BIOLOGICAL ASSESSMENT OF SELECTED EGYPTIAN CHICK PEA CULTIVAR

[1]REHAM HASSAN MEKKY, [2]MOHAMED ROSHDI EL-GINDI, [3]AZZA R. ABDEL-MONEM, [4]MARÍA DEL MAR CONTRERAS, [5]ANTONIO SEGURA-CARRETERO, [6]MOSTAFA R. FAYEDAND, [7]ESSAM ABDEL-SATTAR

[1,2] Pharmacognosy Department, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo-Suez road, 11829, Cairo, Egypt; [3,7]Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, 11562, Cairo, Egypt; [4]Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avenida Fuentenueva s/n, 18071, Granada, Spain; [5]Research and Development Functional Food Centre (CIDAF), Bioregi´on Building, Health Science Technological Park, Avenida del Conocimiento s/n, 18016, Granada, Spain, [6]Pharmacology and toxiclogy Department, Faculty of Pharmacy, Egyptian Russian University, Badr, City, Cairo-Suez road, 11829, Cairo, Egypt
E-mail: reham.hassan@gmail.com, mrelgindi@yahoo.com, azzaramy@yahoo.com, mmcontreras@ugr.es, ansegura@ugr.es, essam.abdelsattar@pharma.cu.edu.eg

Abstract- Chickpea (Cicer arietinum) is a legume of the family Fabaceae, subfamily Faboideae. In Egypt, chickpea seeds are usually consumed at the raw green and tender stage, or in the form of mature dry seeds. Based on the results of chemical analysis of seven Egyptian cultivars, Giza 1 was selected for further biological studies. The extract showed a strong hepatoprotective activity based on the measurement of liver enzymes (AST, ALT and AST), levels of albumen, globulin, total protein and lipid profile (total cholesterol, high density lipoprotein, triglycerides, and low density lipoprotein). In addition, Giza 1 extract showed a moderate cytotoxic activity against nine human cancer cell lines (MCF7, HEP2, HEP2, HCT116, HepG2, PC-3, A-549, HELA and CACO) and a moderate antimicrobial activities. The antioxidative stress was also tested by measuring catalase, reduced glutathione, superoxide dismutase and malondialdehyde levels. The low acute toxicity (up to 2 g/Kg) and low cytotoxicity indicates the safety profile of potential of the extract.

Key words- Chickpea; Cicer arietinum; hepatoprotective; antioxidative stress; cytotoxicity; LD50.

I. INTRODUCTION

Chickpea (Cicer arietinum) is the fifth major important legume in the world on the basis of total grain production and because of their nutritional quality. Chickpea seeds are grown mainly in the Mediterranean area, the Near East, Central Asia and America (Singh et al., 1991). Chickpeas are rich sources of complex carbohydrates, protein, vitamins and minerals (Wang et al., 2010). In addition, chickpeas are rich also in secondary metabolites such as saponin glycosides and isoflavonoids. Chickpeas have shown numerous health benefits, e.g. lower glycemic index for people with diabetes (Chillo et al., 2008), increased satiation and cancer prevention as well as protection against cardiovascular diseases due to their dietary fiber content (Goni ET AL., 2003). In a previous study, the combination of a solid–liquid extraction and
RP-HPLC-DADESI-QTOF-MS analysis, using a, enabled to perform a comprehensive metabolic profiling of seven Egyptian cultivars of chickpea (Mekky et al., 2015). Giza 1 cultivar showed the highest total phenol content, highest amount of isoflavonoids and hydroxybenzoic acids and antioxidant activity.

II. OBJECTIVE

In present study, Giza 1 cultivar was selected to assist its hepatoprotective activity, antioxidative stress, cytotoxicty and antimicrobial activity.

III. STUDY DESIGN

Acute toxicity Study
Normal rats were randomized into 5 groups of (6 animals each) and received chickpea extract at five doses (250, 500, 1000 and 2000 mg/Kg) and rats were observed over 72 hours.

In vivo hepatoprotective effect of chick pea extract
Normal rats (male Wister albino rats) will be randomized into 5 groups (8 animals each) and treated for seven days as follows (Raj and Gothandam, 2014):
Group 1: normal control.
Group 2: CCl4 group.
Group 3: positive control group (silymarin group + CCl4).
Group 4 and 5: extract (250 or 500 mg/kg) + CCl4.
The hepatoprotective activity was assessed by measuring liver enzymes (AST, ALT and ALP), levels of albumen, globulin, total protein in serum and lipid profile (total cholesterol, high density lipoprotein, triglycerides, and low density lipoprotein) and hepatic index (HI).

Antioxidative stress effect of chick pea extract
The antioxidative stress was assessed by measuring glutathione reduced and malonliadehyde levels, superoxide dismutase and catalase activities
In addition, the anti-inflammatory marker (TNF-□) was determined and a histopathological examination was done to confirm the hepatoprotective effect.

In vitro cytotoxic activity of chick pea extract

The cytotoxicity of Giza 1 extract was tested against nine human cancer cell lines (MCF7, HEP2, HEP2, HCT116, HepG2, PC-3, A-549, HELA and CACO) according to method reported by Gangadevi and Muthumary (2007).

The antimicrobial activity of chick pea extract

Giza 1 extract was screened for its antibacterial activity against Staphylococcus aurues, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli. The antifungal activity was tested against Aspergillus fumigatus, Syncephalastrum racemosum, Candida albicans, Geotricum candidum.

IV. RESULTS AND CONCLUSION

Acute toxicity Study

No mortality was detected in all the groups, indicating the safety of extract up to 2000 mg/Kg.

In vivo hepatoprotective activity

The oral administration of chickpea Giza 1 extract exerted significant hepatoprotective activity by reduction of the aforementioned markers (AST, ALT and ALP) comparable to the reference drugI. silymarin(Fig. 1). On other hand, the oral administration of the higher dose of chickpea Giza 1 extract significantly restored the levels of serum albumin and globulins (Fig. 1) to the control group indicating hepatoprotective activity.

Antioxidative stress

The liver endogenous antioxidant enzymes GSH, CAT, SOD and MDA as markers of lipid peroxidation were significantly restored to nearly the normal values at both doses, which indicated the antioxidant activity (Fig. 2). The higher dose of chickpea Giza 1 extract also significantly reduced the level of TNFα more effectively than the standard silymarin.

The lipid profile

The higher dose of Giza 1 extract significantly restored the levels of serum total cholesterol, LDL, HDL and triglycerides (Fig. 3) to the control group indicating hepatoprotective activity.

Histopathological Examination

Upon the treatment with the 500 mg/Kg dose of the extract of group 5 (extract + CCl4) showed a more normal architecture of the liver, with fewer hepatocytes showing fatty change. A fewer number of hepatocytes surrounding the central vein had necrobiosis (Fig 5 e1-e2) when compared to CCl4 group (Fig 5 b1 and b2). The histopathological examination is complying with the results reported by Banda et al. (2013).

CONCLUSION

Chick pea (Cicer arietinum) represents the ffth most important crop in Egypt (FAO-statitstics, 2012). It represents well recognized source of dietary proteins, carbohydrates, minerals and trace elments (Juknti et a., 2012). However, little is known about their secondary metabolites and documentation of its biological activities. Our previous results showed that Giza 1 cultivar has the highest in vitro antioxidant activity and phenolic content among the seven cultivars analysed. The extract of Giza 1 cultivar reduced CCl4 induced hepatotoxicity by increasing antioxidant enzyme activities, inhibiting lipid peroxidation and decreasing the levels of hepatic markers. Our work substantiated the well known correlation between the hepatoprotective activity and antioxidant activities. In addition Giza 1 cultivar showed low acute toxicity and cytoxicity against nine human cancer cell lines. In addition Giza 1 cultivar showed a moderate antimicrobial activity against panel of gram positive and gram negative and fungi.

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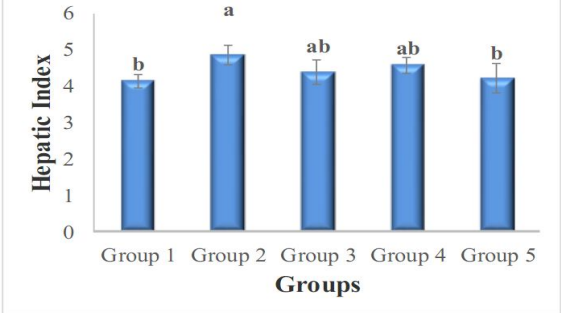
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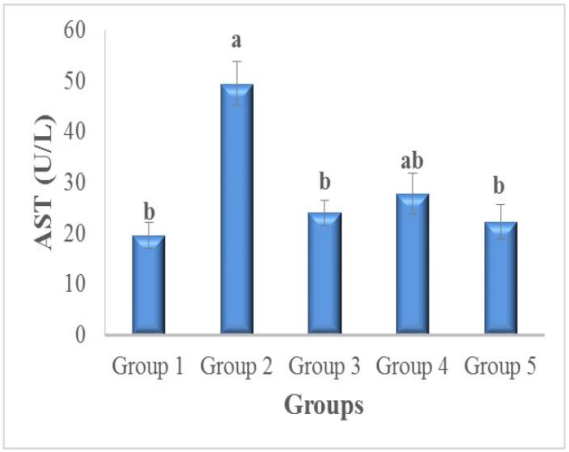
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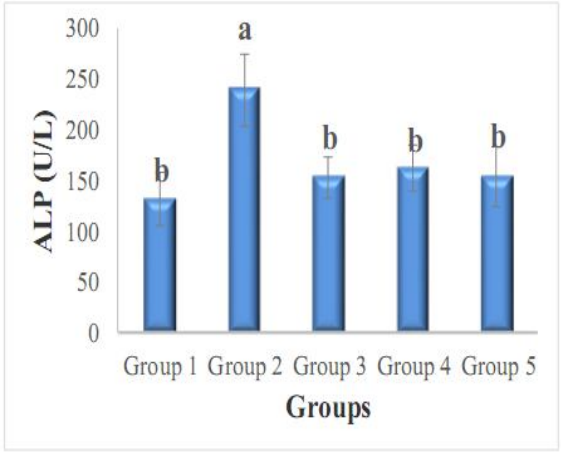
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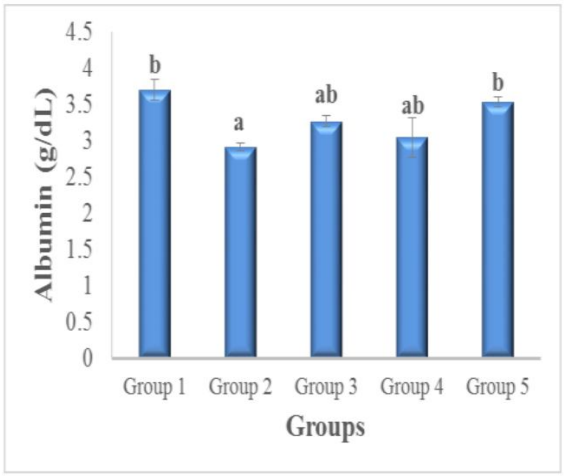
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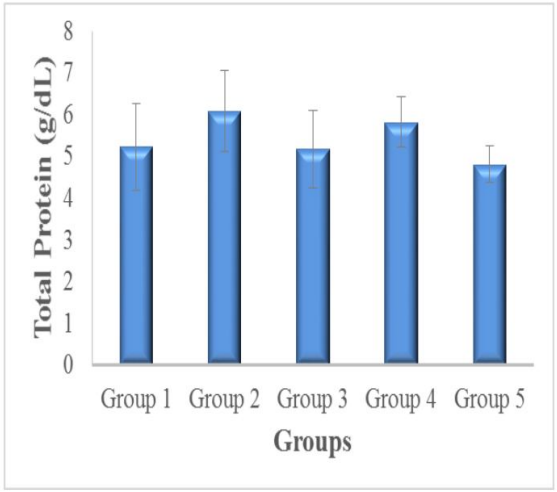
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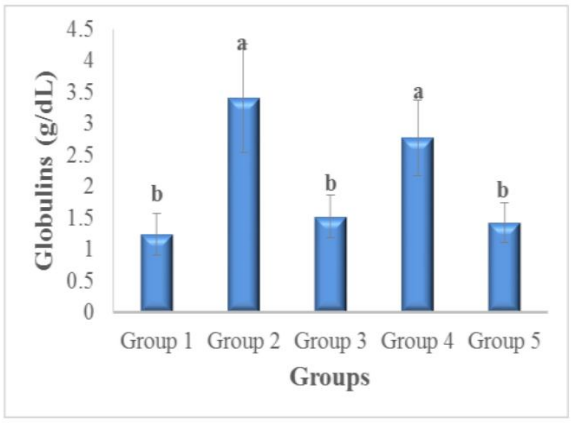


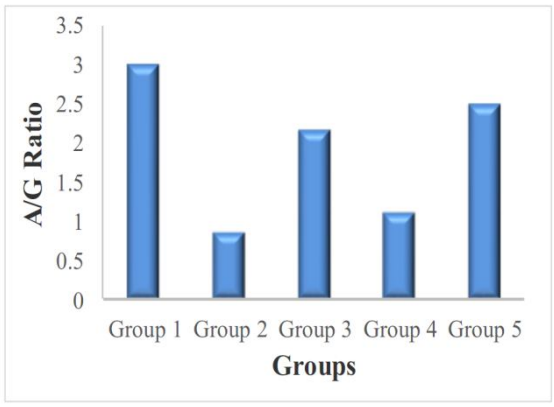












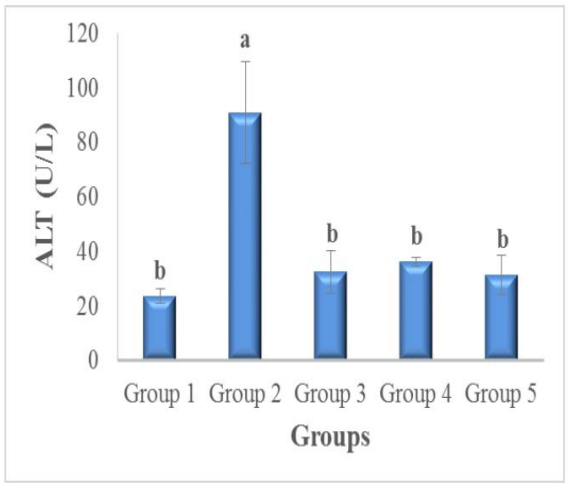
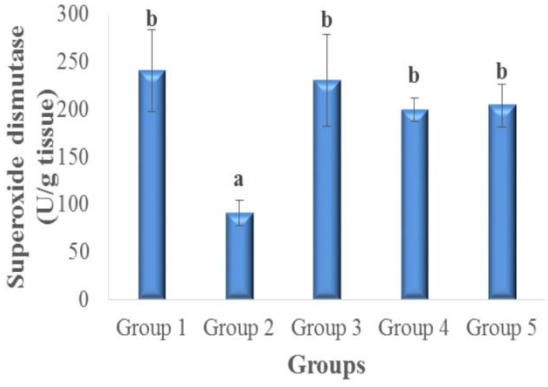


Fig. 1. Evaluation of the protective effect of extract of chick pea seeds (Giza 1 cultivar) on the biochemical parameters of liver in CCl4-induced hepatic damage in male albino Wister rats



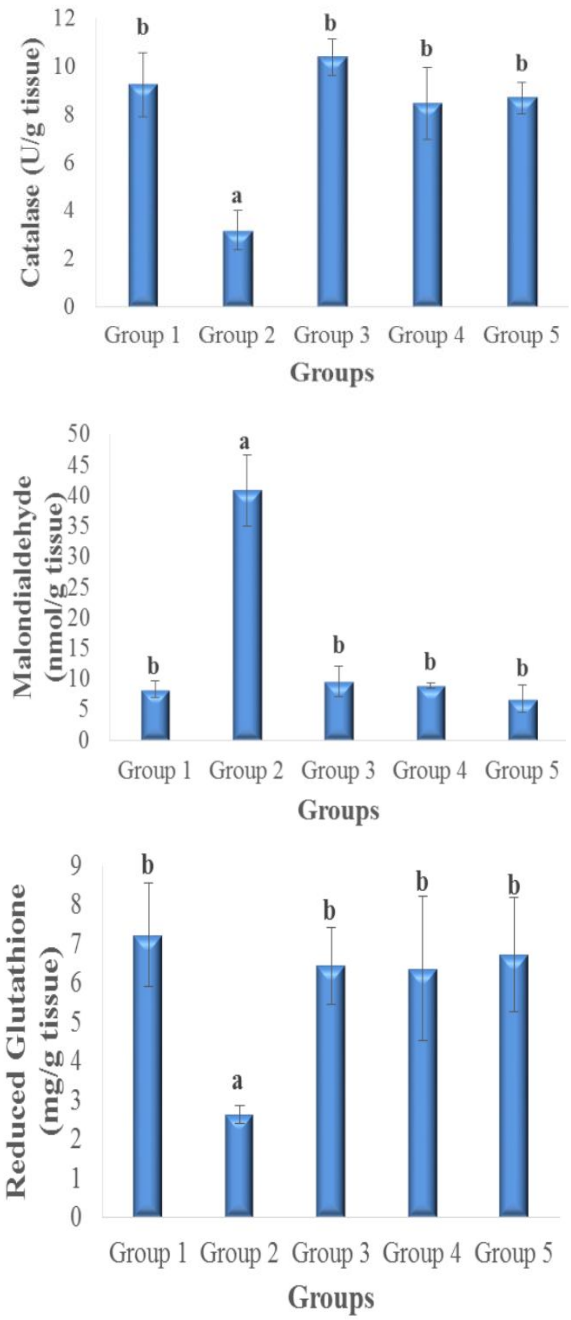
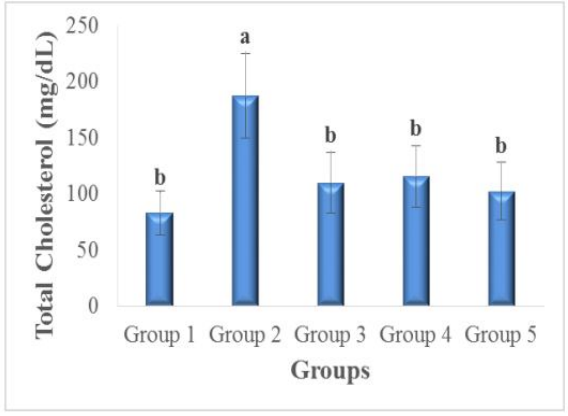


Fig. 2. Evaluation of the effect of extract of chick pea seeds (Giza 1 cultivar) on the hepatic oxidative stress markers in CCl4-induced hepatic damage in male albino Wister rats



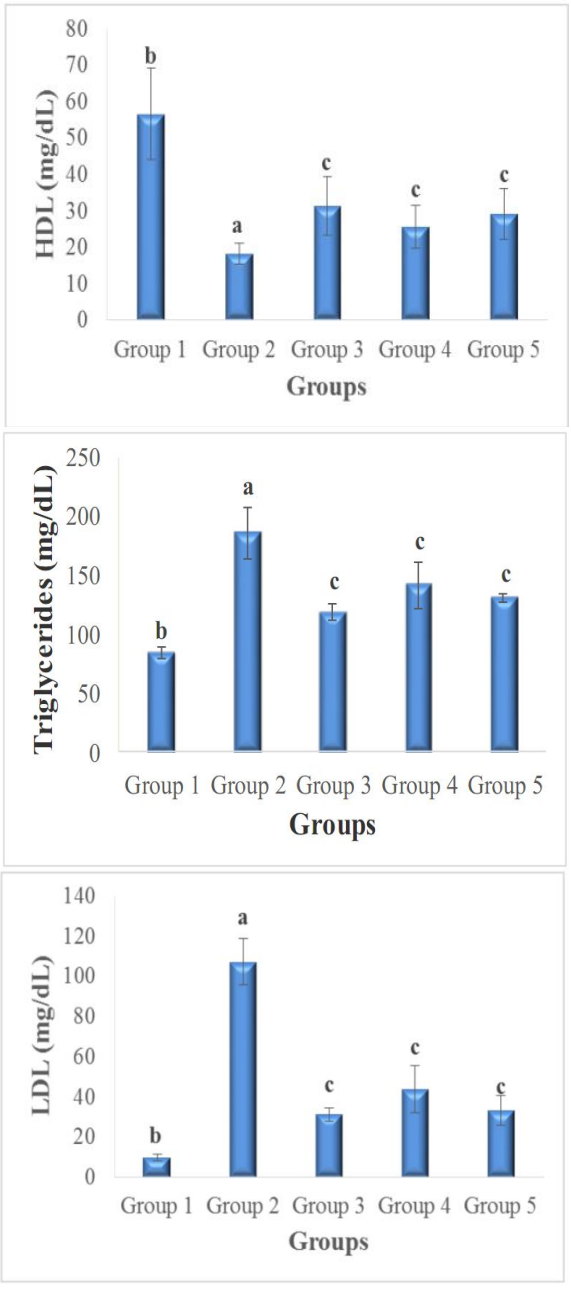


Fig. 3. Evaluation of the effect of extract of chick pea seeds (Giza 1 cultivar) on the lipid profile in CCl4-induced hepatic damage in male albino Wister rats

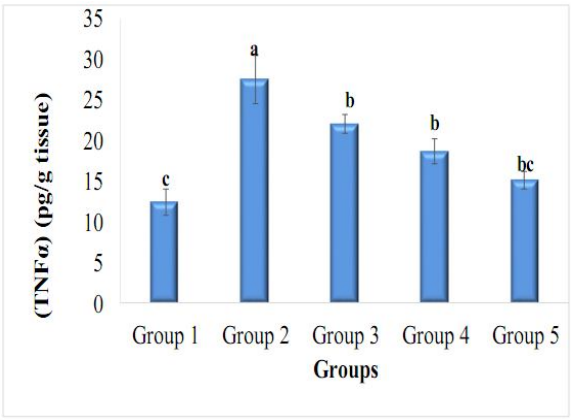


Fig. 4. Effect of extract of chick pea seeds (Giza 1 cultivar) on inflammatory marker (TNFα, pg/g tissue).

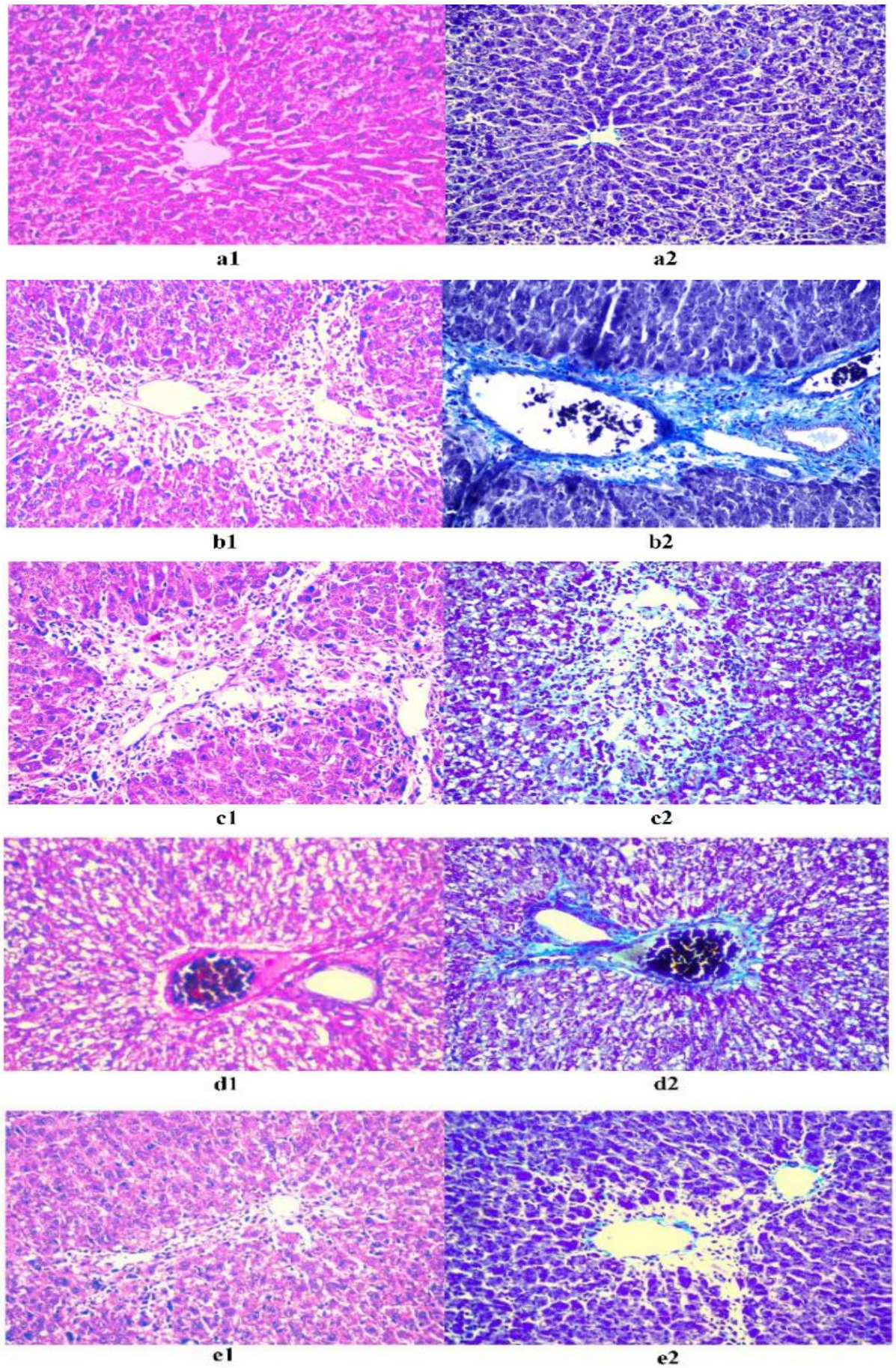


Fig. 5. Effect of extract of chick pea seeds (Giza 1 cultivar) on CCl4-induced histopathological alterations in hepatic tissues.

Table 1. Antibacterial and antifungal activities of seeds of chick pea extract (Giza 1 cultivar).

Microorganism	Antimicrobial activity			
Gram positive bacteria	Giza 1 cultivar		Ampicillin	
	IZ (mm)	MIC (µg/mL)	IZ (mm)	MIC (µg/mL)
S. aurues (RCMB 010028)	21.30 ± 1.2	1.95	27.15 ± 1.2	0.49
B. subtilis (RCMB 010067)	23.40 ± 0.58	0.98	32.40 ± 1.2	0.24
Gram negative bacteria			Gentamicin	
P. aeruginosa (RCMB 010043)	13.60 ± 1.5	62.5	22.60 ± 1.5	0.98
E. coli (RCMB 010052)	20.40 ± 0.72	3.9	22.3 ± 0.71	0.98
Fungi			Amphotericin B	
A. fumigatus (RCMB 02568)	18.30 ± 1.5	7.81	27.70 ± 0.58	0.98
S. racemosum (RCMB 05922)	16.50 ± 1.2	15.63	21.90 ± 0.72	0.98
C. albicans (RCMB 05031)	NA*	NA*	19.80 ± 1	3.9
G. candidum (RCMB 05098)	22.40 ± 0.58	0.95	22.40 ± 0.58	0.49

*NA : no activity; IZ: inhibitory zone; MIC: minimum inhibitory concentration

Table 2. Results of cytoxtoxicity (IC50) of Chick pea seeds extract (Giza 1 cultivar) against nine human cancer cell lines.

Minimum inhibitory concentration, IC_{50} (µg/mL)															
HepG-2		MCF-7		HCT-116		PC-3		A-549		HELA		HEP-2		CACO	
G-1 C	Dox.	G-1 C	Dox.	G-1 C	Dox.	G-1 C	Dox.	G-1 C	Dox.	G-1 C	Dox.	G-1 C	Dox.	G-1 C	Dox.
43.1	0.47	41.6	0.43	33.1	0.46	38.2	0.71	37.9	0.93	>50	0.9	>50	1.13	46.7	0.72

G-1 C: Giza 1 cultivar; Dox: doxorubicin; HEPG2: liver carcinoma; MCF-7: breast carcinoma; HCT-116: colon carcinoma; PC-3: prostate carcinoma; A-549: lung carcinoma; HELA: cervical carcinoma; HEP2: larynx carcinoma; CACO: intestinal carcinoma.

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