrosis of hepatocytes (N). (H&E) (400X)

At the concentration of 10000 ppm; the histopathological changes were similar for that concentration of (5000) but the necrosis of hepatocytes was marked as it shown in (figure 12). National Toxicity Program concluded in (2002) when DEA administrated orally can be absorbed slightly less efficiency than dermal route, DEA can simplify its own absorption; in higher doses more completely absorbed than lower doses, but its distribution was similar in all administration routes. When DEA absorbed once; it will distributed in all tissues in the same way of different routes of administration, as a parent compound firstly in liver, kidney, spleen and brain (Mathews *et al.*, 1995). Diethanolamine concentrations can be absorbs in tissue organs 150 to 250 higher than blood, because the half- life of DEA in blood longer than the liver; but in repeated administration these properties will decrease (Mathews *et al.*, 1997). It accumulates in repeating exposure; but the highest doses can be cleared from DEA in half- life of 6 days (but not in repeatingdoses); while first DEA excretion can be found in urine as a parent molecuole, with less metabolites like O- phosphorylated and N- methylated metabolies (NTP, 2002). Mathews *et al.* (1997) found that ratio of DEA in rats' liver 50 times higher than in blood after exposure for 8 weeks to DEA. National Toxicity Program (2002) described this mode is very special for this small polar molecule which can be preserved by soma developing biological mechanisms to preserved related– ethanolamines which considered common ingredients of phospholipids. In mice administrated by DEA; the hepatic levels of choline metabolites were decreased while no changes observed in rats liver (Stott *et al.*, 2000; Lehman *et al.*, 2002). Lehman *et al.* (2002) found that the pregnant mice especially were sensitive to DEA through administration which caused in decreased in choline concentrations in liver and increasing apoptosis. Diethanolamine can stimulate tumours in mouse liver by a mechanism comprised to cause choline deficiency (Leung *et al.*, 2005). Food and Drug Administration (2013) reached to the fact that DEA decreased gap junctional hepatic and choline metabolites and S- adenosylmethionine (SAM) levels in mice, phosphatidyle choline synthesis by closing choline cellular uptake in vitro (but not in excess of choline); in the same time it stimulated tumors in mice, transformation in Syrian hamster embryo, cells and increased S- phase DNA synthesis in hepatocytes in mouse. In comparison, DEA absorption by rodents higher than humans, DEA has varieties in absorptive properties among species also related with ability of choline deficiency; so mice and rats being more able to absorb DEA than humans (Sun *et al.*, 1996). Diethanolamine hypomethylation is considered an epigenetic mechanism of carcinogenesis and number of genes can be activated involved oncogenes (Eden *et al.*, 2003). Kamendulis and Klauning (2005) found in their study that DEA inhibits choline uptake that leads to depletion of cellular choline with decreasing of choline levels; minimizes the potential methylation reaction and alteration of DNA methylation resulting in affecting gene expression encompassed in cell growth regulation.

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