Biochemical and histological studies on the possible protective impact of the herb basil (Ocimum basilicum) on adriamycin induced toxicity in rats. I. Influence on the liver.

Bayomy* M.F.F., Sakr, S.A., Gendia S.E. M.
Zoology Department & Faculty of Science Menoufia University, Shebin El-Kom, Egypt

(* Corresponding author e mail. mffbayomy@yahoo.com)

Abstract

The purpose of this work was to evaluate the effect of basil (Ocimum basilicum) against hepatotoxicity induced in albino rats by the anticancer drug adriamycin (ADR). The biochemical results showed that adriamycin caused significant elevation in serum ALT (Alanine aminotransferase) and AST (Aspartate aminotransferase) enzymes after 4, 6 and 8 weeks of treatment. It also caused an increase in malondialdehyde (lipid peroxidation marker) and decrease in activities of the antioxidant enzymes, superoxide dismutase and catalase. This drug has resulted in various histological changes in the liver. These changes include impairment of the normal structural organization of the hepatic lobules, congestion and dilatation of blood vessels, cytoplasmic vacuolization of the hepatocytes, leucocytic infiltrations and fatty infiltration. Treating animals with ADR and basil (Ocimum basilicum) led to an improvement in both biochemical and histological changes induced by ADR. There are significant decreases in ALT and AST activity. Moreover, Ocimum basilicum reduced the level of malondialdehyde and increased the activity of superoxide dismutase and catalase. In conclusion the results of the present work indicated that Ocimum basilicum had a protective effect against liver damage induced by adriamycin and this is due to antioxidant activities of some substances found in water extract of Ocimum basilicum.

Keywords: basil, adriamycin, liver, aminotransferases, antioxidant enzyme, histology.

1 Introduction

Adriamycin (Doxorubicin) is a drug used in the treatment course of cancer (Rizk et al., 2014). It is obtained from a bacterial species (Streptomyces peucetius) and it was introduced in 1969 for tumour treatment. It is an anthracycline antitumor antibiotic and has a highly effective role in many human tumours including certain types of bladder, lung, breast, ovarian and stomach cancer (Ogawa, 1985), Hodgkin's lymphoma (Hodgkin's disease), non-Hodgkin's lymphoma and acute leukemia (Blum and Carter, 1974). Its use is limited due to the development of hepatotoxicity, nephrotoxicity and cardiotoxicity (Tallaj et al, 2005). Hepatotoxicity is one of the main side effects associated with ADR treatment. Extensive investigations have been conducted on the hepatotoxicity as well as general organ toxicity of adriamycin (Deepa and Varalakshmi, 2003; El-Sayyad et al., 2009).

Human beings have probably used medicinal plants for thousands of years for cure of their various ailments and still continue to do so (Hill, 1989). These natural products have many biologic and pharmacologic properties (Hosseinimehr, 2014). Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs (Ogbera et al., 2010). Basil or sweet basil (Ocimum basilicum) is an important medicinal plant and a culinary herb widely cultivated in many countries that contains several antioxidants compounds and considered as a major volatile oil crop (Grayer et al., 1996). This plant is belonging to the family Lamiaceae (Ghosh, 1995). It is widely used in folk medicine to treat a wide range of diseases due to its numerous pharmacological activities. Many studies have reported that basil leaf extracts have potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties (Akujobi et al., 2004; Chiang et al., 2005; Bozin et al., 2006; Manosroi et al., 2006; Almeida et al., 2007). The present study was conducted to reveal the influence of Ocimum basilicum on the hepatotoxicity of the anticancer drug, adriamycin in albino rats.
2 Materials and Methods

Chemicals
Adriamycin (Doxorubicin): Doxorubicin (Adriablastina produced by Carlo Erba) was purchased from a local pharmacy in the form of 10 mg/ampoule.

Basil extract: A fresh leaves of Basil (Ocimum basilicum) was collected from the garden in Faculty of Science - Menoufia University, Shebin El- Kom, Egypt. The leaves were rinsed with clean water to remove any foreign matter. Leaves were blended with distilled water. The mixture was strained, the merc pressed and the mixture was filtrated using filter paper. The aqueous extract was used at a dose level of 20 ml/kg Ocimum basilicum (Offiah and Chikwendo, 1999).

Animals and treatments
Healthy adult male albino rats (Rattus norvegicus) weighing 120 ± 5 g were used. They were obtained from the breeding center of experimental animals, Helwan, Egypt. Animals were kept in the laboratory under constant temperature (24±2°C) for at least one week before and throughout the experimental work. They were maintained on a standard diet composed of 55% corn starch, 20% casein, 15% corn oil, 5% salt mixture and 5% vitaminized starch (Egyptian Company of Oils and Soap Kafr El Zayat, Egypt). Water was available ad libitum. All the experiments were done in compliance with the guide for the care and use of laboratory animals (National Research Council, 1985).

Animals were divided into 4 groups:
Group 1: These animals (20 rats) were served as normal controls
Group 2: These animals (20 rats) were treated with oral aqueous O. basilicum extract at a dose level of 20 mg/kg for 5 days/wk through 8 weeks.
Group 3: Animals of this group (20 rats) were injected intraperitoneally with ADR at a dose level of 2 mg/kg body weight in sterile saline, once per week for 8 weeks.
Group 4: Animals of this group (20 rats) treated with ADR at the same dose level as those of group 3 followed by oral administration aqueous O. basilicum extract at a dose level of 20 mg/kg for 5 days/wk through 8 weeks.

Biochemical assays
For biochemical assays blood was collected and centrifuged at 3000 rpm for 10 minutes and stored at - 20 °C. Liver function enzymes, ALT and AST, were determined in serum according to the method of Gella et al. (1985). malondialdehyde was determined by the method of Ohkawa et al. (1979) and superoxide dismutase was assayed using the method of Rest and Spitznagel (1977). Catalase activity in the sera was determined according to the method of Aebi et al. (1974).

Histological preparations
Immediately after decapitation, the animals were dissected, their livers were removed from treated and control animal groups and fixed in 10% formalin. After fixation, the specimens were dehydrated using an ascending series of alcohol, cleared in 2 changes of xylene, and embedded in molten paraffin (melting point: 50-58°C). Sections of 5 mm thickness were cut using rotary microtome and mounted on clean slides. For histopathological examination, the sections were stained with Ehrlich hematoxylin and counter stained with eosin.

Statistical analysis
The data were expressed as mean ± standard error. Data were analyzed using Student’s t-test and homogeneity of variances (Levene test) using statistical program of social science (SPSS) software for windows. P < 0.05, P < 0.01 and P < 0.001 values were used.

3 Results
Change in total body weight
Data in (Fig. 1) showed that treating animals with ADR for 8 weeks caused significant decrease in body weight of rats compared with control group. On the other hand, administration of O. basilicum and ADR caused significant increase in body weight of rats compared with ADR group. When O. basilicum was given to animals, insignificant change in body weight was recorded.

Biochemical results
Treatment with ADR for 8 weeks caused a highly significant elevation (P < 0.001) in the activity of ALT and AST as compared to those of the control animals. On the other hand, treatment with ADR and O. basilicum extract showed a reduction in the activity of these two enzymes (Figs. 2, 3). Both control and animals given O. basilicum showed no significant differences in serum activity of ALT and AST. Lipid peroxidation marker (MDA) increased significantly in ADR–treated animals when compared to the control group. Animals treated with ADR and O. basilicum showed a significant decrease in MDA when compared with ADR group (Fig. 4). Data expressed in (Fig. 5) and (Fig. 6) show that there are no significant differences in levels of SOD and CAT in both control and O.basilicum extract groups. Animals treated with ADR revealed that levels of SOD and CAT decreased significantly (P < 0.001) after 4, 6 and 8 weeks of treatment. On the other hand, treating animals with ADR and O. basilicum extract led to an increase in these antioxidant enzymes. This increase was highly significant (P < 0.001) after 8 weeks of treatments.
Histological results

Liver of control rat showed normal lobular architecture. The hepatic cells were found arranged in strands around the central vein and sinusoids appeared containing Kupffer cells (Fig. 7). Liver obtained from rats treated with basil extract exhibited the normal structure (Fig. 8). Whereas animals treated with ADR for 4 weeks revealed that the hepatic tissue was injured and the hepatic blood vessels were enlarged and congested (Fig. 9). After 6 weeks, the hepatic tissue lost its normal organization and Most of the hepatocytes showed cytoplasmic vacuolation with pyknotic nuclei (Fig. 10). After 8 weeks of treatment, these changes became intensive. The hepatic architecture was lost, clearly fatty infiltrations and masses of leukocytic infiltration were observed (Fig. 11, 12). After treatment with both ADR and *O. basilicum*, an improvement was recorded in the hepatic tissue. In these specimens, the hepatocytes appeared normal with an increase of binucleated cells (Fig. 13).

Fig. 2: Effect of Adriamycin and/or basil on serum ALT.

Fig. 3: Effect of Adriamycin and/or basil on serum AST.

Fig. 4: Effect of Adriamycin and/or basil on serum MDA

Fig. 5: Effect of Adriamycin and/or basil on serum SOD

Fig. 6: Effect of Adriamycin and/or basil on serum CAT

Fig. 7: Section in the liver of a control rat showing the basic structure: central vein (CV), hepatocytes (H), blood sinusoids (S) and Kupffer cells (K) (X400).

Fig. 8: Section in the liver of a rat treated with basil extract showing the hepatic architecture appeared normal, where the hepatic cells (H) arranged around central vein (CV) in
cords, Blood sinusoids (S) and Kupffer cells (K) were also observed (X400).

Fig. 9: Section in the liver of a rat treated with adriamycin for 4 weeks showing congested and dilated blood vessel (arrow), disruption in hepatocytes architecture with pyknotic nuclei (P) (X400).

Fig. 10: Section in the liver of a rat treated with adriamycin for 6 weeks showing loss of the characteristic hepatic architecture, cytoplasmic vaculation of the hepatocytes (C) and pyknotic nuclei (P) (X400).

Fig. 11: Section in the liver of a rat treated with adriamycin for 8 weeks showing loss of the characteristic hepatic architecture and fatty infiltration (F). (X400).

Fig. 12: Section in the liver of a rat treated with adriamycin for 8 weeks showing congested and enlarged portal vein (PV), enlarged and proliferated bile ductule (BD) and leucocytic infiltration (Li).

Fig. 13: Section in the liver of a rat treated with ADR and O. basilicum showing advanced degree of improvement, restoration of structure of the liver tissue and binucleated cell (Bi) is observed (X400).

4 Discussion

Adriamycin is a very important agent in the treatment of cancer patients although its use may be complicated by the presence of acute and chronic side effects. It has been shown that free radicals are involved in adriamycin - induced toxicities (Yagmurca et al., 2004). It has been reported that adriamycin caused severe damage in some organs like liver, testes, heart and kidneys (Shivakumar et al., 2012). Adriamycin was found to cause the generation of free radicals and the induction of oxidative stress that correlates with cellular injury (Saad et al., 2001). The present results revealed that ADR exerted deleterious action on the liver of rats both biochemically and histologically. Among the most sensitive and widely used liver enzymes are the aminotransferases. They include alanine aminotransferase (ALT) and aspartate aminotransferase (AST). These enzymes are normally predominantly contained within liver cells and to a lesser degree in the muscle cells. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, raising the AST and ALT enzyme blood levels and signaling liver disease. Adriamycin was found to affect serum transaminases (ALT, AST). Significant increase in ALT and AST levels of sera of ADR treated rats was recorded in the present study. In agreement with this result Roomi et al. (2014) found that administration of ADR to old male BALB/c mice resulted in a marked increase of hepatic ALT and AST. Several investigators also obtained similar results. In this concern, Ito et al. (2001) observed increase in the levels of ALT and AST in the serum of adriamycin administered rats. Similarly, Guo et al. (2016) reported that administration of ADR at dose of 20 mg/kg increases the levels of ALT and AST. Swamy et al. (2011) proved that administration of ADR for two weeks elevated the activity of serum marker enzymes ALT and AST. A high lipid peroxidation with a decrease in the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were recorded in liver of adriamycin-treated rats. Similarly, Swamy et al. (2012) observed that administration of ADR increased the MDA levels and decreased SOD and CAT.
levels compared to the level of the normal group. Also, a study investigated that male Wister rats given a single i.p. (intraperitonal) injection of 15mg/kg/body weight of ADR caused increased the MDA levels and decreased SOD and CAT levels (Al-Sowayan and Mahmoud, 2014). Koti et al. (2009) recorded an increase in the levels of MDA and decrease in the levels of SOD and CAT after administration ADR. Many histological alterations were observed in liver of ADR treated rats. The most marked signs of tissue impairment were congestion of blood vessels, leucocytic infiltrations, cytoplasmic vaculation of the hepatocytes and fatty infiltrations. In agreement with these results, Gokcimen et al. (2007) reported that ADR increased mononuclear cells infiltration, congestion of blood vessels and necrosis. Marked changes in liver of male albino rats such as congestion of blood vessels, leucocytic infiltrations and fatty infiltration after treatment with ADR was observed by Sakr and Abo-El-Yazid (2012). Abbas (2011) noticed changes in hepatic tissue including inflammatory cell infiltration, necrosis and vacuoluation after ADR administration. Also, Taher et al. (2013) revealed that higher doses of ADR caused massive hepatotoxicity including dissolution of the hepatic cords, focal inflammation, apoptosis and necrosis of the hepatic tissues with fibrosis around the portal area. Lower doses exhibited abnormal changes including vacuolation of the hepatocytes with widening of the sinusoidal capillaries in addition to congestion and vasodilatation of the central veins.

The present findings demonstrated that O. basilicum improves the biochemical and histological changes induced in the liver by ADR. This indicated the effectiveness of O. basilicum in prevention of ADR hepatotoxicity. In agreement with these observations the hepatoprotective effects of O. basilicum have been shown in many studies on experimental liver damage. Yamamoto et al. (2005) proved that O. basilicum suppressed hepatic fibrosis and protected liver against parenchymal damage induced by CCl4. The aqueous extract of Ocimum americanum leaves at doses of 200 and 400 mg /kg p.o. has significant hepatoprotective ability against paracetamol – induced hepatic damage in rats. The levels of some serum biochemical parameters such as ALT and AST in rats intoxicated with paracetamol alone had their serum AST and ALT levels significantly increased (Bayomy and Salah Eldin, 2015). The pretreatment of rats with aqueous extract of O. americanum leaves caused a significant decrease in the serum levels of ALT and AST (Aluko et al., 2013). Oral doses of basil extract of 200 mg/kg b.w. significantly decreased the higher levels of serum of ALT and AST induced by CCl4 (Yacout et al., 2012). Lahon and Das (2011) reported that O. sanctum have hepatoprotective effect against paracetamol toxicity in rats. The hepatoprotective effects of O. basilicum have been shown in studies on experimental liver damage. Sakr et al. (2011) reported that treating animals with CCl4 and aqueous leaf extracts of O. basilica led to an improvement, in both histopathological and biochemical alterations induced by CCl4. A study by Arghohro et al. (2012) indicated that aqueous leaf extracts of O. gratissimum has anti-hepatotoxic action against cisplatin induced hepatic toxicity in rats. The ameliorating effect was further evident through marked decrease in the activities of the marker enzymes ALT and AST and decreased histopathological alterations of liver tissues. Mahboub and Arisha (2015) showed that treating rats with diazinon and O. basilicum extract improved the histological structure of the liver and caused significant decrease of ALT and AST. These results suggested that O. basilicum protects the hepatocytes from injuries and improves liver function. Rasekh et al. (2012) decided the safety of Ocimum basilicum administration and found that microscopic examination of liver in control and treated animals with O. basilicum showed no toxic effects on this organ and supported safety of O. basilicum data was inferred from biochemical parameters. Chinnasamy et al., (2007) reported that the protective action of ocimum was attributed to its antioxidant action. They added that this protection may be also due to the anti-inflammatory property of ocimum which reduces formation, release, and activity of inflammatory mediators such as cytokines, histamine, prostaglandins, and leukotrienes. Ocimum treatment was found to exhibit hepatoprotective effect as recorded by enhanced activity of the antioxidant enzyme, SOD and CAT and diminished amount of lipid peroxidation against the adriamycin-induced hepatotoxicity in rats. These results are in agreement with other studies which proved the antioxidant effects of O. basilicum extract. Sakr and Abdel samie (2015) reported that lipid peroxidation marker, MDA, was increased while the antioxidant enzymes namely SOD and CAT were decreased after Diazinon treatment. Treating animals with diazinon and O. basilicum extract caused a reduction of MDA level and elevation of both SOD and CAT activity. Kath and Gupta (2006) showed that hydroalcoholic extract of ocimum sanctum leaves lowered levels of MDA and increased levels of SOD in animal models of peptic ulcer. Ramesh and Satakopan (2010) reported that administration of Ocimum sanctum extract before and after cadmium intoxication resulted in a significant decrease in lipid peroxidation (LPO) levels and significant increase in SOD and CAT levels. Therefore, the results of the present work indicate that O. basilicum has protective effect against hepatotoxicity induced by adriamycin and this is may be attributed to its antioxidant activities of some substances found in water extract of Ocimum basilicum.

5 References


