

Antioxidant Effects of 1,8- Cineole Against Long Term DL-Polychlorinated Biphenyls (PCBs) Toxicity in Domestic Hen's Liver

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ABSTRACT

Current study was aimed to investigate 1,8-Cineole effectiveness as antioxidant defense against dioxins like compound polychlorinated biphenyls (dl-PCBs) toxicity in hen's liver. Domestic hens (n=45) were divided randomly and equally into 3 equal groups. 1st group (control) was given weekly for 6 months orally by gavage corn oil as carrier and feed on normal chicken pellets, 2nd group were given orally by gavage weekly for 6 months PCBs at dose 2 µg /kg weekly dissolved freshly in corn oil and evaporated to nitrogen, 3rd group (PCBs+cineole) and acetone mixed 2 µg/ kg B.W./weekly PCBs + Cineole 1000mg/kg/ weekly for 6 months. Half of liver sample were taken for histopathological examination at end of experiment and the other half liver for biochemical assay. In liver thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and catalase (CAT). Results showed that significant increase $P < 0.05$ in TBARS at 2nd group when compared with control and 3rd group, while CAT and GSH indicated significant $P < 0.05$ decreased at 2nd group compared with control and 3rd group. Histopathological changes in the hen's liver at 6 months revealed no important pathological changes in control and 3rd group, while 2nd group (PCBs) showed necrosis, heterophiles and mononuclear cell infiltration, severe granulation tissue in hepatocyte, disorganization of liver and liver adenoma.

Keywords: 1,8-Cineole, dl - polychlorinated biphenyls (dl - PCBs), hen's liver.

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INTRODUCTION

In the past, cases of polychlorinated biphenyls (PCBs) contamination, exceeding limits in food from animal origin (eggs, meats and milk) mainly caused by feed in industrially produced food (1). PCBs are widely recognized environmental and food contamination (2 & 3). PCBs uses and due to their weak life - cycle control and prevention causing excessive contamination of technosphere (4). Human via food especially animal- derived like eggs, meat, fish & dairy product will exposure to PCBs (5). Animal's feeds and feed additives are major source of PCBs contamination for food of animal origin (2). Eggs from laying hen's housed outdoors are mainly sensitive indicators of PCBs, soil contamination and a relevant exposure source for humans (6,7 &8).

Monitoring study found that more 50% of the eggs from 60 small flocks in Netherlands exceeded the EU maximum limited (9). Hens and broilers ingest on average 11g and up to 30 g soil /days (10, 11), about 2-4ng TEQ/g fat (6, 8, 9, 12), so chicken is the most sensitive exposure for PCBs.

Cineole also known as Eucalyptol, it has strong odor, colorless present in large amount in *Rosmarinus officinalis* plant and various species of Eucalyptus gender (13). *Rosmarinus officinaus* were reported it effects upon pharmacological like Smooth muscle relaxant, antioxidant, decreased blood pressure, anti-inflammatory (13), So current study was aimed to present by biochemical and histopathological analysis the antioxidant effectiveness of cineole against hen's liver damage induced by PCBs.

MATERIALS AND METHODS

Chemicals

PCBs- dioxin like compound dl(PCBs) purity > 99% and cineole was supplier from Sigma chemical Co. (St Louis Missouri, USA) with highest grade available.

Animal husbandry

Forty-five (45) adult (sexually mature) domestic hens chosen randomly from local market from Baghdad province, Iraq at 60 weeks of age were located and adapted in the animal house, Baghdad veterinary medicine, University of Bagdad, Iraq. Hens were adapted about 10 days before Intoxicant and feed on

commercial corn- soy prepared , water and feed were supplied, the temperature of experiment maintain at $23\pm C^{\circ}$ and humidity $70\% \pm 5\%$ with $0.35 \text{ ft}^3/ \text{ min}/ \text{ bird}$ ventilation and $6.25 \text{ ft}^3/ \text{ min}/ \text{ bird}$ dark light cycle 12:12h. Hens were examined at adaptation period before experiment and sick one was excluded. The experiment was done according to the Institutional Animal care and Use committee in all animal procedures.

A total of 45 sexually mature brown hens about 1000 gm in weight and aged 60 weeks were obtained, then divided equally and randomly into 3 equal groups (n=15 in each group). 1st group (control) served as negative control and received weekly only corn oil by gavage for 6 month with normal chicken pellet, 2nd group (PCBs) group contain (n=15 hens) received weekly for 6 months orally by gavage PCBs at dose of $2 \mu\text{g}/\text{kg}/ \text{ weekly}$ (14), stock solution of PCBs freshly dissolved in acetone and diluted with corn oil after acetone evaporated under nitrogen, 3rd group (PCBs+ Cineole) group weekly by gavage administrated orally PCBs ($2 \mu\text{g}/\text{kg}$) mixed with $1000\text{mg}/\text{kg}$ B.W. Cineole suspended by corn oil (15).

Liver samples for biochemical analysis and histopathological changes:

Liver samples were taken from abdominal cavity of hens in all groups after 6 months after recorded the abnormal necropsy finding in liver like size, color and adhesion. 50% of liver samples for histopathological examination after fixed with 10% neutral buffered formalin for 3days and then stained by H&E (16), other 50%

of liver samples from all groups homogenized were done in teflon glass and homogenized with $150\mu\text{g}$ Kcl 1:10 (w/v), pH 7.4, then centrifuged at $18000 \times g(4^{\circ}\text{C})$ for 30 min. and prepared for biochemical analysis (TBARS, CAT and GSH) level in liver tissue.

Biochemical analysis:

1- Thiobarbituric acid reactive substance (TBARS) nmol/g tissue in hen's liver: TBARS to lipid peroxidation index by assay thiobarbituric acid reaction and the product at 532nm in Spectrophotometer analysis the homogenization (17).

2- Catalase (CAT) k/mg protein in hen's liver, H_2O_2 enzymatic decomposition directly decrease in absorbance at 240nm. (18).

3- Reduced glutathione (GSH) nmol/mg protein in hen's liver measured at 412nm. (19).

Statistical analysis:

All the grouped data were statistically read by SPSS program, Version 17 software (2010). Testing methods including one way ANOVA for comparisons among groups. P values of less than <0.05 were considered statistical significance. All data were expressed as means \pm standard error (SE) (20).

RESULTS

Biochemical assay:

The table 1 showed significant increase ($P<0.05$) in Thiobarbituric acid reactive substance (nmol/g tissue) in 2nd compared with 3rd & control group.

Table 1: Thiobarbituric acid reactive substance (nmol/g tissue) in hen's liver tissue after 6 months.

| groups | 6 months |
|--|-------------------|
| 1 st group (control) | 132.5 ± 4.8 b |
| 2 nd group (PCBs) | 270.8 ± 3.1 a |
| 3 rd group (PCBs+Cineole) | 111.2 ± 4.9 b |

n:15 with $P<0.05$

while in the table (2) showed significant decrease ($P<0.05$) in Catalase (k/mg protein) activity in 2nd compared with 3rd & control group.

Table 2: Catalase (k/mg protein) activity in hen's liver tissue after 6 months.

| groups | 6 months |
|--|-------------------|
| 1 st group (control) | 9.71 ± 0.39 b |
| 2 nd group (PCBs) | 3.95 ± 0.46 c |
| 3 rd group (PCBs+Cineole) | 12.2 ± 0.44 a |

n:15 with $P<0.05$

The table (3) showed significant decrease ($P<0.05$) in GSH (nmol/mg protein) in 2nd compared with 3rd & control group.

Table 3: GSH (nmol/mg protein) in hen's liver tissue after 6 months.

| groups | 6 months |
|--|--------------------|
| 1 st group (control) | 2.64 ± 0.23 a |
| 2 nd group (PCBs) | 1.12 ± 0.02 b |
| 3 rd group (PCBs+Cineole) | 1.93 ± 0.10 ab |

n:15 with $P<0.05$

Histopathological changes:

No important histopathological changes were observed at 1st and 3rd groups, while 2nd group showed after 6 months of experiment liver fatty change characterized by swelling of liver cells with reticulation of the cytoplasm with fatty change present as single or multiple large fat droplet of varying in size which sometimes displaces the nucleus to the periphery of the cells (hyperlipoproteinemia) fig(1). Necrosis of hepatocytes with multinucleated hepatocytes and broad multilayered trabeculae liver cells are separated by hemorrhage with mitotic figure and eosinophilic homogeneous fibrinoid degeneration in the central vein wall (fig. 2). Other section revealed extensive heterophiles infiltration with hemorrhage and degeneration of hepatocytes (fig. 3), the same lesion present in (fig.4) with edema and fibroblast in dilated bile duct. Portal tract shows hyperplasia of duct with severe lymphocytic

infiltration some are granuloma with interstitial edema (fig. 5), Fibroblast invasive the interstitial layer of hepatocyte with mononuclear infiltration mostly lymphocytes (fig. 6). All hepatocytes were necrotic with dark eosinophilic cytoplasm liver parenchymal tissue separated by large area of fibrous tissue (granulation tissue) and band causing distortion of hepatocytes with moderate infiltration of lymphocytes (fig. 7). Broad multilayered trabeculae of neoplastic liver cells were separated by bands of capillary stroma and sinusoid with extent of cellular pleomorphism and number of mitotic (fig. 8), All hepatocytes disorganized homogenous with severe dense lymphocytic aggregation (fig. 9). Increase in kupffer cells with hepatocytes has considerable amount of eosinophilic cytoplasm (neoplastic cells) with very uniform around basophil nuclei with fibrin network with on bile duct (liver adenoma) (fig. 10).

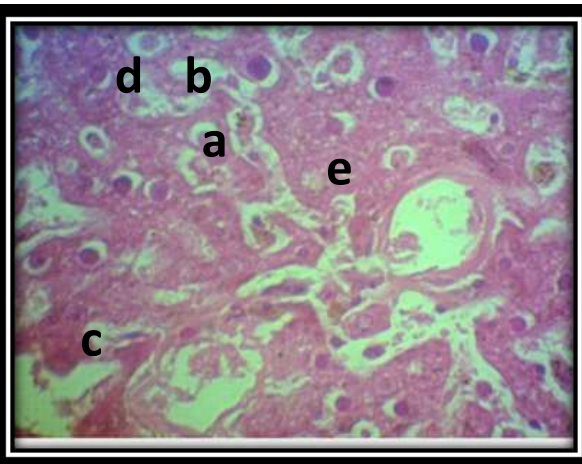
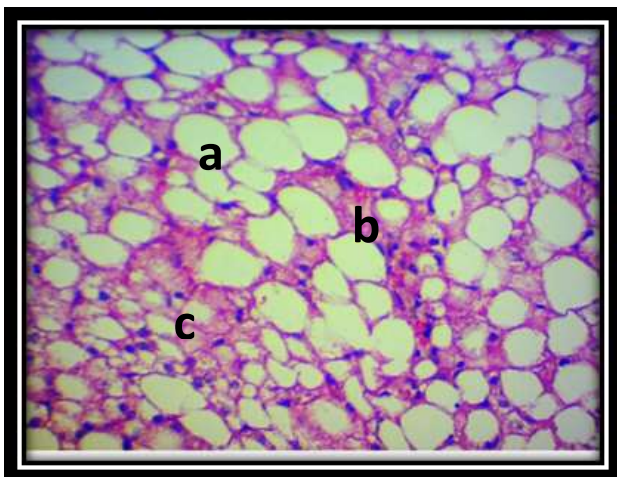


Figure (1): Liver of hen's 2nd group after 6 months showed: a) swelling of hepatocytes b) reticulation of cytoplasm c) fat droplet varying in size displace the nucleus periphery.
(X40 H&E).

Figure (2): Liver of hen's 2nd group after 6 months showed: a) necrosis hepatocytes b) multinucleated hepatocytes c) broad trabeculae (sinusoids) d) mitotic figure e) eosinophilic homogenous fibrinoid degeneration.
(X40 H&E).

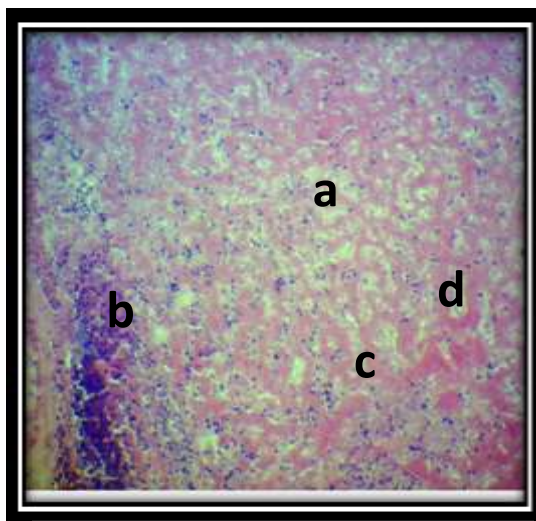


Figure (3): Liver of hen's 2nd group after 6 months showed: a) degeneration of hepatocyte b) zone of heterophiles c) necrotic hepatocytes d) hemorrhage.

(X20 H&E).

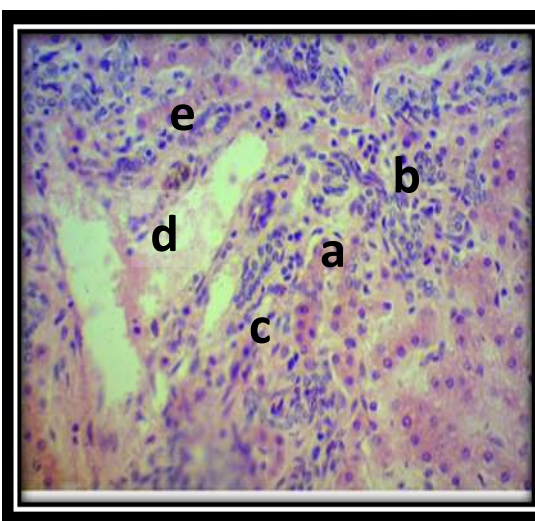


Figure (4): Liver of hen's 2nd group after 6 months showed: a) acute cellular swelling b) increase in Kupffer cells c) fibroblast cells d) edema e) hemorrhage.

(X40 H&E).

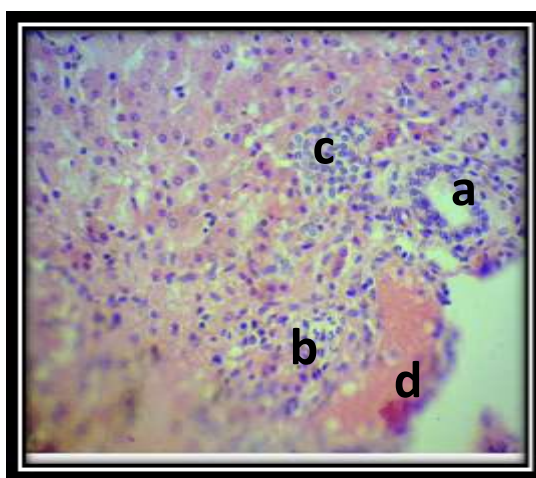


Figure (5): Liver of hen's 2nd group after 6 months showed: a) the portal tract shows hyperplasia b) lymphocytic infiltration c) lymphocytic granuloma d) interstitial edema.

(X40 H&E).

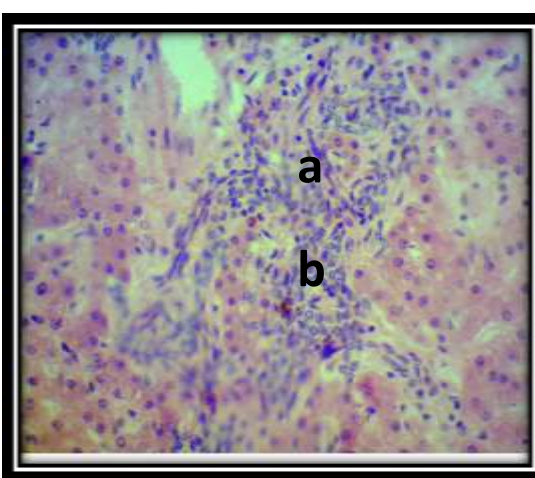


Figure (6): Liver of hen's 2nd group after 6 months showed: a) fibroblast b) aggregation of mononuclear cells mostly lymphocytes.

(X40 H&E).

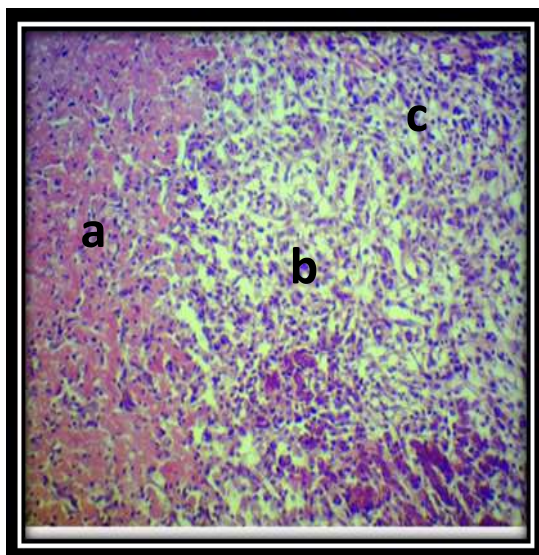


Figure (7): Liver of hen's 2nd group after 6 months showed: a) necrotic hepatocyte b) large areas of fibrous tissue (granulation tissue) c) lymphocytic infiltration.

(X40 H&E).

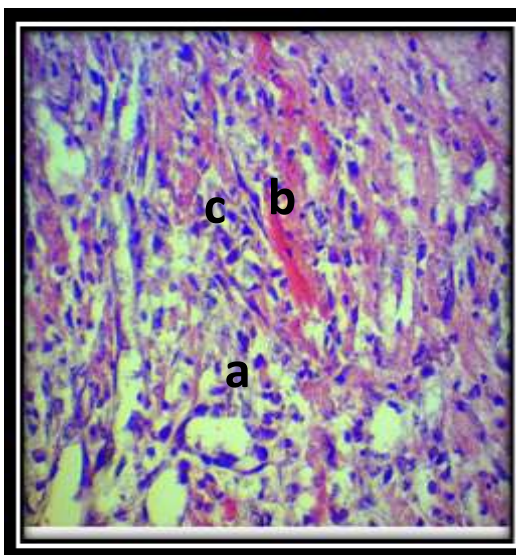


Figure (8): Liver of hen's 2nd group after 6 months showed: a) multilayered trabeculae of neoplastic hepatocytes b) band of capillary stroma c) pleomorphism hepatocytes & mitotic figure.

(X40 H&E).

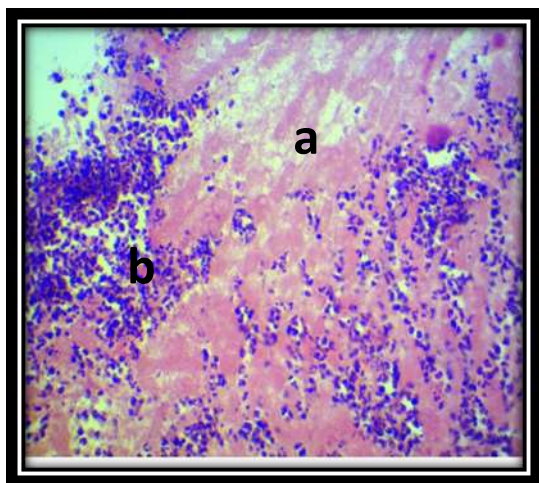


Figure (9): Liver of hen's 2nd group after 6 months showed: a) disorganization hepatocyte b) dense lymphocytic aggregation & infiltration.

(X40 H&E).

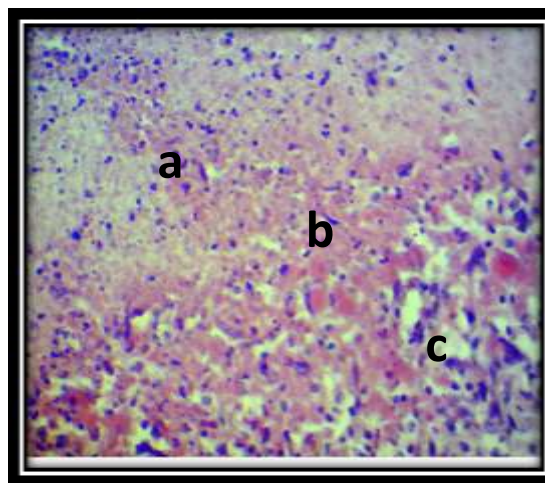


Figure (10): Liver of hen's 2nd group after 6 months showed: a) fibrin network b) kupffer cells c) neoplastic hepatocytes with basophilic nuclei (hepatocyte adenoma.

(X40 H&E).

DISCUSSION

Biochemical assay in hen's liver:

The TBARS showed in hen's liver significant increased $P < 0.05$ in (PCBs) group when compared with control and (PCBs+Cineole) group after 6 months, while at same time a significant decline in the level of GSH and CAT at same groups above. Liver is the principal organ for toxic effect characterized by pathological and biochemical alteration (21- 23). Other studies reported that similar increases in TBARS formation in the liver, kidney, thymus and brain tissue in TCDD dioxine. (24-26), also the results are in agree with (27) who showed significant decrease $P < 0.05$ in (PCBs) group for GSH & CAT after exposure to toxic material (acrylonitrile) in albino male rats. There is no study on the effect of Cineole on level of TBARS, GSH and CAT. Cineole-containing plants are potent inducer of detoxifying enzymes and there by prevent inducers of damage, also it is effective protect bacterial and human cells against oxidative damage (28 & 29), So the current results above indicates that Cineole has protects hen's liver from oxidative damage induced by PCBs.

Histopathological changes:

No important pathological changes observed in 1st and 3rd groups, while 2nd group (PCBs) showed sever liver damage characterized by necrosis, fatty change, sever granulation tissue, hetrophiles and mononuclear infiltration with hepatocytes disorganization (pelomorphisum, nuclei, mitotic figure) and liver adenoma hyperplasia of bile duct.

The results in 2nd group are in agree with (30 & 31) who showed macrovascular hepatic fatty change, liver cells present single or multiple large fatty droplets of varying size displace the nucleus to the periphery of the cells mostly hyperlipoproteinemia with mononuclear cells infiltration mostly lymphocytes, liver abscess consist of liquefactive necrosis surrounded with dead basophilic, neutrophils and zone of fibrous connective tissue, acute hepatitis with confluent necrosis liver with centrolobular necrosis, mast cells aggregated in the liver parenchyma forming a foci causing hepatocytes atrophy and hemorrhage.

Liver is the major site for detoxify toxins and polluted materials. Dioxins and dioxin like compound transported from external to adipose tissue or liver through plasma lipid and bind to aryl- hydrocarbon receptor (AhR) in cystol of hepatocytes and translocated to the cell nucleus & bind to the dioxin response element on DNA (dioxin responsive element, DRE) this compound is a signal for transcription of cytochromes P450 and 448 (aryl hydrocarbon hydroxylase- AHH) genes, the highest concentration of AHH, the enzyme responsible for dioxin conversions occur in endoplasm

reticulum of hepatocyte, while inflammatory of dioxins effects limited by reducing the level of TNF, So Cineole administration as antioxidant blocks cyclooxygenase -II enzyme (Cox-2) and deactivates the AhR receptors. Accumulation of dioxin, at 6 months in liver tissue modify the metabolism of cholesterol and hormones, it is directly change blood picture and biochemical response to inflammatory reaction (32-36). Experiment indicated that dioxins induced carcinogenicity mostly hepatoma or liver adenoma, So the International Agency for Research on Cancer (IARC) classified dioxins as group 1 carcinogen (37 & 38).

CONCLUSION

The TCDD showed intoxicant and may be carcinogenic in hens according to the time and dose depended on manner, also the cineole considered strong antioxidant against the TCDD and other toxins.

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