Eruca sativa and Raphanus sativus Oils Enhance Hepatic and Renal Tissues Regeneration in White Mice

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Abstract
Regeneration is a process of tissue repairing in the body, and according to this process the cells of the body are divided into three types: labile, stable and permanent cells. The aims of this study are to evaluate the ability of two herbal seed oils [Eruca sativa and Raphanus sativus] to enhance regeneration and repair in the liver and kidney in the irradiated mice. And to investigate which herbal oil is more effective. Four groups of mice were used in this study. The first three groups were exposed to radiation while the fourth was used as a control. After irradiation the first and second groups were treated with local Eruca sativa and Raphanus sativus oils respectively. While the third group used as a control. Then histopathological investigation was done. Results: Histopathological examination in irradiated groups exhibited that both seed oils could induce regeneration in both hepatic and renal tissues but the activity of Raphanus sativus oil was more effective than Eruca sativa especially in renal tissue. Meanwhile, poor regeneration process appeared in the third group [control]. In conclusion, both local herbal oils had ability to enhance regeneration in the examined tissues but R. sativus seed oil exerted more activity than E. sativa seed oil.

Keywords: Eruca sativa oil, Raphanus sativus, hepatotoxic, nephrotoxic, ultraviolet irradiation.

Introduction
Previous civilizations were used the herbs to get the comfort, but it's complicated to point out the precise time whenever initiate use as a medicine[1] to prevent diseases or support ordinary medication as in case of cancer disease or use as synergist part[2]. In spite of their illustration in the food, Eruca sativa [ES] and Raphanus sativus [RS] part of these medicinal plants from Brassicaceae family, which are, used all parts [roots, leaves and seed] in several medications. Greek used the leaf of E. sativa to treat stomach, scurry and as diuretic plant[3]. Its used as antimicrobial as well as Eruca seed was used as aphrodisiac drug in Arabian countries to enhance sexual craving in infertile men. E. sativa records high level of antioxidants compound and glucosinolates in the leaves and seed populates to be anticancer herb[4]. Phytochemical
components of *E. sativa* inhibit melanoma tumor growth according to Khoobchandania *et al.* [5] Ishrat R, *et al.* 2010 authentic the effect of methanolic extract of seed against *Enterobacter agglomerans* and *Hafnia alvei*. Methanolic extract also found to be affected against *Penicillium funiculosum Paecilomyces variotii* respectively[6]. Mona S, Nehal M, 2001 showed *Eruca sativa* seed oil stimulates spermatogenesis among male rat in low doses[7] however its regenerate liver and kidney function to normal condition which damaged by aflatoxin B1 among rabbits[8].

*Raphanus sativus* [RS] has been illustrated in many countries as food consumption for a long time but its phytochemistry constituents of each part capacitate to use in medicinal field as antimicrobial, antifungal agent, treat hepatic and respiratory diseases, stimulate digestion and bile secretion[9][11]. Anti–influenza effect from leave extract, whilst root juice exhibit antimicrobial effect against numerous types of bacteria as Aqueous extract of the whole plant presents activity against *Sarcinia lutea*and *Staphylococcus epidermidis* [12]. Seed of *R. sativus* regenerate hepatic tissue damaged by Carbonetetrachloride and regulate level of AST and ALT activities due to compeitition of its antioxidant contents with oxidized agents.

Suh *et al.* authenticate that *R. sativus* extract depressed the characteristics appearance of atherosclerosis and restenosis[13]. In study in Ain Shams University, Egypt concludes that its juice diminishes nephrotoxicity and hepatotoxicity caused by oxidative damage due to dimethoate[DM] among mice by improve enzyme status through effect of antioxidant content[14] as the same result from Jalila A, *et al.* 2015 who explain the protective effect of *Raphanus sativus* extract against immunotoxic and biochemical alterations in rats of Cadmium [Cd] initiated by Reactive oxygen species formation[15]. Chromatographic analysis of R. seed oil demonstrate the chemical composition of certain oil complete similar to those of cotton seed oil[16] The antioxidant components stimulate repair the pancreas, liver and kidney tissue with rebounding of β-cells of islets of Langerhans to output insulin and improve their immunohistochemical finding of diabetic rat induced by Streptozotocin[17]. Aly *et al.* confirm the same result when used Egyptian radish but radish oil appears partial recovery of pancreas tissues exposures to Streptozotocin effects[18]. All these previous studies focused on a great amount of antioxidant constituents of experimental herbs in all parts of plants. The present study was aimed to compare impact of two important herbs seed oils in folk medicine [*Eruca sativa* and *Raphanussativus*] on renal and hepatic tissue injured by ultraviolet radiation exposure.

**Materials and Methodologies**

**Herbs oils**

*Eruca sativa* and *Raphanussativus* oils used in this study were purchased from the local Iraqi markets in Baghdad as manufactured by Emad factory for producing herbal oils /Mousl./ Iraq. Placed in dark bottles.

**Animals**

Thirty five white mice at 9- 12 weeks old and 32-37 g weight were utilized in the accurate study, allocated into ample stainless style cages in the animals breeding center of College of Medicine - University of Baghdad necessity to all duration of trail. Experimental animals were providing with water and balanced nutritional feed. All animals subjected to equal duration of dark and light in temperature 20-25 °C.

**Design of experiment**

The mice were divided into two main groups as following:

- Control group consisted of five mice did not expose to radiation and lived in normal conditions that was considered as a control group for irradiation.

- UVC group consisted of 30 mice were subjected to study the impact of UV radiation, *E. sativa* oil and *R. sativus* oil on hepatic and renal tissues. All mice in this group were exposed to UVC irradiation for one hour/day and leave to rest at the second day. The UVC lamp from industrial fiber optics of 250 nm wave length and a power of 20 Watt was used.
The UVC source placed at 30 cm above mouse cage where the final UVC power at the mouse skin surface 1.2 mW for 1 hour. After resting time they were divided into three equals' subgroups and treated as following:
First group treated orally with 0.5 ml *Eruca sativa* oil at 100% in concentration for 21 days. Second group treated orally with 0.5 ml *Raphanus sativus* oil at 100% in concentration for 21 days. Third group treated orally with 0.5 ml normal saline for 21 days and used a control.

**Sampling**
The mice in all groups were killed after 21 days to extirpation of kidneys and liver which wash by normal saline solution and fixed separately in 10% formalin. These fixed parts were processed and stained with H and E for histopathological examination[23].

**Results**

**Histopathological changes in the liver**

*1-The mice exposed to UV radiation*
The architecture of hepatolobules were distrorded and the hepatocytes appeared smaller in size with losted their hexogonal shape, their cytoplasm became more eosinophilic with either fint or fragmented or disappeared neucleus (Figure 1). Milde to slightly moderate hemosidrosis were appeared (Figure 1D) and in some sections few cells look like oval cells can be detected (Figure 1E).

**Figure 1**: The effect of UV radiation on the hepatic tissue. A) the architecture of liver lobule has been lost X10, B) Smaller hepatocytes with more eosinophilic cytoplasms and fint neuclei, [C] Necrotic area of liver characterized by all the cell in the section lost their nuclei X40, D) moderate hemosidrosis [arrows] and E) Some oval cells are scattered in the section [arrows] X40.
2-The mice treated with E. sativa oil
Different degree of regeneration were appeared in the liver of mice treated with E. sativa, i.e. the nuclei of hepatocytes exhibited large, irregular shape and hyperchromic. Meanwhile the oval cells were commonly seen in all part of hepatic lobule and few endothelial cells are clearly seen (figure 2).

**Figure 2**: liver of mice treated with *E. sativa* after exposure to radiation. A) low magnification shows high cellularity near portal area X10, B, C, D and E) High magnification shows high cellular density of the liver stem cells [oval cells] [black arrows] and the hepatocytes contain large irregular hyperchromic nuclei [head arrows] X40, E, G and F) few endothelial cells present [arrows] associated with oval cells and hepatocytes X40 [H & E stain].
3-The Mice treated with R. sativa oil
The microscopical appearance of the hepatocytes was characterized by presence of very large, irregular shaped hyperchromic nuclei which occupied most of the cell size. This appearance was associated with distributed oval cells in the hepatic lobule and also endothelial cells were present in somed sections (Figure 3).

![Figure 3: Hepatic regeneration in mice exposed to radiation then treated with R. sativa oil. A and B) Hepatocytes have huge irregular nuclei with very scant cytoplasm [green arrows] large number of liver stem cell [oval cells] diffuse in the liver [black arrows] X40, C) Endothelial cells appear in the tissue [red arrows] associated with oval cells [black arrows] and hepatocytes [green arrows] which have the same characteristics in the A and B X40 [H & E stain].](image)

Histopathological Changes in the Kidney
1-The group of mice exposed to UV radiation
After 21 days from exposure to the radiation, the renal tissue appeared eosinophic and the characteristic features of the kidney were lost, therefore neither glomeruli nor tubule could be recognized. The cells lost their nuclei and underwent necrosis meanwhile no indication or evidence on the regeneration process could be identified (Figure 4).
2-The group of mice treated with E. sativus oil after exposure to UV radiation

After 21 days of treatment with E. sativus oil in the animals exposed to radiation. Different degree of regenerative process could be recognized. However in some sections the cells organized in tubule structure, while in other sections the tubule appeared still distorted but the presence of stem cells were predominant in all sections (Figure 5).

Figure 4: Microscopic appearance of the kidney in the mice exposed to radiation. A) Eosinophilic appearance of the renal tissue X10, B and C) distortion and necrosis in the renal tubules are seen X40 [H&E stain].

Figure 5: Histopathological changes in renal tissue of mice exposed to radiation then treated with ESO for 21 days. A) and B) distortion of most tubules with moderate number of stem cells are found [arrows], C) Most cells are arranged in tubules. The tubular cells are low cubic to flat in shape, D) the epithelial cells of tubules are high cubic in shape and organized in tubules. Stem cells are seen [arrows] X40 [H & E stain].
3-The group of mice treated with R. sativus after exposure to radiation

Repair clearly appeared in the renal tissue of the mice treated with R. sativus oil, it was characterized by hypercellularity of the tissue associated with hyperchromacia in most of the cells and hyperatrophied cells of the renal tubules which appeared tall cubic to columnar in shape associated with numerous number of stem cells.

![Microscopic appearance of the renal tissue in mice exposed to radiation then treated with RSO for 21 days. A) low magnification of kidney shows high cellularity X10, B) high magnification, appears hypertrophied cells [tall cubic to columnar in shape] which have hyperchromic nuclei and organized as tubules. Stem cells are found X40, C) the cells of tubules contain condensed chromatin X40 [H&E stain].](image)

**Discussion**

The results in this study exerted that the UV radiation had harmful effects on both hepatic and renal tissues. It acted by some way to induce injurious effect on these tissues. That indicated the pathological effects of radiation extend to the internal organs which consistent with Svobodova et al. who reported that both skin and non-skin tissues can be affected by UV radiation[19] and also consistent with other researchers whose confirmed that the real feature of renal tissue [glomeruli and tubules] was absent in exposure to UV[20][21]. The injurious effect of UV in the cell is attributed to the production of free radicals through its radiolysis of water molecules that interfere with biochemical processes of cells causing changes in their structures and or functions[22]. Free radicals have affinity to cell components seeking stability through attacking the normal biological reaction producing newly molecules. Thereby generate chain of these unstable electrons causing cell damage[23]. In normal conditions, free radicals as reactive
Oxygen [ROS] and Nitrogen species [RNS] are produced by mitochondria in normal biological processes through cellular respiration and redox. However, in some cases under internal factors like hormones, cytokines [pro-inflammatory] or external factors like ultraviolet irradiation, detoxification enhance production of oxidative factors or deletion of antioxidant in the cell resulting in cell damage through modification of protein or cell, DNA deterioration and lipid peroxidation[24].

Our results showed few numbers of oval cells in the liver of mice exposed to the radiation that means the liver has ability to induce regeneration after damaging with UV but this ability probably is limited as mentioned by Ozaki M, et al. 2003 who reported that is hard to induce hepatic regeneration under pathological conditions[25].

Current experimental study exhibited that both E. sativa and R. sativus had ability to enhance repairing process in the tissues which had been damaged by UV radiation; their action appeared by enhancing the regeneration activities in the liver and kidney. In the liver, they acted by somehow to activate hepatic progenitor cell [liver stem cell] that also called oval cell. The liver stem cells or oval cells are resident in the normal adult liver and under certain conditions, these cells replicate and differentiate to hepatocytes[26]. Regeneration in the liver mainly occurs by two forms; the first form is done by replication and proliferation of mature hepatocytes to regenerate the liver and the hepatic regeneration due to injury by drugs, toxin, and viral diseases occurs by this way. This form of regeneration acts as a front-line of defense[27].

Meanwhile the second form is done by replication and identification of liver stem cells (oval cells). Oval cells are derived from canal of Hering (terminal part of bile duct)[27] and may have an extrahepatic origin such as bone marrow[28]. These cells have ability to replicate and differentiate to give two types of liver cells, hepatocytes and cholangiocytes, therefore oval cells are similar to hepatoblast in the embryonic liver. Usually the replication and differentiation of oval cells to hepatocytes is occurred only when injury is sever or when the capability of mature hepatocytes to replication have been lost or blocked[27].

However, bone marrow involvement in liver regeneration is controversial[29], our results showed that there was activation in endothelial cells, this activation appeared in the group treated with R. sativus oil and with little extend in the group treated with E. sativa this observations probably improve the theory says that bone marrow implicated and participated indirectly in liver regeneration. Therefore, the effect of these oils on the liver also may be done through bone marrow activation.

Our observations agree with other research, which reported that the R. sativus has protective and therapeutic effects on the liver, so the levels of liver enzymes that associated with hepatic damage such as AST and ALT become low after treatment with R. sativus[30]. However, these effects can be attributed to the antioxidant components of R. sativus, which lead to reduce oxidative activity and improved lipid profile and liver enzymes[17] and prevent hepatic cirrhosis, by suppression of inflammation[31].

Regeneration in the renal tubules also was found in our experimental study in both treated groups of mice, but the group of mice treated with R. sativus exerted more active in the regenerating process than in the group treated with E. sativa, i.e the ability of R. sativus oil to enhance activity of regeneration was more than E. sativa. This effect is probably done by amount and type of antioxidants compounds present in the R. sativus oil[32][33]. Three repairing theories try to explain the origin of the stem cells that may contribute to renal regeneration (1). Renal stem or progenitor cells [there is suggestion these cells are found outside of nephron migrate into the damaged nephron and the repair the tubules. (2). Dedifferentiation of injured tubular cells [following detachment of injured epithelium] the basement membrane of tubules is covered by mesenchymal cells that repair tubular damage (3). Immigration of bone marrow derived cells to the kidney and participates in renal regeneration[34]. These results are confirmed with other research that showed the
protective impact of *E. sativa* against nephrotoxicity[35][36].

Greaten herbs compound, unlike recognized medicines, are a complicated admixture of chemical components and often a complete characterization of the bioactive compounds from an herbal is unknown[37]. The therapeutic effects of both *E. sativa* and *R. sativus* in this study occurred due to various phytochemical presents in these herbal oils such as vitamin C,E,A,phenolic compounds, flavonoids, sulfur containing compound ...etc that have a competitive effect with free radicals due to their antioxidant characteristics[38]. Antioxidant is biodefense means against free radicals and reactive chains. Its bioactive occurs in some order may be lowering free radical energy leading to interrupt new molecules at first or cross oxidation chains to prevent damage tissues. Antioxidant defenses divide into two classes, [Endogenous antioxidants] enzymatic and [endogenous factors] non-enzymatic, first one involves catalase glutathione peroxidase [GPx], superoxide dismutase [SOD], etc. while the second class involves vitamin C, vitamin E, carotenoids, flavonoids, etc. All these play role in scavenging of free radical in some ways[39]. Flavonoids the main polyphenol compounds that inhibit lipid peroxidation or elevate level of GSH as antioxidants[40] and they also decrease tocopheroxyl and elevated uric acid[41] that limit the reaction of Hb with peroxides, inhibit erythrocyte lysis and good scavenger of ROS and hydroxyl radicals[42]. However Glucocerucin of *E. sativa* can activate glutathione S-transferases (GSTs)[43] and other important detoxifying and antioxidants enzymes[35][36][38].Therefore our experimental oils may be acted as non-enzymatic endogenous antioxidant and also induce the activity the enzymatic endogenous antioxidants.

The activity of experimental oils in tissue regeneration in addition to previous study explanation maybe there is a synergetic effect between these different types of antioxidants. If two or more antioxidant combined to protect each other or to regenerate antioxidant by others or to potent antioxidant who less activity like ascorbic acid and tocopherols[ ascorbic acid is a synergest and tocopherol is primary] or may synergic two or more component to protect other anti-oxidant. Also carotene and tocopherols its complicated type of synergism[44].Renal regeneration in the group of mice treated with *R. sativus* exerted more active in regeneration than in group treated with E.sativa. Each of two oils possess potent antioxidant , free radicals scavenger and repair DNA damaged by irradiation but may be some components in certain concentrations found in RSO not found in *E. sativa* which affect the accurate results.

**Conclusion**

It could be conclude that the local preparation of *R. sativus* and *E. sativa* oils have ability to repairing action on damaged renal and hepatic tissues by enhancing the regenerated processes in these organs in irradiated white mice. And the oil of *R. sativus* appeared more effective than *E. sativa*.

It could be to recommended advance study on phytochemical of herbs to investigate their bioactivity and therapeutic effect.

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