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Role of *Sepia* sp. Extract Against Toxicity Induced By Benzene in Male Rats

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Abstract:

Benzene is a highly toxic chemical agent, therefore, the present study was carried out to investigate the protective effects of cephalopoda extract (*Sepia* sp.) against benzene toxicity in male white albino rats. Twenty four white male rats were used, these animals were divided into four groups, each group contain six animals as a following: the first group (control group) treated orally with normal saline (1 ml/ kg^{-1}) for two weeks, the second group (benzene group) injected I.P. with benzene 0.5 ml/kg at first day of each week for two week the third group treated intraperitoneally (I.P.) with *Sepia* sp. extract (720 µg/ rat) for two week, , and the fourth group (benzene + extract) injected I.P. with benzene 0.5 ml/kg at first day of each week for two week, then injected I.P. with extract 720 µg/animals for two weeks. The result indicated non-significant increase in the level of the AST, and a significant increase in levels of ALT, cholesterol, triglyceride, LDL, VLDL of male rats treated with benzene compared with control group. Also, there was a significant decreased in HDL in the second group compared with the control group. Protective activity of *Sepia* sp. extract against toxicity of benzene observed in decreasing of ALT, AST, cholesterol and triglyceride, LDL and VLDL levels, and increased in the HDL level.

Keywords: *Sepia* sp., Benzene, Liver enzymes, Lipid profile.

Introduction:

Benzene, a toxic aromatic hydrocarbon can alter the microanatomy and physiology of different organs (Ozturk *et al*., 1997). Benzene, is one of carcinogenic substances to which gasoline station workers are most likely exposed via inhalation (Tunsaringkarn *et al*., 2012). It's widely used in different industries like rubber, drugs, detergents etc. (Fatima *et al*., 2004). A large number of industrial workers from petroleum, rubber, paint, shoe making, printing, solvent and other chemical industries are occupationally exposed to benzene (Sul *et al*., 2005; Tompa *et al*., 2005). Moreover, the individuals working at the petrol filling stations, tanker crew, motor mechanic and traffic policemen are also at a potential risk to benzene exposure occupationally (Verma *et al*., 2003; Carrieri *et al*., 2006). People working in those areas where

benzene is present in high concentration may suffer from some metabolic disorders. It can creates cancer also (Kohn *et al*., 2005). However, we expose to benzene through auto-exhaust and other smokes (ASTDR, 2007). Benzene can also able to generate reactive intermediates, mainly reactive oxygen species (ROS) that can cause cellular damage (Farooq *et al*., 2006).

Marine species comprise approximately half of the total global biodiversity, and thus the sea offers many potential sources for discovery of novel compounds, the potential of marine organisms as a source of new substances is huge and has been barely investigated (Aneiros and Garateix, 2004). Among the molluscs, the cephalopods are found in all the oceans on earth. Cuttlefish are marine animals of the order *Sepiida* belonging to the class Cephalopoda (which also includes Squid, Octopuses, Nautiluses) (Rajaganapachy *et al*., 2000; Nithya *et al*., 2011).

Marine biotechnology is the science in which marine organisms are used in full or partially to make or modify products, to improve plants or animals or to develop microorganisms for specific uses. With the help of different molecular and biotechnological techniques, humans have been able to elucidate many biological methods applicable to both aquatic and terrestrial organisms (McCarthy and Pomponi, 2004). Discovered bioactive compounds in molluscs were identified essentially as peptide, depsipeptide, sterols, sesquiterpene, terpenes, polypropionates, nitrogenous compounds, macrolides, prostaglandins and fatty acid derivatives, miscellaneous compounds and alkaloids which presented specific types of activities (Balcazar *et al*., 2006; Blunt *et al*., 2006). In recent years the ocean has been considered as a rich source of compounds possessing novel structures and biological activities, biologically active molecules isolated from marine flora and fauna have applications in pharmaceuticals, nutritional supplements, cosmetics, agrochemicals, molecular probes, enzymes, and fine chemicals (Faulkner, 2002). The aim of this study to investigate the effect of marine cephalopoda extract (*Sepia* sp.) on some biochemical parameters against toxicity induced by benzene in male rats.

Materials and Methods:

Samples collection:

Sepia sp. was freshly collected from Arab gulf water, south of Iraq. Samples were washed several times with tab water. These species were classified according to Barnes
(1968).

Preparation of extract:

To prepare crude extract of *Sepia* sp. according to method by Li *et al.* (1962). Homogenize *Sepia* sp. (whole body) in electrical blender with an equal volume of 50% acetic acid, and the homogenate was put on magnetic stirring for 24 hour, the pH was adjusted to 5.0 using pH meter, Centrifuge at 2000 g/h and remove sediment. Dialyze against distilled water for 48 hour was carried out (in refrigerator) and the water was replaced each that 6 hour and lyophilize by freeze drier.

Qualitative chemical tests:

Extract subjugate to varies chemical tests to get acquaint chemical family such as proteins, peptides , free amino group, alkaloids, saponins, carbohydrate, glycosides, aldehyde, keton, flavonoids, and phenolic compounds (Degaim, 2009).

Laboratory animals: White albino male rats (130-155 g) aged 6-10 weeks were obtained from the animal house of department of biology, college of science, University of Thi-Qar, Iraq. The rats were housed in standard metal cages (6 rats/cage). The animals were housed in a well ventilated 12 hrs light and 12 hrs

dark cycles with pellets *ad libitum*. The rats were divided into four groups comprising six animals in each group. All treatments were given intraperitoneally to experimental rats as follows:

1- The first group (control group) injected I.P. with normal saline (1 ml/kg^{-1} for two weeks).
2- The second group (benzene group) injected I.P. with 0.5 ml/kg at the first day of each week for two weeks (once a week).
3- The third group injected I.P. with *Sepia* sp. extract 720µg/animal for two weeks.
4- The fourth group injected I.P. with 0.5 ml/kg at the first day of each week for two week (once a week), then injected with *Sepia* sp. extract 720µg/animal for two weeks.

The effective dose of *Sepia* sp. extract (720 µg) was determined according to Litchfield and Wilcoxon (1949).

Biochemical parameters:

At the end of the experiment, the animals must be fasted for one night, then sacrificed under light ether anesthesia. Five ml of blood samples were collected by heart puncture, and put in tubes without EDTA and centrifuged at 3000g for 10 minutes for obtained serum. The biochemical parameters included liver function tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT)), and lipid profile (cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL)).

Results:

The chemical analysis of *Sepia* sp. extract reveal that it contains proteins ,amino acids and carbohydrates. The results indicated non-significant difference of AST level in the second group compare with the first group, while there was a significant increase ($p<0.05$) in AST compare with the third and the fourth groups. Also, there was a significant decrease of AST in the third group compare with the fourth group. ALT level increased significantly ($p<0.05$) in the second group compare with the first, third and fourth groups, while there was non-significant difference between the first, third and fourth groups (table 1).

Table (1): Effect of *Sepia* sp. extract on the AST and ALT of male rats

Animal groups	AST (U/L)	ALT (U/L)
First group	30.3±0.33^{a}	6.1±0.13^{b}
Second group	33±4.1^{a}	10.3±1.33$_{a}$
Third group	24.6±0.88^{b}	6.6±0.33^{b}
Fourth group	19.3± 3.17^{c}	6.3±1.20^{b}

-Values are expressed as Mean ± SE, n = 6.
- Different letters indicated a significant differences at $p<0.05$

The results showed a significant increase ($p<0.05$) of cholesterol and triglyceride values in the second group compare with other groups. Also, there was a significant increase ($p<0.05$) in cholesterol of the third group compare with the first and the fourth groups, while TG decreased significantly ($p<0.05$) in the third group compare with the first and the fourth groups (table 2).

Table (2): Effect of *Sepia* sp. extract on the Cholesterol and Triglyceride levels of male rats

Animal groups	Cholesterol (mmol/L)	Triglyceride (mmol/L)
First group	64±4.66^{c}	41±5.77^{b}
Second group	89.4±3.14^{a}	48.6±5.43^{a}
Third group	75.3±6.37^{b}	35.2±1.70^{c}
Fourth group	68.2±4.13^{c}	41±0.57^{b}

-Values are expressed as Mean ± SE, n = 6.
- Different letters indicated a significant differences at $p<0.05$

The result indicated a significant increase ($p<0.05$) of LDL in the second group compare with other groups which don't appear a significant difference between them. HDL decreased significantly ($p<0.05$) in the second group compare with other groups which don't appear a significant difference between them. The results showed non-significant difference in VLDL in all groups (table 3).

Table (3): Effect of *Sepia* sp. extract on the LDL, HDL and VLDL levels of male rats

Animal groups	LDL	HDL	VLDL
First group	32.3±1.20 ^{b}	32.3±2.08 ^{a}	9.06±6.66 ^{a}
Second group	54.1±0.88 ^{a}	20.6±2.40 ^{b}	8.1±0.65 ^{a}
Third group	22.9±1.86 ^{b}	53.2±2.13 ^{a}	7±0.34 ^{a}
Fourth group	24.9±3.01 ^{b}	48.1±3.54 ^{a}	6.5±0.24 ^{a}

-Values are expressed as Mean ± SE, n = 6.
- Different letters indicated a significant differences at $p<0.05$

Discussion:

The chemical analysis of extract revels that it composed from protein, amino acid, and carbohydrate (Baslow, 1977; Sikorski and Kolodziejska, 1986) showed the proximate composition of squid meat is 75-84% water, 13-22% crude protein, 0.1-2.7% lipids, and 0.9-1.9% minerals. The biological activity of this extract possibly due to the presence of protein and amino acid especially proline (Degaim, 2009). The current results was agreement with some researches such as (Mohammed *et al*., 2006) which found that Stylisin 1 was Proline-rich cyclic heptapeptide that was isolated from *Stylissa caribica*, and (Dahiya *et al*., 2009) were isolated Phakellistatins are group of proline rich cyclic heptapeptides .

Assessment of liver toxicity is done by measuring the marker enzymes such as ALT and AST which are originally present in high concentration in the cytoplasm. When there is hepatic injury these enzymes leak into blood stream inconformity with extend of hepatotoxicity. Elevated level of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978), but the elevated levels of enzymes are decreased to nearness with normal levels after treated with extract indicates that it offered protection by preserving the structural integrity of the hepatocellular membrane against benzene.

The results revealed increase of total cholesterol, triglyceride , LDL and VLDL levels in toxicity rats, but the HDL level reduced in this group. The administration of the extract lead to reduce the levels of all parameters except HDL in the fourth group which treated with benzene and extract. Farooq *et al*. (2006) showed benzene caused damage to the liver. The enzyme levels involved in major carbohydrate metabolism and antioxidant levels have also been depicted when benzene induced rats were compared with control

group (Khan and Yusufi, 2009). Significant alterations of LDH and other enzymes was observed in benzene induced rats (Banday *et al*., 2008), our present study was restricted to assess the protective role of extract on benzene induced alterations of rat liver especially the levels of some major enzymes.

Conclusion:

Benzene has adverse effects on human health, and benzene is capable of inducing marked alterations in biochemical parameters. So the administration of *Sepia* sp. extract after benzene exposure, which could be beneficial for alleviating benzene toxicity.

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