Histological Studies on the Possible Protective Effect of Ginger Extract against Gasoline Exposure Induced Liver Toxicity in Adult Male Albino Rats

1Heba Nageh Gad ELHak, 2Mahmoud Ezzat Mohallal, 3Nour EL-Din Hussein Saleh, 4Hend Maarof Tag, 5Mohamed Salah EL-Deen Mahmoud EL-Naggar

1Assistant Lecturer
Zoology department
Faculty of Science, Suez Canal University, Ismailia, Egypt

Abstracts - Ginger (Zingiber officinale) is used traditionally for many therapeutic purposes. The aim of this study was to investigate the possible hepatoprotective role of ginger against leaded gasoline induced hepatotoxicity in rats. Sixty male adult albino rats (120-150 gm) were divided into 10 groups (n=6). Control group. Groups (2-5) inhaled leaded gasoline with nominal concentration 18.18 ppm for exposure times 3, 6, 9 and 12 hrs/days for 14 successive days. Group (6) orally received 100 mg/kg ginger per day for 14 days. Group (7-10) inhaled gasoline in same conditions and same exposure times of groups (2-5), in addition to orally receiving 100 mg/kg ginger during exposure for 14 days. After sacrificing, the liver of the rats was taken for histological preparation. The results of the present study on the liver showed that subchronic exposure to gasoline produced changes in some hepatocytes mainly hydropic degeneration, necrosis and occasional fatty change were seen, congestion of the central vein and infiltrations of the inflammatory cells in the portal area. It can be concluded that Ginger administration (100 mg/kg) showed mild hepatoprotective action against gasoline-induced histological liver damage in rats.

Keywords - leaded gasoline, ginger, liver, histology

I. INTRODUCTION
Petroleum vapors generated from increasing activities of petroleum and the related industries contribute an appreciable percentage of pollutants in the environment (Zahlsen et al., 1993). Gasoline vapors are released to the air during refueling of gasoline-powered vehicles, bulk transfer of gasoline at distribution terminals, leaks from storage containers, loading equipment, during removal and maintenance of underground storage tanks. Volatile hydrocarbons in gasoline spilled on soil or surface water will rapidly evaporate, contributing to air contamination (Van Gelder-Ottway, 1976; Phillips and Jones, 1978; Irving and Grumbles, 1979; McDermott and Vos, 1979; Kearney and Dunham, 1986; Shamsky and Samimi, 1987 and Kawai, et al. 1991). Hydrocarbons, other constituents of petroleum and the related products, like other xenobiotics, are metabolized primarily in the liver (Murray, 2003).

Plant derived products have been used for medical purposes for centuries. At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments. Also they are widely used in phytotherapy, which is using plants and their chemical constituents to eliminate certain health problems. Ginger obtained from the rhizome of Zingiber officinale (Family Zingiberaceae) has been used as a spice and in traditional medicine due to its characteristic pleasant flavor, spicy taste and health beneficial properties (Zorate, and Yeoman, 1996; Balladin et al., 1998; Bartley and Jacobs, 2000; Azian et al., 2004; Ravindran and Babu, 2004 and Young et al., 2005).

The aim of this study was to investigate the possible protective role of ginger on histological changes of the liver induced by gasoline exposure.

II. MATERIAL AND METHODS
Gasoline sample:
Gasoline was obtained from Misr petroleum station octane 95 (lead content 0.037mg/l) according to the company manuscript.

Preparation of aqueous extract of rhizome of Zingiber officinale
The ginger rhizome was purchased from a local store and then grounded in an electronic grinder. Ginger powder (100 mg/kg) were dissolved in the boiled water (5 ml) and their filtrates were administrated by oral gavaging using intragastric syringe (Fatehi-Hassanabad, et al. 2005).

Experimental Animals
For this study sixty male albino rats (weighting 120-150 gm) at the beginning of the study were divided into 10 groups (n=6). The rats were acclimated for 7 days prior to the exposure. Water and food were supplied ad libitum.
Group 1: control group. Groups (2-5): inhaled leaded gasoline with nominal concentration 18.18 ppm for exposure times 3, 6, 9 and 12 hrs/days for 14 successive days. Group (6): orally received 100 mg/kg ginger per day for 14 days. Group (7-10): inhaled gasoline in same conditions and same exposure times of groups (2-5), in addition to orally receiving 100 mg/kg ginger during exposure for 14 days.

Inhalation exposure of rats to gasoline and chamber operation (based on Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals No. 412).

Rats were housed individually in plastic cages within a 10 L and stainless steel wire mesh at the bottom and the top of the cage. At the top of the cage covered with piece of glass contain fans. Under this cage, the gasoline container of 10 L (18.18 ppm) was placed. Exposure chambers were operated dynamically in a laminar hood with volume (60 x 90 x 45) cm$^3$ at a calibrated airflow rate of approximately 550 L/min with a complete air change in per min. During inhalation through the chamber the flow is constant. Chamber size and airflow rates were adequate for an animal-loading factor below 5% and an oxygen level above 19%. Rats were exposed whole body concentration for nominal concentration 18.18 ppm and actual concentration is from 0.1 ppm to 17.8 ppm in exposure times 3, 6, 9 and 12 hrs/days for 14 days after $t_{99}$ times 30 sec. in a dynamic inhalation chamber. $T_{99}$ is the time which the chamber has reached 99% of its experimental concentration. During chamber operations, the airflow through the chamber was kept constant. The concentration is calculated by the actual volume/ rate of air. Animals did not receive food or water during the exposure period.

Histological preparation

After decapitation, rats were rapidly dissected, tissue of liver, were excised and cut into small pieces. Tissues of these organs fixed with (10%) neutral formalin for 24 hours, washed with distilled, transfer through two changes of 20 % chloral hydrate 24 hours each (Lhotka and Ferreira’s, 1949), washed in distilled water and then preserved in 70 % ethanol. The specimens were then dehydrated in ascending grades of ethyl alcohol, cleared in terpineol, then washed in benzene and embedded in pure paraffin wax. Serial transverse sections of liver were cut (at 5 microns thickness). Then were stained with Harris Hematoxylin and Eosin (Lillie and Fuller, 1976), cleared in Xylene and mounted in DPX. The sections of selected organs in the different groups were examined carefully and microphotographs were required.

Results:

Histological study:

A-the liver

The liver of control rat which exposed to air only (Fig 1-A) and that which received 100 mg/kg ginger for 14 successive days showed normal histological appearance (Fig 2-D).

Exposure of rat to 18.18 ppm gasoline in different exposure time (3 hr., 6 hr., 9 hr. and 12 hr.) for 14 successive days caused some histological changes in rat liver. Their portal areas are infiltrated with inflammatory cells and most of the hepatocytes are destructed (Fig 3-H). The central veins for the four exposure time are congested and dilated. The hepatocytes showing hydrobic degeneration (where the cells are enlarged and their nucleus become pyknotic or karyorrhexis) (Fig 1-B). It has been observed only the hepatocytes of one rat which exposed 3 hr. and another rat which exposed to 12 hr. gasoline for 14 successive days formed macrovesicular fatty degeneration which is represented by vacuoles or empty spaces in their cytoplasm and appearance of apoptotic cells (Fig 1-C).

Exposure of rats to 18.18 ppm gasoline in different exposure time (3 hr., 6hr., 9 hr. and 12 hr.) and received 100 mg/kg ginger for 14 successive days during exposure caused some histological changes in rat's liver. Their portal areas are infiltrated with inflammatory cells and destructed most of the hepatocytes around it (Fig 3-I) and their central veins are mild congested (Fig 2-E). Rats exposed to gasoline for 6, 9 and 12 hours showed focal necrosis (Fig 2-E and 2-F).
Figure 1: Liver section from a control rat showing hepatocytes (HC) radiating around the central vein (CV). The cells alternate with blood sinusoids (S) contain Van kupffer cells (K) (HX & E., 400X) (A). Liver section from a rat which exposed to 18.18 ppm gasoline 9 hour/day for 14 successive days showing severe hydrobic degeneration (HD) of the hepatocytes surround the congested central vein (CV) (HX & E., 360X) (B). Liver section from a rat which exposed to 18.18 ppm gasoline 3 hour/day for 14 successive days showing the hepatocytes forming macrovesicular fatty degeneration (FD) surround congested central vein (CV) (HX & E., 360X) (C).

Figure 2: Liver section from a rat which received 100 mg/kg ginger for 14 successive days showing normal hepatocytes (HC) radiating around the central vein (CV). The cells alternate with blood sinusoids (S) contain Van kupffer cells (K) (HX & E., 720X) (D). Liver section from a rat which exposed to 18.18 ppm gasoline 6 hour/day and received 100 mg/kg ginger for 14 successive days showing focal necrosis (N) increased near to the central vein (CV) (HX & E., 400 X) (E). Liver section from a rat which exposed to 18.18 ppm gasoline 9 hour/day and received 100 mg/kg ginger for 14 successive days showing increase of the focal necrosis (N) near to the central vein (CV) (HX & E., 400 X) (F).
III. DISCUSSION

The liver is one of the most important organs that performs high activity in metabolism and has a chief role in detoxification process and withdrawal of many toxic substances which enter the body (Yamazuki and LaRusso, 1988).

The liver of rat which exposed to 18.18 ppm gasoline in different exposure time (3 hr., 6 hr., 9 hr. and 12 hr.) for 14 successive days and rats which received ginger during exposure to gasoline showed inflammatory infiltration in the portal areas and most of their hepatocytes are destructed, the central veins were dilated and contain red blood cells. The hepatocytes of rat which exposed to gasoline only showed hydropic degeneration which are characterized by excess water accumulation inside the cells due to mitochondrial damage and hence decreased energy production which leads to the accumulation of sodium inside the cells and followed by entry of the water (Oudea et al., 1973 and fatty degeneration appeared to hepatocytes of rats which exposed to gasoline, these degenerative changes didn’t appeared to the hepatocytes of rats which received ginger during exposure to gasoline. these results is agreement with the previous finding of Uboh et al., (2005) found that exposure of upgraded concentrations of petrol fumes in albino Wistar rats for 4 days daily for two weeks induced degenerative changes in the integrity of the hepatic cells; Janker and El-Nouri, (2009) noticed that administration orally of lead acetate to Albino mice for two and four weeks induced hydropic degeneration, fatty degeneration, necrosis in some hepatocytes, congestion within central veins, hemorrhage between hepatic cords and infiltrations of inflammatory cells to the liver cells; Uboh et al., (2010) found that the exposure of upgraded concentrations of gasoline to male rats for 6 hours/ 5 days per week for 10 weeks induced degenerative changes in the integrity of the hepatic cells; Patrick–Anyawu et al., (2011) who noticed that chronic exposure of Wistar albino rat with petrol contaminated diet induced significant degenerative changes in the structural integrity of the hepatic cells and also Jarrer and Tiab, (2012) showed that chronic exposure Wistar albino rats to lead acetate trihydrate altered in the hepatocytes were mainly anisokaryosis, nuclear vesiculation, binucleation, cytoplasmic inclusions, cytoplasmic swelling, hydropic degeneration, necrosis and reduction in glycogen content in addition portal area mild infiltrated with inflammatory cells, kupffer cells hyperplasia and occasionally fatty change were found.

Focal necrosis appeared in some hepatocytes of rats liver treated with ginger during exposure to gasoline. This result is similar with finding by Amer et al., 2013 that the liver of mice which received 100 mg/kg of ginger caused also focal necrosis. Ritter, (1977) reported that liver cell necrosis may be due to inhibition of synthesis of DNA needed for the growth and maturation of the liver. In the other hand, Mannem, (2014) noticed that ginger with dose (200 and 300 mg/kg) treated male rats received lead acetate for eight week attenuated the histological alterations to the liver tissues induced by the lead acetate.

Johar et al., (2004) suggested that lead could interact with proteins and enzymes of the hepatic interstitial tissue interfering with the antioxidant defense mechanism and leading to reactive oxygen species generation which in turn may initiate an inflammatory response. Also Bucci, (1991) suggested that congestion of the central vein may be due to failure of the heart which produces changes in different organs via two ways. Firstly, excessive blood in venous system increases blood pressure in the
veins and capillaries which may exert undue pressure on the neighboring structures. Secondly, this is usually accompanied by a corresponding reduced arterial blood.

**IV. CONCLUSION**

Ginger cannot be considered as a protective against histological changes induced by exposure to the leaded gasoline to the liver cells. It only decrease the histological alterations induced by leaded gasoline and didn’t return completely to the control pattern especially in short time exposure to gasoline.

**REFERENCES**


