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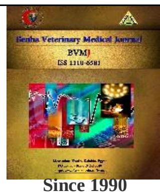

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Original Paper

The possible impacts of rosemary and hops ethanolic extracts on hepatocellular carcinoma experimentally induced in rats

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ABSTRACT

Hepatocellular carcinoma (HCC) constitutes a major health hazard in Egypt and globally for its high prevalence. This study is designed to evaluate the anti-carcinogenic effect of rosemary and hops on hepatocellular carcinoma experimentally induced in rats using cisplatin as standard chemotherapy. Fifty rats were randomly allocated into five equal groups: control group: didn't receive any treatment; groups (2-5) received three doses of diethyl nitrosamine (DEN) 200 mg/kg b.wt. by intraperitoneal injection with two weeks intervals then DEN + cisplatin group injected intraperitoneally with 1.5 mg/kg b. wt. Cisplatin; DEN + rosemary group orally administrated 200 mg/kg b.wt. rosemary ethanolic extract; DEN + hops group administrated 150 mg/kg b.wt. hops ethanolic extract orally. Groups 3-5 received their treatments for 4 weeks. Blood samples were collected at the end of the 10th week of experiment for biochemical evaluation of hepatic functions. Results revealed that DEN markedly increased alpha-fetoprotein (AFP) and all hepatic enzymes activities evaluated and decreased serum albumin and total protein. On the other hand, cisplatin, rosemary, and hops mitigated to a large extent all the biochemical alterations caused by DEN in different degrees. Notably, rosemary extract was more effective in preserving hepatic function than hops when compared to cisplatin. Our results indicate that rosemary and hops may have anti-carcinogenic potentials compared to cisplatin which provides natural, safe, feasible alternative to chemotherapy.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) prevalence is among the highest in the globe. Up to 90% of liver tumors are this type. Hepatocellular carcinoma is a noteworthy public health concern in Egypt as it is responsible for 33.63% of males and 13.54%, in females, of all malignancies. It has a poor prognosis after discovery. Hepatitis C and B, as well as alcoholic and nonalcoholic cirrhosis, are frequently associated with hepatocellular cancer. This was closely related to the hepatitis C virus epidemic, which had afflicted 10 and 15 % of Egyptian society during the previous three decades (Elghazaly et al., 2018).

Liver is the body's most vital organ. The liver's primary role is to remove harmful chemicals from the body. When toxins accumulate and interfere with the liver's ability to metabolize them, hepatic injury can result (Bigoniya et al., 2009; Bhakuni et al., 2016). The most frequent malignant tumor of the hepatobiliary system is HCC (Sung et al., 2021). Diethyl nitrosamine (DEN) is a substantial health hazard substance that generates reactive oxygen species (ROS) leading to oxidative stress and causes a perturbation in nucleic acid repair mechanisms (Khan et al., 2017).

Cisplatin, cis-diamminedichloro-platinum (II), is a well-known antineoplastic medication used to treat various human malignancies, including those of the bladder, head, and neck, ovary, lung, and testicles. It works well against a number of cancer types, including sarcomas, lymphomas, germ-cell tumors, and carcinomas (Dasari and Tchounwou, 2014). As one of the anti-cancer drugs, cisplatin may injure healthy cells; therefore, scientists search for safer and more effective strategies to minimize the unintentional side effects of chemotherapy.

Rosemary (*Rosmarinus officinalis*) is one of the *Lamiaceae* (*Labiatae*) family is a famous aromatic plant. It is used in processing food for enhancing flavor in addition to using it in medicinal purposes (Bilto and Alabdallat, 2015). Rosemary leaves have a variety of bioactivities, including anti-cancer, anti-inflammatory, antioxidant, anti-headache, and anti-fibrotic effects (Said et al., 2019).They have flavonoid and polyphenolic compounds. Flavonoids belongs to the three subclasses (flavones, flavonols, and flavanones) are identified in rosemary products (Lopez-Novoa et al., 2011). Rosemary is highly effective at scavenging free radicals such as nitrogen species also reactive oxygen (Azab and Albasha, 2015).

Hops are the flowers of *Humulus lupulus* (Liu et al., 2015). Hops are used in herbal drugs in a way similar to valerian for treating anxiety, agitation, and insomnia. It also possesses anti-bacterial, antifungal, antibiotic, antioxidant,

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wound healing, and antiviral activities (Karabín et al., 2016).

The purpose of the current study is to evaluate the potential impact of rosemary and hops on experimental model of HCC using cisplatin as a standard chemotherapeutic drug.

2. MATERIAL AND METHODS

Ethical statement

The experiment was conducted at Laboratory of Biochemistry and Molecular Biology Department, Faculty of Veterinary Medicine, Benha University. The Veterinary Medicine Faculty Ethical Committee approved all protocols used in this inquiry (BUFVTM 23-09-22.).

2.1. Experimental animals:

In our work, fifty male albino rats (aged 4-5 weeks) with average body weights of 100–120 g were provided by Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University. Animals were housed in separate metal cages with a 12-hours cycle of light and darkness, excellent ventilation, and humidity. All rats were kept for 7-days for acclimatization before the experiment. The rats were given a diet of standard pellets and were given unlimited access to clean, fresh drinking water.

2.2. Chemicals and drug

Diethyl nitrosamine ((DEN) 1 gm/1ml vial) was purchased from Sigma Chem. Co. (St. Louis, U.S.A). Cisplatin (Cisplatin®; 50 mg/50ml vial) was obtained from Pfizer, Egypt. Rosemary and hops were purchased from El-Captain Co for extracting natural oils, herbs, and cosmetics; El-Obour City, Cairo, Egypt.

2.3. Preparation of plant ethanolic extract

The two plants were crushed into powder. Each powder was combined with ethanol in a sealed container, and the mixtures were left to stand for three days at room temperature with intermittent vigorous shaking. The mixture underwent filtration to produce liquid extract. A rotary evaporator was used to evaporate the alcohol and concentrate the extract at 50 °C and low pressure. These procedures were repeat three times to extract nearly all the constituents from each plant. The extract was then weighed and kept at -20 °C until use (Said et al., 2019).

2.4. Experimental design

In the first six weeks, rats were divided into two main groups. Control group (10 rats) received no treatment, and HCC group (40 rats) received three doses of diethyl nitrosamine (DEN) 200 mg/kg b.wt. by intraperitoneal injection in normal saline with two weeks intervals (Khan et al., 2011). Then for the next four weeks of our experiment, the HCC group was subdivided into 4 subgroups, each containing 10 rats. DEN group: didn't receive any treatment; DEN + cisplatin group injected intraperitoneally with 1.5 mg/kg b. wt. Cisplatin (Abass et al., 2018); DEN + rosemary group orally administrated 200 mg/kg b.wt. rosemary ethanolic extract (Alnahdi, 2012); DEN + hops group administrated 150 mg/kg b.wt. hops ethanolic extract orally (Khakpoor et al., 2004).

2.5. Sampling

After overnight fasting, blood samples from all animal groups were drawn from the retro-orbital venous plexus and placed in sterilized tubes to clot for 30 minutes. The serum was separated by centrifugation for 15 minutes within 1267 *g*. The parameters that were determined in serum were ALT and AST activities (Schumann et al., 2002), Albumin (Doumas et al., 1971), Total protein (Koller and Kaplan, 1984), AFP level (Engall, 1980), ALP activity (Tietz et al., 1983), LDH activity (Pesce, 1984), and GGT activity (Szasz, 1969).

2.6. Statistical analysis:

One-way analysis of variance (ANOVA) was used to examine the collected data, and Duncan was used as the post-hoc test. The Windows version of SPSS 25 (SPSS Inc., Chicago, USA) was used to conduct the analysis. Results were presented as the mean ± standard error (SEM). At P values < 0.05, the data were statistically significant.

3. RESULTS

The intraperitoneal injection of DEN exhibited a significant increase in the level of AFP and activities of all liver enzymes under investigation when compared with those of control (Figure 1&2).

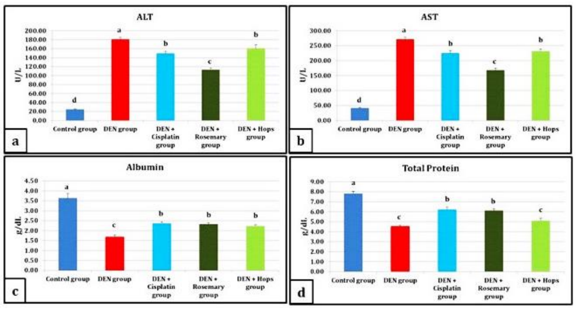

Figure 1: Effect of cisplatin, rosemary and hops on liver enzymes, albumin and total protein in DEN-administered rats.
a: ALT Activity (U/L); b: AST Activity (U/L); c: Serum Albumin level (g/dL); d: Serum Total protein level (g/dL).

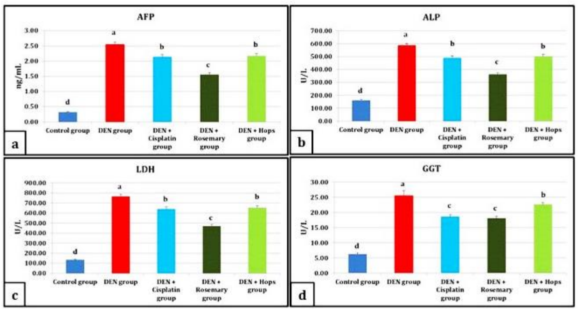

Figure 2: Effect of cisplatin, rosemary and hops on tumor markers in DEN-administered rats.
a: AFP level (ng/mL); b: ALP ctivity (U/L); c: LDH Activity (U/L); d: GGT Activity (U/L).

Comparing to control, serum albumin and total protein were found to be decreased significantly (Figure 1 c&d). On the other hand, the administration of cisplatin significantly reduced all the elevated levels of AFP (15.9%) and hepatic enzymes (ALT: 17.4%; AST: 16.9%; ALP: 16.3%; LDH: 16.2%; GGT: 27.4%) toward control and improved the lowered albumin and total protein. Moreover, rosemary extract showed considerably lowered enzyme activities (ALT: 37.3%; AST: 37.8%; ALP: 38.2%; LDH: 38.3%; GGT: 29.4%) and AFP (38.7%) level and increased albumin and total protein levels than that of DEN group. Notably, rosemary administration significantly decreased AFP level (Figure 2a), and all hepatic enzymes under

investigation except GGT (Figure 2d) showed a non-significant decrease.
Additionally, serum albumin and total protein levels showed non-significant change compared to the group treated with cisplatin (Figure 1 c&d). Meanwhile, rats treated with hops extract exhibited a statistically significant decrease in the activities of (ALT: 11.0%; AST: 14.2%; ALP: 14.4%; LDH: 14.4%; GGT: 11.6%) and AFP (14.6%) with significant increase in serum albumin and total protein compared to DEN group (Figure 1 c&d). Compared with the group treated with cisplatin, hops showed non-significant changes in almost all tested parameters except a significant decrease in total protein (Figure 1d) and a significant elevation in GGT activity (Figure 2d).

4. DISCUSSION

Hepatocellular carcinoma is one of the most severe tumors in the world, causing death of approximately a million people annually (Islam et al., 2019). Rapid infiltration growth, early-stage metastasis, high-grade malignancy, and poor treatment efficacy define HCC a complex illness. It happens in stages, starting with changes at the early stages, then progressing to malignant transformation through promotion and development (Hassan et al., 2015).
An increase in transaminases (ALT and AST) was the most obvious indicator of cell membrane leakage and loss of functional integrity (Al-Rejaie et al., 2009). Our results showed that these hepatic enzyme activities significantly increased in response to DEN injection (Figure 1 a&b), indicating hepatocellular damage. The GGT is an enzyme mostly located in the canalicular area of the hepatocyte plasma membrane. One of the most accurate indications of liver injury was thought to be the serum GGT activity. This enzyme's release into the serum is a sign that the liver and hepatic cells have been damaged (Kadasa et al., 2015). However, the substantial bile duct obstruction, intrahepatic cholestasis, and infiltrative liver disorders, especially due to abnormalities in the permeability of cells, could be blamed for the increase in ALP activity in the DEN group (Lou et al., 2016).
An important blood marker for detecting HCC is AFP. It serves as an immunosuppressive mediator, inhibits cell death, and encourages cell proliferation (Mizejewski, 2013). Jagan et al. (2008) observed that rats treated with DEN had significantly higher levels of AFP than the control group (Figure 2a), which is consistent with our findings. Additionally, the liver's functional and excretory capacities can be determined using the serum level of albumin. As a result, changes in this serum markers' levels indicate the severity of hepatic disease (Kadasa et al., 2015). According to prior research (Ding et al., 2017), DEN has a persistent hepatotoxic effect that has been linked to cancer development in rats. This significant disturbance in the investigated biochemical parameters was attributable to this effect.
Cisplatin has cytotoxic activity through the creation of DNA adducts. Further replication is stopped in such a large way (El Miniawy et al., 2017). Comparing cisplatin treated and untreated HCC rats, the increased AFP level somewhat decreased, which could be attributed to its anti-cancer activities. This outcome was consistent with earlier findings (Niu et al., 2017). Our experiment observed a partial alleviation in biochemical disturbances induced by DEN after post-treatment with cisplatin. These improvements could be attributed to the cytotoxic effect of cisplatin on the altered hepatic tissue preventing its uncontrolled proliferation. This effect preserves to an extent the integrity of hepatic cellular membrane.
The current study's findings demonstrated that administering rosemary minimized to a large extent, most, if not all, the biochemical alterations caused by DEN. These results concurred with those of Xiang et al. (2013), who claimed that carnosic acid prevented liver damage, as shown by a decrease in the levels of enzymes related to liver functions in the serum. According to Rašković et al. (2014), the fundamental reason why liver injury results in a rise in AST and ALT activity is because these enzymes leak out of injured hepatocytes and into the bloodstream. These hepatotoxic markers were reduced by administration with rosemary ethanolic extract. They attributed this reduction to the active ingredients of rosemary, particularly carnosic acid, have been shown to have many effects as anti-inflammatory, anti-free radical, and muscle relaxant that also has positive effects on avoiding hepatotoxicity by minimizing the extent of lipid peroxidation and resulting cell membrane damage. Additionally, treatment of the active component of rosemary, rosmarinic acid, stimulated hepatocyte proliferation, suggesting that rosmarinic acid may be helpful in promoting liver regeneration (Lou et al., 2016). Adopting the same principle, Said et al. (2019) concluded that rosemary protective efficacy against thioacetamide-induced hepatic encephalopathy to its constituents.
Regarding hops, its flavonoids have shown in the past that they can scavenge reactive nitrogen and oxygen species by preventing neuronal nitric oxide synthase from functioning, enhancing renal and liver functioning (Stevens et al., 2002). Prenylated flavonoids found in hops have been demonstrated to have strong immunotherapy, in addition to cytotoxic effects on human tumor cell lines (Plazar et al., 2008). Examples of these compounds are isoxanthohumol and xanthohumol. Inhibiting metabolic activation has been proposed as one of their defense mechanisms in addition to activation of antioxidant enzymes and detoxification enzymes. Consequently, xanthohumol displayed the maximum activity in both singlet oxygen absorbance capacity and total oxygen radical absorbance capacity (Yamaguchi et al., 2009). Additionally, a group of bitter chemicals called hop bitter acids, which are generated from the hops have been shown to have a variety of impacts both *in vitro* and *in vivo*. By reducing cell growth and angiogenesis, causing apoptosis, and upregulating the cytochrome P450 detoxification enzyme, they may have anti-cancer properties (Van Cleemput et al., 2009).
Relying on these previous studies on rosemary and hops, we can explain our results by the constituents of these herbs. Moreover, the notable and moderate ameliorating potency of rosemary and hops, respectively, that overcomes the alterations in most parameters when compared with DEN + cisplatin may be related to the active ingredients and their concentration in both herbs.

5. CONCLUSION

The current findings confirm that rosemary and hops can alter biochemical disturbances caused by DEN toward control. Their effects may be attributed to their content of active constituents that have antioxidant and anti-inflammatory characteristics, which require further investigation on the possible mechanisms by which rosemary and hops exert their action.

CONFLICT OF INTEREST
No conflicts of interest exist, according to the authors.

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