Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Properties of Crocus sativus Saffron on DEN-induced Hepatocellular Carcinoma in Rats

Reda Fikry , Nabila Zein and Asmaa Faozan

Chemistry Department-Faculty of Science, Zagazig university, Egypt

ARTICLE INFO

Saffron, DEN, CCl4, HCC, caspase and MMP

ABSTRACT

The purpose of this study was to investigate the possible mechanisms of Crocus sativus saffron against diethylnitrosamine (DEN)-induced liver cancer in rats. Administration of Crocus sativus saffron at a dose of 300 mg/kg/day was started 2 weeks prior to the DEN and CCL_4 injection and was continued for 42 days. Crocus sativus saffron significantly reduced the DEN-induced increase in the number and the incidence of hepatic dyschromatic nodules, saffron restored the superoxide dismutase, catalase and malondialdehyde levels and increased the expression of active caspase 3 and inhibited the formation of MMP9.

INTRODUCTION

Hepatocellular carcinoma is the fifth most common cancer and the third leading cause of cancer mortality in the world. Most cases of HCC as a result of either viral hepatitis infection (hepatitis B virus or hepatitis C virus), metabolic toxin such as alcohol or aflotoxin. Other factors that contribute to the formation of HCC include exposure to environmental carcinogens, iron overload and fatty liver disease **(Amin et al., 2011)**.

Current studies have shown the dietary factors such as carotenoids and antioxidants play a key role in the development of cancers. Anticancer activity and protective effects of natural products has extensively been studied such the activity of the saffron against cancer has been also reported **(Bathaie et al., 2013)**.

Saffron, the dried stigma of *Crocus sativus* L. belonging to The Iridaceae Family cultivated in Iran, Europe, Turkey, Centra Asia, India, China and Algeria. Saffron has been widely used as a herbal medicine, Spice,food coloring and a flavoring agent since ancient times. It can increase the bioavailability and enhance absorption of other drugs **(Bolhassani et al., 2014)**.

Over along period of time, many health benefits of saffron have been known in traditional medicine and it has been used in various aliments including bronchospasms, asthema menstruation, insomenia, pain relief and cardiovascular disease. In the last years, scientists have focused on pharmacological activity of saffron and its purified constituents. Results of these *in vivo* and *in vitro* studies have demonsterated anticancer, anti-oxidant,

Corresponding author: **Reda Fikry,** Chemistry Department-Faculty of Science, Zagazig university, Egypt

anti-convulsant, anti-depressent, anti-anxiety and memory improvement properties of this golden spice **(Amin and Hosseinzadeh, 2012)**.

In this study, the potential antitumor effect of saffron was tested.

MATERIALS AND METHODS

Chemicals:

Diethylnitrosamine (DEN), carbonate tracholride (CCL4) were purchased from Sigma Alderish. Csapase 3 assay kit (Casp-3-c), enzyme linked immunosorbent assay kit for MMP9 from Sigma alderish for biochemical analysis.

Plant material:

Crocus sativus L. stigmata were collected from local market.

Extract preparation:

270 gm of saffron sooked in 4.5 litre distilled water for 3 days in the fridge and then filtered for 24 hours. This aqueous extract was lyophilized (freeze-drying) by lyophilizer. Final extract was 33 gm of saffron extract **(Premkumar et al., 2003)**.

Animals:

30 adult male Spraguse-Dawely rats weighing 150-200 gm were purched from Animal House of the national Research center, Dokki, Giza, Egypt. They were kept individually in stainless steel wire bottomed cages at room temperature. Rats had free acess to food and water.

Experimental design:

Animals were randomly divided to three groups of thirty rats as follow:

Group 1: Control group, rats were treated with distilled water (5ml/kg weight).

Group 2: HCC group, Rats induced by DEN and CCl4

Group 3: Saffron group, Rats were treated by gavage with saffron (300 mg /kg) 2 weeks prior to hCC intiation and continued for 6 weeks.

Preparation of HCC model:

Hepatocarcinogenesis was induced in rats by injection of a single intraperitoneal dose of diethylnitrosamine at a dose of 200 mg/kg body weight followed by weekly subcutaneous injections of CCl4 at a dose of 3ml/kg body weight for 6 weeks **(Abdel Aziz et al., 2011)**.

Sampling of blood:

At the end of the experiment, the blood samples were collected from the retro-orbital venous plexus and direct cardiac puncture, under ether anesthesia samples for biochemical analysis into plain sample tubes. Serum was separated by centrifugation at 600x g for 10 min.

Biochemical analysis:

1-Measurement of serum catalase (CAT) activity according to (Aebi., 1984)

2- Measurement of serum serum superoxide dismutase (Marklund et al ., 1979)

3- Measurement of serum serum reduced glutathione(Elham., 1959)

4- Determination of Active Caspase 3 and Matrix metalloportinase 9 (MMP9)

Histopathological analysis:

The harvested liver tissues were fixed in 10% formaline then the specimens were dehydrated in a graded series of ethanol cleared in Xylene and embebed in paraffin wax. Tissue blocks were sectioned into 4.0 thick using rotary microtome. Sections were stained by hematoxylin and eosin . stained sections were examined by light microscope.

Statistical anaylsis:

Statistical analysis of data was performed by using SPSS 14.0 version T-test (2-tailed) was applied to compare between groups and one-way analysis of variance (ANOVA) followed by post-hoc test using Graphpad Prismm-5 software. Numerical data were expresses as mean SD , P-values < 0.05 were considered to be statistically significant.

RESULTS

-Effect of Crocus sativus saffron on GSH, SOD, CAT

GSH, SOD and CAT activities were decreased in the DEN

group compared with the control group ($p < 0.01$) Saffron

group had significantly increased serum GSH and SOD and CAT activity compared with the DEN group ($p < 0.05$).

- Effect of Crocus sativus saffron on MMP9 level:

There is a significant increase in serum concentration of MMP9 in HCC group as compared to Control group. In the saffron group, saffron blocked the increase in hepatic MMP9 in rats.

- Effect of saffron on active caspase 3:

Saffron treated group rats showed significant increase of active caspase 3 compared to HCC group and control group. This confirms that saffron regulates multiple components of the cell death pathway driving the cancer cells towards apoptosis.

- Histopathological study:

Histopathological examination showed dysplastic foci have cellular atypia and tissue disorganization were seen scattered with hepatic paranchyma in animals treated with DEN-CCl4 alone.

The formentioned dysplastic cells were large with vaculated nuclei. The latter were hyperchromatic with fragmented periphery situated chromatin and prominent nudeoles beside mitotic figure.

The remaining hepatic pranachyma suffered from different types of cell injuries varied from acute cell swelling diffuse steatosis to coagulative necrosis. Congested blood vessels, focal hemorrhage and hemosedrosis were evident.

However, in saffron-treated group, a few scattered hepatic foci from dysplastic cells with karyomegaly encirded by inflammatory reactions were absorved.

The majority of cells which exhibited neoplastic features suffered from apoptosis. Focal microsteatosis of some hepatic cells have periphery lobular zone distributions without necrotic changes in the hepatic parenchyma were common

A few strands collagen deposits containing mononuclear cells and bile canaliculi could be seen in portal area and interlobular tissues.

DISCUSSION

Saffron contains more than 150 volatile and aroma-yielding compounds. It also has many nonvolatile active components**(Abdullaev., 2002)** many of which are carotenoids, including zeaxanthin, lycopene, and various α- and β-carotenes. However, saffron's golden yellow-orange colour is primarily the result of α-crocin. This crocin is trans-crocetin di-(β-D-gentiobiosyl) ester; it bears the systematic (IUPAC) name 8,8-diapo-8,8-carotenoic acid. This means that the crocin underlying saffron's aroma is a digentiobiose ester of the carotenoid crocetin. Crocins themselves are a series of hydrophilic carotenoids that are either monoglycosyl or diglycosyl polyene esters of crocetin**(Abdullaev., 2002)** Crocetin is a conjugated polyene dicarboxylic acid that is hydrophobic, and thus oil-soluble. When crocetin is esterified with two water-soluble gentiobioses, which are sugars, a product results that is itself water-soluble. The resultant α-crocin is a carotenoid pigment that may comprise more than 10% of dry saffron's mass. The two esterified gentiobioses make α-crocin ideal for colouring water-based and non-fatty foods such as rice dishes(McGee., 2004)

The bitter glucoside picrocrocin is responsible for saffron's flavour. Picrocrocin (chemical formula: C16H26O7; systematic name: 4-(β-D-glucopyranosyloxy)-2,6,6-trimethylcyclohex-1-ene-1-carboxaldehyde) is a union of an aldehyde sub-molecule known as safranal (systematic name: 2,6,6-trimethylcyclohexa-1,3-diene-1-carboxaldehyde) and a carbohydrate. It has insecticidal and pesticidal properties, and may comprise up to 4% of dry saffron. Picrocrocin is a truncated version of the carotenoid zeaxanthin that is produced via oxidative cleavage, and is the glycoside of the terpene aldehyde safranal **(Leffingwell ., 2002).**

When saffron is dried after its harvest, the heat, combined with enzymatic action, splits picrocrocin to yield D–glucose and a free safranal molecule. Safranal, a volatile oil, gives saffron much of its distinctive aromaSafranal is less bitter than picrocrocin and may comprise up to 70% of dry saffron's volatile fraction in some samples. A second molecule underlying saffron's aroma is 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one, which produces a scent described as saffron, dried hay-like**(Leffingwell., 2002)** Chemists find this is the most powerful

contributor to saffron's fragrance, despite its presence in a lesser quantity than safranal**(Leffingwell., 2002)**, Dry saffron is highly sensitive to fluctuating pH levels, and rapidly breaks down chemically in the presence of light and oxidising agents. It must, therefore, be stored away in air-tight containers to minimise contact with atmospheric oxygen. Saffron is somewhat more resistant to heat.

HCC is the main form of liver cancer and classified as the fifth leading cause of cancer mortality due to it's bad prognosis (Abouzeid et al., 2015).

The common denominator in HCC of different etiology is the induction of oxidative stress by inflammatory cells resulting in chronic hepatic injury and cell death followed by oncogenic transformation of surviving hepatocytes and compensatory proliferation that lead to tumorgenesis **(Youssef et al., 2012)**.

Diethylnitrosamine (DEN) is a representive chemical of a family of carcinogenic n-nitroso compounds . N-nitroso compounds can acts as alkylating agents either directly or after metabolic activation by cytochrome P450 enzymes which are the key enzymes in tumorgensis **(Youssef et al., 2012)**.

DEN plays an important role in induction of hepatic carcinogenesis via increased generation of ROS and decreased antioxidant enzymes in liver **(Youssef et al., 2012)**.

ROS play a major role in tumor promotion through intraction with critical macromolecules including lipids causing lipid peroxidation and DNA causing DNA adducts.

The current results were in accordance with many studies: **Li et al. (2007)** who showed that treatment with DEN induced DNA damage, mutation and induction of HCC. It was revealed by **S.srigopalram.I et al. (2014)** that treatment with DEN induced cell proliferation associated with DNA adducts and lipid peroxidation.

CCl4 which is well known hepatotoxin that is widely used to induce toxic liver injury in a range of laboratory animals.

CCl4 induced hepatotoxicity is believed to involve two phases:

The initial phase involves the metabolism of CCl4 by CYP450 to CCl3 and /or CCl3OO which lead to membrane lipid peroxidation and finally to cell necrosis.

The second phase of CCl4 induced hepatotoxicity involves the activation of kupffer cells which is accompanied by the production of proinflammator mediators **(Eidi et al., 2012)**.

In agreement, **Ghada et al. (2013)** demonstrated the alteration of biochemical markers, hepatic antioxidant status and hepatic nucleic acid content may therefore manifestation of oxidative stress and cellular DNA damage caused by CCl4.

Cellular defense against free radical injury is provided by enzymatic (catalase (CAT), superoxide dismutases (SOD), and glutathione peroxidase) and nonenzymatic (gluthatione (GSH), a-tocopherol, vitamin C, and urate) free radical scavenging systems, present in the cell. (Samarghandian et al., 2013)

We found that saffron increased glutathione level, catalase, and superoxide dismutase activities in the saffron group compared with the DEN group as presented in table (1) SOD is responsible for removal of superoxide radicals and catalase decomposes hydrogen peroxide to water and oxygen; thus, these enzymes may contribute to the modulation of redox state of plasma.48 This observation perfectly agrees with those of Kianbakht et al. (2011)

In the current study, we focused on the possible chemotherapeutic effects of saffron that could be mediated by modulating the expression of MMP9 and Caspase3 in HCC animal model.

Saffron, a spice and a food colorant present in the dry stigmas of the plant (*Crocus sativus* L.) has been used as a herbal remedy for various aliments including cancer by the ancient Arabian,Indian and Chinese cultures .

Recent investigations present verifications that saffron exert an important chemoperventive effect against liver cancer due to inhibition of cell proliferation and induction of apoptosis.

The exact mechanisms underlying this effect are largely unknown.

Different hypotheses for anticarcinogenic and antitumor effects of saffron and its ingredients have been proposed including inhibition of DNA and RNA synthesis not protein,ability of scavange of free radicals,involvement in the metabolic of conversion of carotenoids to retenoids,mediation of interactionof carotenoids with topoisomerase 2 , an enzyme involved in cellular DnA-Protien interaction and promotion of inductions mediated by lectins **(Ragab et al., 2013)**

Several studies demonstrated the role of oxidative stress in liver pathogenesis and proved the role of natural antioxidants in its ametoration.

In the multiple stages of these processes, the degredation of enviromental barriers such as the extracellular matrix (ECM) and basement membrane is the initial step and several proteolytic enzymes participate in the degredation of these barriers. Among these enzymes Matrix metalloproteinases play a major role.

MMPs are group of enzymes which degrades the macromolecules of connective tissues, ECM and basement membrane.

This degredation results in removing physical and structural barriers which promote cell migration and invasion. In general MMP9 has been most consistently detected in a malignant tumor progression. Thus down regulation the level of MMp9 is important for the preventation of malignant tumor progression.

In our study, saffron treatment significantly decreased the level of MMP9 comparing with HCC and control group thereby decreased the local spreading of tumors within the liver and suppressed tumorgensis.

According to these results, **Marzieh et al. (2014)** investigated the inhibitory effect of saffron on MMp9 gene expression level.

It was also revealed by **Gopalakrishnan et al. (2008)** that sylimarine treatment significantly decreased the level of MMP9 thereby decreased the spreading of tumors within the liver.

Apoptosis or programmed cell death represents the regulated activation of a pre-exisiting death program encoded in the genome.

It is a highly orchestrated form of cell death and plays a central role in the control of tissue cell numbers in organ development, hemeostasis and normal functioning such as cell proliferation and differentiation.

Dysregulation of apoptosis may be involved in the pathogenesis of diseases.

It has been proved that occurance of cancers is due to the loss of control of normal apoptosis and the disturbance of balance between cell apoptosis and cell proliferation **(Chodon et al., 2007)**.

The apoptosis was quantified by examination of the activity of caspase 3. Caspase 3 plays a central role in mediating nuclear apoptosis including chromatin condensation and DNA fragmentation as well as cell bledding **(Porter and Janicke, 1999)**.

Activation of caspase 3 plays a central role in the induction of apoptosis , caspase activation occurs through the release of cytochrome C from the mitochondria and hence , mitochondrial outer membrane permability and cytochrome c release is directly and activates effector caspases as caspase 3 and 7 which execute the apoptotic program **(Bahashwan et al., 2015)**.

The apoptosis was quantified by examination of the activity of caspase 3. Caspase 3 plays a central role in mediating nuclear apoptosis including chromatin condensation and DNA fragmentation as well as cell bleeding **(Ramakrishnan Gopalakrishnan et al., 2013)**.

Activation of caspase 3 plays a central role in the induction of apoptosis, caspase activation occurs through the release of cytochrome C from the mitochondria and hence, mitochondrial outer membrane permability and cytochrome c release is directly and activates effector caspases as caspase 3 and 7 which execute the apoptotic program **(Bahashwan et al., 2015)**.

With regard to the Caspase 3, **Amin et al. (2011)** indicated that the antiproliferative activity of saffron was also associated with the

induction of apoptosis as evidenced invitro by caspase 3 cleavage.

The present study showed a significant increase in the level of active caspase 3 in saffron treated group.

Our results coincide with many studies:

Ramakrishnan et al. (2013) showed the significant level of actove caspase 3 as indicator of apoptosis when they used the slymarin as natural remedy for treating HCC by decreasing cell proliferation and increasing apoptosis. **Also, Shatadal et al. (2015)** used curcumin as a herbal antioxidant of HCC, they reported that curcumin increased the level of caspase 3 which mean the increasing induction of apoptosis

The histological results obtained in the current study, showed that DEN caused gross structural alterations in rat liver, with dysplastic foci have cellular atypia and tissue organization were seen scattered with hepatic parenchyma.

In accordance, the study of **Magda Ismail et al. (2012)** showed that DEN induced necrosis. This liver necrosis which has been found in many of animals causes collapse of the paranchymal framework of the liver which can resemble fibrotic changes.

Also, **Mekky et all. (2014)** demonstrated that administration of DEN induced morphological deformation in the liver pronounced with degeneration of hepatocytes and liver neoplasic cellular alteration.

With regard to the Saffron, the present study showed that the majority of cells which exhibited neoplastic suffered from apoptosis in the group of rats were treated with saffron.

This also can be explained by **Amr Amin et al. (2011)** whom reported that the groups of rats were treated with saffron have a significant reduction in the number and size of the nodules induced by DEN an large number of regular hepatocytes were observed.

Also **Saleh et al. (2014)** showed that livers of rats treated with crocin (Saffron) and CCL4 revealed better reservation of the normal liver architecture and rare generalized vacuolization of the cytoplasm of hepatocytes, with apparently normal nuclei very few inflammatory cells infilteration.

Shatadal et al. (2015) reported that curcumn treatment to HCC rats models showed a considerable improvement in liver morphology.

Conclusion:

In summary, the data presented here show the saffron dramatically inhibited the number and the incidence of hepatic nodules in livers of DEN- treated rats.

This inhibition was associated with induced apoptosis, reduced cell proliferation, decreased oxidative stress and down regulation of inflammatory markers.

References

1) **Amin A, Hamza AA, Bajbouj K, Ashraf SS, Daoud S (2011):** Saffron: A potential candidate for a novel anticancer drug against hepatocellular carcinoma. Hepatologys; 54: 857-867.
2) **Abdullaev, F. I. (2002):** Cancer Chemopreventive and Tumoricidal Properties of Saffron (Crocus sativus L.), Experimental Biology and Medicine, 227 (1)
3) **Bathaie SZ, Miri H, Mohagheghi MA, Mokhtari-Dizaji M, Shahbazfar AA, Hasanzadeh H (2013):** Saffron aqueous extract inhibits the chemically induced gastric cancer progression in the wistar Albino Rat Iran J Basic Med Sci; 16: 27-38.
4) **Bolhassani A, Khavari A, Bathaie SZ (2014):** Saffron & natural carotenoids: Biochemical activities and anti-tumor effects. Biochemia et Biophysica Acta 1845 (2014): 20-30.
5) **Amin B, Hosseinzadeh H (2012):** Evaluation of aqueous & ethanolic extracts of saffron, *Crocus sativus* L., & its constituents, Safranal & Crocin in allodynia & hyperalgesia induced by chronic constriction injury model of neuropathic pain in rats. Fitoterepia; 83: 888-895.
6) **Premkumar K, Abraham SK, Santiya ST, Ramesh A (2003):** Phytother. Res., 17: 614-617.
7) **Abdel Aziz MT, El-Asmar MF, Hazem M. Atta, Mabfouz S, Fouad HH, Roshdy NK, Rashed LA, Jabry D, Hoassouna AA, Taha**

FM (2011): Journal of Experimental & Clinical Research 2011, 3: 49.

8) **Abouzeid MM, Eltahir HM, Abdel Aziz MA, Ahmed AS, Abd El-Ghany AA, Abd El-Aziz EA, Abd El-Aziz HO (2015):** Curcumin ameliorate DENA-induced HCC via modulating TGF-β, AKT, and Caspase-3 expression in experimental rat model. Tumor Biol. 2015; 36: 1763-1771.
9) **Youssef MI, Maghraby H, Youssef EA, & El-Sayed MM (2012):** Expression of ki 67 in hepatiocellar carcinoma induced by diethylinitrosamine in mice & its correlation wioth histopathological alterations. Journal Pharmaceutical Science; 02 (03): 52-59.
10) **Li X, Shi ZM, Fing P, Wen ZY, Wang XJ (2007):** Effect of Qi-protecting powder (Hugi san) on expression of C-jun, C-Fos & C-myc in diethylnitrosamine-mediated hepatocarcinogenesis. World J Gastroenterol 2007; 13: 4192-4198.
11) **Srigopalram I, Jayraay A, Balesswuran B, Balamurugan K, Ranjithkumar M, Kumar TK, Park JI, Nou IS (2014):** Ellargic acid normalizes mitochondrial onter membrane permeabilization & alternuates inflammation-mediated cell proliferation in experimental liver cancer. Appl. Biochem. Biotechnol; 173: 2254-2266.
12) **Eidi A, Mortazavi P, Tehrani ME, Rohanii AH, Safi S (2012):** Hepatoprotective effect of pantothenic acid on carbon-tetrachloride-induced toxicity in rats. EXCLI Journal; 11: 748-759.
13) **Ragab GM, El-Denshary ES, Hassan AM, Abdel-Azeim SH, Hassan NS, Mannaa FA, Abdel-Wahhab MA (2013):** Grape (*Vitis vinifera*) Seed Extract Inhibits the Cytotoxicity and Oxidative Stress in Liver of Rats Treated with Carbon Tetrachloride. Global Journal of Pharmacology; 7: 258-269
14) **Zhang Z, Zhiwang C, Wen XD, Shoyama Y, Yuan CS (2013):** Role of saffron & its constituents on cvancer chemoprevention. Pharm Biol; 51 (7): 920-924.
15) **Bahashwan S, Hassan MH, Aly H, Ghobara MM, El-Beshloishy HA, Busat I (2015):** Crocin migrates carbon tetrachloride-induced liver toxicity in rats. Journal of Taibah University Medical Science; 10 (2): 140-149.
16) **Ghosh S, Bhattacharyya S, Rashid K, Sil PC (2015):** Curcumin protects rat liver from Streptototocin-induced diabetic pathophysiology by counteracting reactive oxygen species & inhibiting the activation of p53 & MAPKS mediated stress response pathways. Toxicology Reports; 2: 365-376.
17) **Gopalakrishnan Ramakrishnan, Sundaram Jagam, Sattu Kamaraj, Pandi Anandakumar, Thiruvengadam Devaki.** Silymarin attenuated most cell requirement therapy decreased the expressions of matrix metalloprotienases in rat liver carcinogenesis. Invest New Drugs 2009; 27: 233-240.
18) **Mohammed A.F. Elewa, Mohammed M. Al-Gayyar, Mona F. Schaalan, Khaled H. Abd El Galil, Mohamed A. Ebrahim, Mamdouh M. El-Shishtway.** Hepatoprotective & anti-tumor effects of targeting MMP-9 in hepatocellular carcinoma & its relation to vascular invasion markers. Clin Exp. Metastasis (2015) 32: 479-493.
19) **Festuccia C, Mancini A, Gravina GL, Scarsella L, Llorens S, Alonso GL, Tatone C, Di Cesare E, Jannini EA, Lenzi A, D'Alessandro AM, Carmona M (2014):** Antitumor effects of Saffron-Derived Cartenoids in prostate cancer cell models. (2014) Biomed Research International; Article ID 135048, 12 pages.
20) **Chodon D, Banu SM, Padmavathi R, Sakthisekaran D (2007):** Inhibition of cell proliferation and induction of apoptosis by genistein in experimental hepatocellualr carcinoma. Molecular and Cellular biochemistry; 297: 73-80.
21) **Porter AG, Janicke RV (1999):** Emerging role of caspase 3 in apoptosis (Review). Cell Death Differ, 6: 99-104.
22) **Ramakrishnan Gopalakrishnan, Jagan Sundaram Kamaraj Sattu, Anandakumar Pandi, Devalki Thiruvengadam(2013),.** Dietary supplementation of silymarin is associated with decreased cell proliferation increased apoptosis & activation of deloxification system in hepatocellular carcinoma. Mol Cell Biochem 377: 163-176.
23) **Leffingwell, J. C. (2002)**, "Saffron", Leffingwell Reports, Leffingwell & Associates, 2 (5).

24) **McGee, H. (2004)**, On Food and Cooking: The Science and Lore of the Kitchen
25) **Ellman GL.** Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70–77.
26) **Kianbakht S, Hajiaghaee R.** Anti-hyperglycemic effects of saffron and its active constituents, crocin and safranal, in alloxan-induced diabetic rats. J Med Plants.2011;10:39–43.
27) **Marklund S, Marklund G.** Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1979;47:469–474.
28) **Aebi H**. Catalase in vitro. Methods Enzymol. 1984;105.

Table (1):

	Control	DEN	Saff.	P-value
GSH(U/gp)	0.6±1.04	0.3±4.1	0.5±3.7	<0.01
SOD(U/gp)	6.5±0.64	4.5±0.18	5.8±0.19	<0.01
CAT(U/gp)	4	2.5	3.5	<0.01

Table (2):

	Control	DEN	Saff.	P-value
MMP9	21.4±1.04	118.6±4.1	61.0±3.7	<0.001
Caspase 3	144.45±3.64	28.26±1.18	79.53±3.19	<0.001

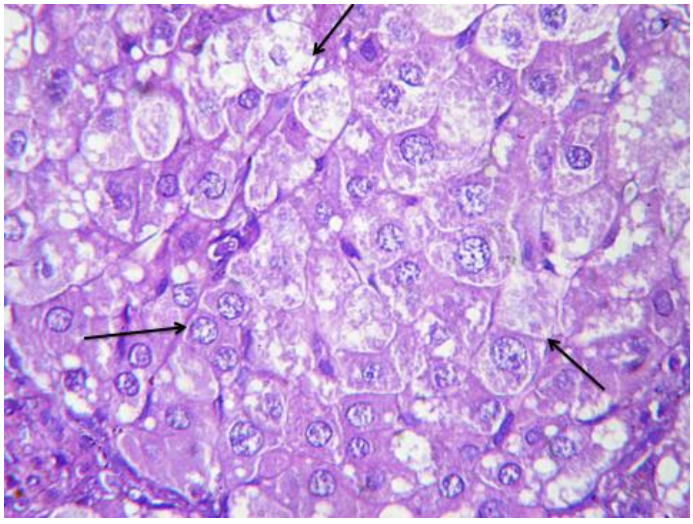

Fig. (1): Liver of rat (DL) showing dysplastic-pleomorphic cells with large vesicular nuclei and marginated chromatin (arrows). H&E (X1500).

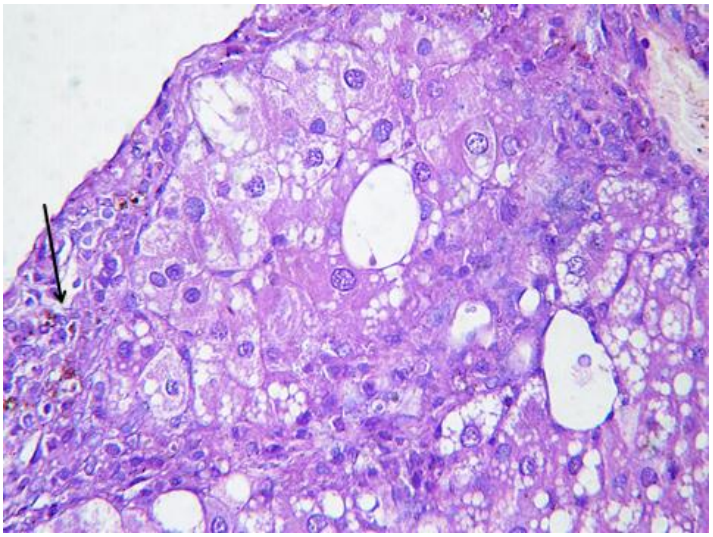

Fig. (3): Liver of rat (SL) showing dysplastic foci encircled by inflammatory reaction (arrow) H&E(X1200).

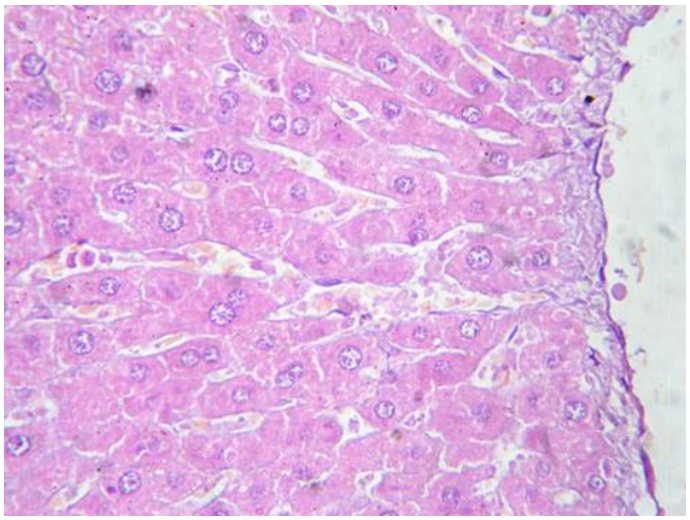

Fig. (6): showing normal hepatic cells cords and sinusoids. H&E(X1200).