ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDY OF HONEY AND WATER EXTRACT OF NIGELLA SATIVA SEEDS EFFECTS ON INDUCED HEPATOCARCINOMA IN RATS.

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SUMMARY AND CONCLUSION

Cancer is a class of disease characterized by uncontrolled cell division and the ability of these cells to invade other tissues, either by direct growth into adjacent tissue (invasion) or by migration of cells to distant sites (metastasis). This unregulated growth is caused by a series of acquired or inherited mutations to DNA within cells which damage genetic information that define the cell function and removing normal control of cell division. Several mutations may be required to transform a normal cell into a malignant cell. These mutations are often caused by chemicals or physical agents called carcinogens. Some mutations occur spontaneously, or they can be inherited

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and it is one of the major causes of death, because of its high frequency and poor prognosis. Hepatocellular carcinoma is now a rather common malignancy in Egypt.

The liver is the first organ to receive blood from the intestinal tract. A primary function of the liver is the biotransformation, detoxification, and excretion of xenobiotics, including carcinogens.

Cancer has become an important topic in medicine since it is a major cause of death in both the developed and developing countries and it is now only secondary to that of myocardial infarction. A great majority of human cancers (about 80%-90%) are attributable to environmental factors, the use of additional treatment such as radiotherapy and chemotherapy has resulted in no more than 5% reduction in the number of deaths. the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades Honey is one of the oldest known medicines. Studies by **Gribel and Pashiniski, (1990)** indicated that honey possessed moderate anti-tumor and pronounced anti-metastatic effects in five different strains of rat and mouse tumors. Furthermore, honey potentiated the anti-tumor activity of chemotherapeutic drugs such as 5-fluorouracil and cyclophosphamide (**Wattenberg, 1986**). Honey contains many biologically active compounds including caffeic acid, caffeic acid phenethyl ester and flavonoid glycones. These compounds have been proved to have an inhibitory effect on tumor cell proliferation and transformation by the down regulation of many cellular enzymatic pathways including protein tyrosine kinase, cycloxygenase and ornithine.

Cancer occurs as a result of abnormal cell proliferation. Cellular proliferation is regulated by cell cycle regulatory proteins. The classic cell cycle consists of several phases: G0, G1, S, G2, and M. Cells in the G0 phase are quiescent. Cells in other phases are said to be cycling because they are undergoing changes related to cell division. Decreased expression of cyclin-dependent kinase (CDK) inhibitors and increased expression of cyclin D family proteins allow cells to enter G1 from G0; and likewise, increased cyclin E expression allows cells in the Gl phase to pass the G1 check point and enter S phase (**Sherr, 1996**).

The black seeds referred to by the prophet Mohammed as having healing powers . In vitro and in vivo studies indicate that both the oil and the active ingredients of N. sativa seeds possess anti-tumor effects. By investigating effect of the volatile oil of N. sativa seeds on different human cancer cell lines, the oil expressed marked cytotoxic effects against a panel of human cancer cell lines **(Islam et al, 2004**) Moreover, oral feeding with N. sativa extract suppressed hepatic tumor in rat induced by diethylnitrosamine or by partial hepatectomy (**Iddamaldeniya et al; 2003**). the crude extract of N. sativa oil, was also found to show in vivo antitumor activity against leukemia and Lewis lung carcinoma (**Kumara and Huat, 2001**), prolonging the life span of the tumor bearing mice.

The purpose of this study is to determine the effect of the honey and nigella sativa on carcinogenesis in the liver. Eight groups of animals were used and fed *ad-libitum*. The first four groups were a control (five animals for each), the 1^{st} control group was fed *ad-libitum*, the 2^{nd} , 3^{rd} and 4^{th} groups were given orally 2g honey/rat/day, 0.2g *Nigella sativa* seed water extract and mixture of honey with *N. sativa* respectively. The

5^{th} , 6^{th} 7^{th} and 8^{th} rats groups were intraperitoneal injected with single dose 150 mg/k body weight for each . After one week the 6^{th}, 7^{th} and 8^{th} groups were given orally 0.2 g *N. sativa* seeds water extract, 2g honey and 0.2 g *N. sativa* seeds water extract with 2 g honey/rat/day, respectively. The effects of honey and *Nigella sativa* on tumor can be detected by histological studies (LM) and (Em) microscopes showed, liver of rats injected with DEN suffered from many lesions such as increase basophile which means hyperchromasia, giant celles, decrease the cytoplasm nucleus ratio cell which lead to hyperplasia and lack of diffrrention and basophilic foci formed nodules. Honey gp showed nearly normal artcheture with inflammation and increase apoptosis. *Nigella* gp showed little normal articeture with several chronic inflammation so *nigella* only have little protective effects but honey and nigella together showed good protection against DEN(no pathological change).

- Immunohistochemical observations:-

A) P53 and PCNA immunohistochemical expression

Control tissues section rats immunostained for p53 and PCNA expression showed very weak positive (less than 5% stained nuclei). In contrast, a large number of strong positive stained nuclei were observed in liver sections of DEN gp as the liver carcinogen. In addition, the liver sections of rats injected with DEN and treated with nigella, honey and mixture of *Niglla* with honey showed the positive stain in some hepatocyte nuclei but less than that of the DEN carcinogen treated animals.

In summary, the present study showed that DEN-induced severe liver injury and carcinogenesis in rat liver were prevented by honey and *Nigella sativa*, suggesting that honey and Nigella is a protective antioxidant against liver toxicity and an anti-tumor agent. Further pre-

clinical and clinical trials are warranted to characterize the inefficacy of honey and *Nigella* in combination with existing therapeutics for chemoprevention and chemotherapy of hepatocellular carcinoma.