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EFFECT OF *OPUNTIA FICUS-INDICA* STEM EXTRACT ON LIVER FIBROSIS INDUCED BY THIOACETAMIDE IN MALE ALBINO MICE

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ABSTRACT

This study aimed to investigate the effect of stem extract of *Opuntia ficus-indica* on experimentally induced liver fibrosis by the hepatotoxicant thioacetamide (TAA) in male albino mice. The experimental mice were divided into four groups. The mice of the first group served as normal control, while the experimental animals of the second group were given 150 mg/kg of body weight of TAA by intraperitoneal injection, twice weekly, for 8 weeks. The mice of the third group were exposed to TAA and supplemented with *O. ficus-indica* stem extract orally. The animals of the fourth group were supplemented with *O. ficus-indica* stem extract only. The plasma levels of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase were measured. The results showed significantly increased levels of the aforementioned enzymes. Histopathological evaluations of hepatic sections from mice treated with TAA showed severe alterations including increase of fibrogenesis processes with structural damage. Concomitant administration of *ficus-indica* stem extract with TAA reduced the extent and development of fibrous septa, liver cells change, and biochemical alterations in mice. The present findings suggest that the supplementation of these extract may act as antioxidant agents and could be an excellent adjuvant support in the therapy of hepatic fibrosis.

Keywords: *Opuntia ficus-indica,* Hepatic cirrhosis, Thioacetamide, Albino mice, Saudi Arabia

INTRODUCTION

Liver is a vital organ responsible for metabolism, blood detoxification, bile production, synthesis and regulation of essential hormones. Various liver diseases have become a global problem associated with high morbidity and mortality levels especially in developing countries. A report from the World Health Organization (WHO) indicates that 10% of the world population has chronic liver disease, in addition about two million people worldwide die each year from hepatic failure [1]. Hepatic fibrosis is a dynamic physiological wound-healing process. When damage is sustained, however, this process becomes exacerbated and irreversible, leading to cirrhosis. Hepatic fibrosis after hepatocyte injury is a pathological process with deposition of extracellular matrix (ECM) proteins such as collagens. Indeed, the gradual replacement of hepatocytes with scar tissue impairs blood circulation in the liver leading to hepatocytes death and loss of liver functions [2].

Thioacetamide (TAA) was originally used to control the decay of oranges and then as a fungicide [3]. TAA is a potent hepatotoxicant which requires metabolic activation by the mixed-function oxidases. For its toxicity, thioacetamide requires oxidation to its S-oxide and then further to reactive S,S-dioxide form which ultimately attacks lipids and proteins [4]. Furthermore, TAA has been widely used in the study of the underlying mechanisms of hepatic fibrogenesis and the therapeutic effects of potential antifibrosis drugs. Additionally, many experimental investigations showed that TAA induced hepatic fibrosis and cirrhosis in rats and mice [5, 6].

Medicinal plants have played an important role in pharmacology and medicine for centuries. Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs [7]. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of many diseases. Medicinal plants and herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost [8,9]. *O. ficus-indica* is a tropical or subtropical plant belonging to the Cactaceae family and is mainly used for fruit production [10]. Additionally, alkaloids, indicaxanthin, neobetanin, and various flavonoids have been isolated from the cactus [11]. It can be used also as a vegetable forage resource for livestock feed in arid and semiarid lands

during periods of drought and shortage of herbaceous plants [12]. Different parts of *O. ficus-indica* are used in the traditional medicine of several countries: the cladodes are utilized for treatment of ulcers, rheumatic pain, wounds, fatigue [13]. This study was carried out to assess the hepatoprotective activity of *O. ficus-indica* against thioacetamide-induced hepatotoxicity in albino mice.

MATERIALS AND METHODS

Animals: male albino mice of the SWR strain, weighing 15.0–25.0 g were taken for the present study. The principles of laboratory animal care were followed throughout the duration of experiment according to the guidelines given by King Abdulaziz University ethics committee [5]. The mice were distributed into four groups (Seven mice per group) and were housed in standard cages at an ambient temperature of 20 ±1 °C with 12-h light:12-h dark cycle and humidity of 65%. The mice were fed ad libitum on normal commercial chow and had free access to water.

Chemicals: Thioacetamide (Sigma-Aldrich, Switzerland) was dissolved in sterile distilled water and injected intraperitoneally to the mice in concentration of 150 mg/kg of body weight.

Extraction of *O. ficus-indica* stem

Fine qualities of *O. ficus-indica* stem were directly collected from the outskirts of Albaha region of Saudi Arabia and identified in Biology department, Albaha University. The collected stems were completely washed, air dried at room temperature and stored in a dry plastic container until extraction processes. The method of Al-Attar and Abu Zeid [14]. was used to prepare the extracts. Briefly, the dried stem of *O. ficus-indica* (50 g) were powdered, added to 2 liters of cold water and mixed using an electric mixer for 20 min. Thereafter, the solutions were gently filtered and evaporated in an oven at 40 °C. With references to the powdered samples, the mean yield of *O. ficus-indica* was 19.3%. Furthermore, these extracts were stored in a refrigerator for subsequent experiments.

Experimental design: The animals were divided randomly into four groups (seven mice per group):

Group 1: Normal control injected with normal saline (0.9% NaCl) twice weekly for eight weeks.

Group 2: injected with TAA intraperitoneally at a dose of 150mg/kg twice weekly for eight weeks.

Group 3: injected with TAA and supplemented with *O. ficus-indica* stem

extract orally at a dose of 150mg/kg/ day for eight weeks.

Group 4: orally received *O. ficus-indica* stem extract only at a dose of 150mg/kg daily for eight weeks.

Biochemical analysis

At the end of experimental period, mice were fasted for 6 hours and anaesthetized with diethyl ether. Blood specimens were collected from orbital venous plexus in vacuum tubes containing EDTA (k3) as anticoagulants. Blood specimens were centrifuged at 200 ×g for 10 minutes, and the clear samples of blood plasma were separated. Plasma ALT, AST, GGT, ALP was estimated using an automatic analyzer (Reflotron Plus System, Roche, Germany).

Histopathological analysis

Liver samples were fixed in buffered formaldehyde (10%) followed by paraffin embedding. Sections were stained with hematoxylin and eosin. All slides were examined by light microscopy and photographed.

Statistical analysis

The data were expressed as mean± standard deviation (SD) and were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 12.0). All values were expressed as mean ± standard deviation. Statistical comparisons were performed by a two-way analysis of variance (ANOVA). The results were considered statistically significant if the P-values were less than 0.05.

RESULTS

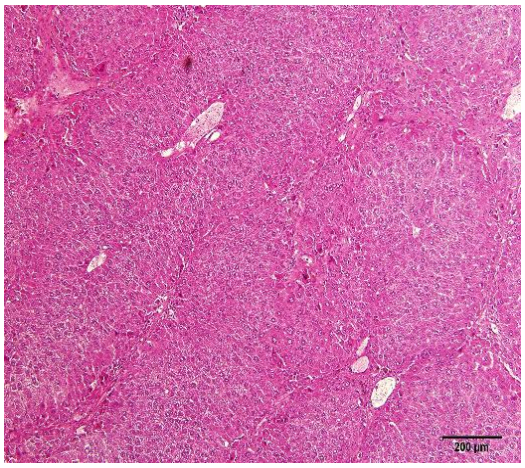
The serum levels of ALT, AST, GGT, ALP of control and treated mice after eight weeks are shown in TAA at the dose of 150 mg/kg body weight induced significant increases ($P < 0.05$) of plasma ALT (+118.7%), AST (+102.6%), GGT (+142.2%), ALP (+53.0%) were statistically decreased ($P < 0.05$) in mice of group 2 compared to the control (group 1), TAA plus *O. ficus-indica* stem extract (group 3), and *O. ficus-indica* stem extract (group 4) treated mice. The level of plasma ALT (+27.9%) was statistically elevated in mice treated with TAA plus *O. ficus-indica* stem extract compared with control and *O. ficus-indica* stem extract treated mice. The level of plasma GGT (+20.6%) was increased in mice treated with TAA plus *O. ficus-indica* stem extract compared with mice supplemented with only *O. ficus-indica* stem extract. Additionally, The levels of plasma AST, ALP were significantly unchanged in mice treated with TAA plus *O. ficus-indica* stem extract. Furthermore, insignificant changes of plasma ALT, AST, GGT, ALP were observed in mice treated with only *O. ficus-indica* stem extract.

Histopathological Examination

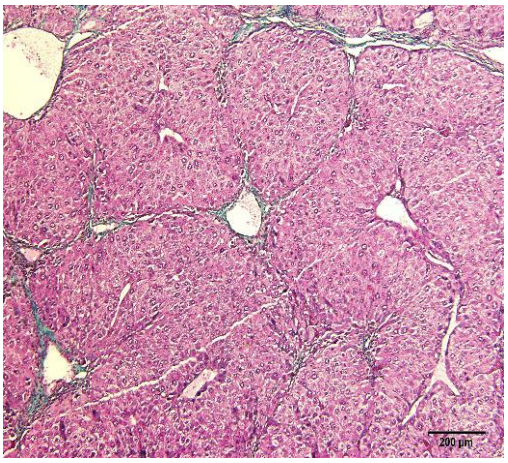
Histopathological examination of liver sections from control mice showed a normal hepatocellular architecture (Fig. 1A). Similar observations were noted in the group of *O. ficus-indica* stem extract stem extract treated mice after eight weeks (Figs. 1F). Liver sections of mice exposed to TAA for eight weeks showed a structural damage with the extracellular matrix collagen and liver cells showed centrilobular necrosis (Fig. 1B and C). Conversely, liver sections from mice subjected to TAA plus *O. ficus-indica* stem extract stem extract (Fig. 1D and E) showed a decreased development of fibrogenesis processes and liver cells showed no necrosis but had only minimal portal inflammation.

Table 1: Plasma ALT, AST, ALP, GGT levels (mean ± SD) of control, TAA, TTA plus *O. ficus-indica* stem extract, and *O. ficus-indica* stem extract treated mice (n = 7). Percentage changes are included in parentheses

Treatments	Parameters			
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
Control	25.61 ± 3.04	40.38 ± 4.10	119.56 ± 8.60	5.79 ± 0.51
TAA	59.00 ± 13.15 (+118.7%)	83.50 ± 23.40 (+102.6%)	180.63 ± 24.28 (+53.0%)	12.55 ± 2.31 (+142.2%)
TAA + *O. ficus-indica* stem extract	34.63 ± 7.35 (+27.9%)	49.87 ±11.64 (+20.5)	127.38 ± 17.78 (+7.4)	4.73 ± 1.33 (+20.7)
O. ficus-indica stem extract	24.75 ± 3.01 (−7.1)	41.00 ± 5.93 (−0.9)	119.13 ± 8.86 (+0.5)	4.54 ± 0.48 (−5.2)



(A)



(B)

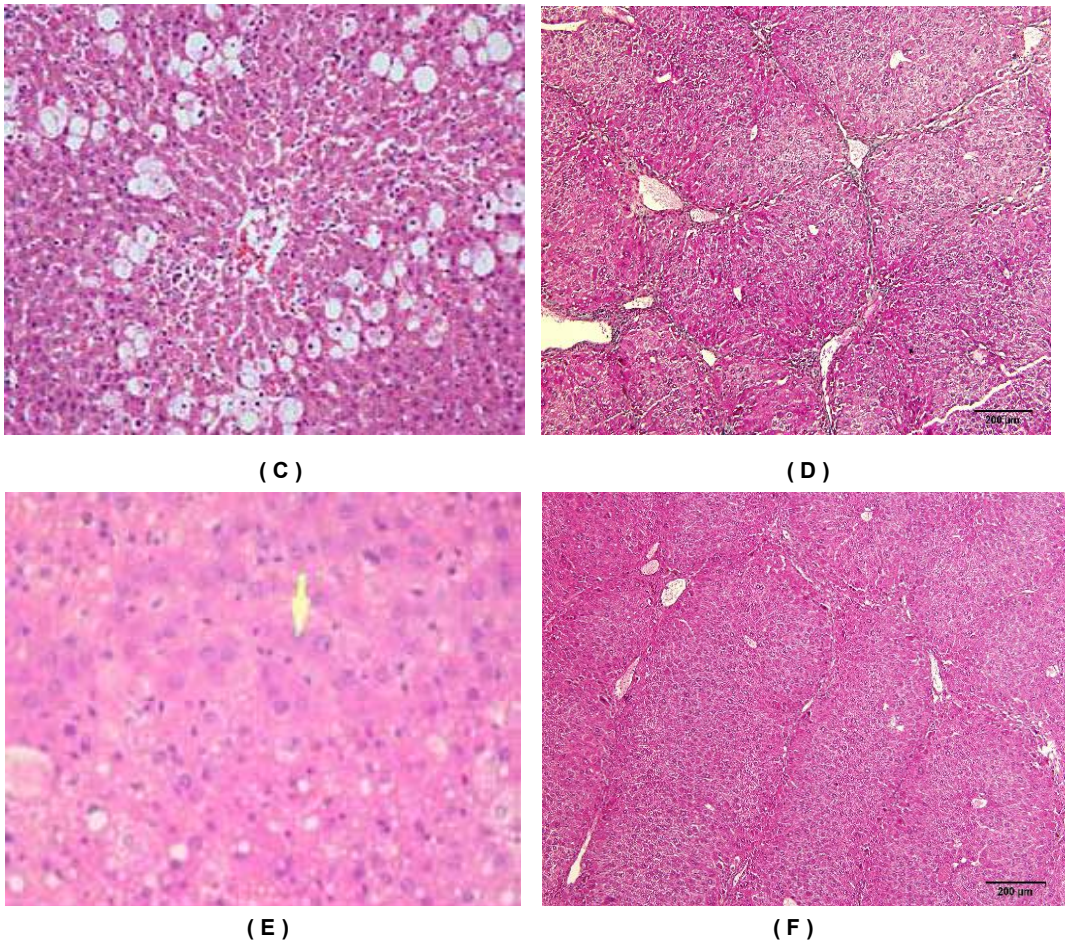


Figure 1: (A– F) Photomicrographs of liver sections in each group. (A) control (X200), (B, C) TAA-treated group (X200, X400), (D, E) TAA plus *O. ficus-indica* stem extract (X200, X400), (F) *O. ficus-indica* stem extract (X200) treated mice for eight weeks

DISCUSSION

Hepatic injuries lead to attenuation of metabolic functions regulated by liver, and have remained one of the serious health problems [15]. Currently, hepatic fibrosis still contributes to the high incidence and morbidity rates of cirrhosis as the latter is irreversible. Thus, researchers are dedicated to find out specific treatment targets that contribute to the development of hepatic fibrosis [16].

The present study is the first experimental investigation designed to evaluate whether supplementation of *O. ficus-indica* stem extract would have protective influences on TAA induced hepatic fibrosis with physiological disturbances and histological injuries in male mice. TAA intoxication has shown significant increases in the levels of serum ALT, AST, GGT and ALP. Similar observations were noted in experimental animals treated with TAA [17-22]. Moreover, necrosis or membrane damage releases these

enzymes into circulation, which agrees with the previously reported results [23].

The mechanism of TAA-induced liver cirrhosis is not fully understood. Certainly, it is a multifactorial process involving Reactive oxygen species (ROS), reduced hepatic antioxidants, and fibrogenic and inflammatory mediators. Thioacetamide is metabolized to toxic metabolites, such as N-acetyl-p-benzoquinone imine (via cytochrome p-450 pathway) and thioacetamide sulfoxide ($TASO_2$). N-acetyl-p-benzoquinone imine reacts with sulfhydryl groups of proteins leading to rapid decrease in intracellular glutathione due to its elimination in urine as conjugates. This reduction of glutathione culminates in increase of oxygen free radicals that cause an oxidative stress and apoptosis [24]. Additionally, $TASO_2$ binds covalently to cellular macromolecules causing protein oxidation, lipid peroxidation, and DNA damage [4].

The present work showed that the treatment of mice with *O. ficus-indica* stem extract reduced the liver fibrosis process and tissues damage induced by TAA administration as verified by the values of liver function markers (ALT, AST, GGT, and ALP) and liver histopathological observations. This indicated the effectiveness of this extracts in prevention of TAA toxicity. The main constituent of the *O. ficus-indica* stem are alkaloids, indicaxanthin, neobetanin, and various flavonoids which are thought to be responsible for pharmacological effects [11]. Furthermore, Chemopreventive effect on oxidative stress and genotoxicity was also recently investigated [13]. Fruit and cladodes of this plant yield high values of important nutrients such as minerals, vitamins as well as further antioxidants [25, 26]. Besides, several studies have reported its efficiency in the treatment of several diseases. These fruits have shown several effects such as antiulcerogenic, antioxidant, anticancer and hepatoprotective activities [27-29]. Stem extract of *O. ficus-indica* has shown a protective effect against liver injuries in Wistar male rad models [30, 31]. They demonstrated that the extract of *Opuntia ficus* possesses hepatoprotective properties against TAA-induced hepatic cirrhosis by inhibiting the physiological and histopathological alterations. Moreover, they suggested that the hepatoprotective effects of *O. ficus-indica* leaves extract may be attributed to its antioxidant activity. Moreover, Smida et al. [32] evaluated the protective effect of *O. ficus-indica* extract against chlorpyrifos-induced immunotoxicity in rats. They suggested that oleuropein possesses

beneficial antioxidant effects against ethanol-induced liver toxicity [5, 22].

CONCLUSION

One of the most important findings in the present study is the observation that the extract of *O. ficus-indica* stem was effective in reducing the TAA induced liver fibrosis, that were proven by physiological analysis and histopathological evaluation. Collectively, the results of this study suggest that the effects of these extracts against TAA-induced hepatic fibrosis possibly due to antioxidant properties of their natural chemical constituents. To the best of author's knowledge, this is the first study that investigates the protective effects of *O. ficus-indica* in liver fibrosis induced by TAA in animal model. Additional physiological, biochemical and histopathological investigations are needed to explore the possible use of different doses of these extracts and their constituents as potential natural therapeutic agents in the treatment of hepatic fibrosis against TAA and may be against other fibrogenic factors.

REFERENCES

[1] Schuppan D, Afdhal NH. Liver cirrhosis. The Lancet, 371(9615), 2008, 838-851.

[2] Ramachandran P, Iredale JP. Liver fibrosis: a bidirectional model of fibrogenesis and resolution. QJM: An International Journal of Medicine, 105(9), 2012, 813-817.

[3] Childs JF, Siegler EA. Uses of thioacetamide in agriculture. Science, 102, 1945, 68-72.

[4] Hajovsky H, Hu G, Koen Y, Sarma D, Cui W, Moore DS, Staudinger JL, Hanzlik RP. Metabolism and toxicity of thioacetamide and thioacetamide S-oxide in rat hepatocytes. Chemical research in toxicology, 25(9), 2012, 1955-1963.

[5] Al-Attar AM, Shawush NA. Influence of olive and rosemary leaves extracts on chemically induced liver cirrhosis in male rats. Saudi journal of biological sciences, 22(2), 2015, 157-163.

[6] Meng Z, Meng L, Wang K, Li J, Cao X, Wu J, Hu Y. Enhanced hepatic targeting, biodistribution and antifibrotic efficacy of tanshinone IIA loaded globin nanoparticles. European Journal of Pharmaceutical Sciences, 73, 2015, 35-43.

[7] Ogbera AO, Dada O, Adeleye F, Jewo PI. Complementary and alternative medicine use in diabetes mellitus. West African journal of medicine. 29(3), 2010, 158-162.

[8] Venkatesh S, Reddy GD, Reddy BM, Ramesh M, Rao AA. Antihyperglycemic

activity of Caralluma attenuata. Fitoterapi, 74(3), 2003, 274-279.

[9] Tohidi M, Khayami M, Nejati V, Meftahizade H. Evaluation of antibacterial activity and wound healing of Pistacia atlantica and Pistacia khinjuk. Journal of Medicinal Plants Research, 5(17), 2011, 4310-4314.

[10] de Cortázar VG, Nobel PS. Biomass and fruit production for the prickly pear cactus, *Opuntia ficus-indica*. Journal of the American Society for Horticultural Science, 117(4), 1992, 558-562.

[11] El Kossori RL, Villaume C, El Boustani E, Sauvaire Y, Méjean L. Composition of pulp, skin and seeds of prickly pears fruit (*Opuntia ficus indica* sp.). Plant Foods for Human Nutrition, 52(3), 1998, 263-270.

[12] Felker P, Paterson A, Jenderek MM. Forage potential of Opuntia clones maintained by the USDA, National Plant Germplasm System (NPGS) collection. Crop science, 46(5), 2006, 2161-2168.

[13] Brahmi D, Bouaziz C, Ayed Y, Mansour HB, Zourgui L, Bacha H. Chemopreventive effect of cactus *Opuntia ficus indica* on oxidative stress and genotoxicity of aflatoxin B1. Nutrition & metabolism, (1), 2011, 73. https://doi.org/10.1186/1743-7075-8-73

[14] Al-Attar AM, Abu Zeid IM. Effect of tea (Camellia sinensis) and olive (Olea europaea L.) leaves extracts on male mice exposed to diazinon. BioMed research international, 2013, 1-6, http://dx.doi.org/10.1155/2013/461415

[15] Wolf PL. Biochemical diagnosis of liver disease. Indian Journal of Clinical Biochemistry, 14(1), 1999, 59-90.

[16] Qin D, Nie Y, Wen Z. Protection of rats from thioacetamide-induced hepatic fibrosis by the extracts of a traditional Uighur medicine Cichorium glandulosum. Iranian journal of basic medical sciences, 17(11), 2014, 879.

[17] Al-Attar AM. Attenuating effect of Ginkgo biloba leaves extract on liver fibrosis induced by thioacetamide in mice. BioMed Research International, 2012, Doi:10.1155/2012/761450.

[18] Al-Attar AM. Hepatoprotective Influence of Vitamin C on Thioacetamide—induced Liver Cirrhosis in Wistar Male Rats. Journal of Pharmacology and Toxicology, 6(8), 2011, 218-88.

[19] Salama SM, Abdulla MA, AlRashdi AS, Ismail S, Alkiyumi SS, Golbabapour S. Hepatoprotective effect of ethanolic extract of Curcuma longa on thioacetamide induced liver cirrhosis in

rats. BMC complementary and Alternative medicine, 13(1), 2013, 56.

[20] Zargar S. Protective effect of Trigonella foenum-graecum on thioacetamide induced hepatotoxicity in rats. Saudi journal of biological sciences, 21(2), 2014, 139-145.

[21] Kim JH, Jeong YJ, Hong JM, Kim HR, Kang JS, Lee WJ, Hwang YI. Chronic vitamin C insufficiency aggravated thioacetamide-induced liver fibrosis in gulo-knockout mice. Free Radical Biology and Medicine, 67, 2014, 81-90.

[22] Al-Attar AM, Alrobai AA, Almalki DA. Effect of Olea oleaster and Juniperus procera leaves extracts on thioacetamide induced hepatic cirrhosis in male albino mice. Saudi journal of biological sciences, 23(3), 2016, 363-371.

[23] Kew MC. Serum aminotransferase concentration as evidence of hepatocellular damage. The Lancet, 355(9204), 2000, 591-2.

[24] Chilakapati J, Korrapati MC, Hill RA, Warbritton A, Latendresse JR, Mehendale HM. Toxicokinetics and toxicity of thioacetamide sulfoxide: a metabolite of thioacetamide. Toxicology, 230(2-3), 2007, 105-116.

[25] Stintzing FC, Carle R. Cactus stems (Opuntia spp.): A review on their chemistry, technology, and uses. Molecular nutrition & food research, 49(2), 2005, 175-194.

[26] Tesoriere L, Fazzari M, Allegra M, Livrea MA. Biothiols, taurine, and lipid-soluble antioxidants in the edible pulp of Sicilian cactus pear (*Opuntia ficus-indica*) fruits and changes of bioactive juice components upon industrial processing. Journal of agricultural and food chemistry, 53(20), 2005, 7851-7855.

[27] Galati EM, Mondello MR, Giuffrida D, Dugo G, Miceli N, Pergolizzi S, Taviano MF. Chemical characterization and biological effects of Sicilian *Opuntia ficus indica* (L.) Mill. fruit juice: antioxidant and antiulcerogenic activity. Journal of Agricultural and Food Chemistry, 51(17), 2003, 4903-4908.

[28] Kuti JO. Antioxidant compounds from four Opuntia cactus pear fruit varieties. Food chemistry, 85(4), 2004, 527-533.

[29] Tesoriere L, Butera D, Pintaudi AM, Allegra M, Livrea MA. Supplementation with cactus pear (*Opuntia ficus-indica*) fruit decreases oxidative stress in healthy humans: a comparative study with vitamin C. The American journal of clinical nutrition, 80(2), 2004, 391-395.

[30] Galati EM, Mondello MR, Lauriano ER, Taviano MF, Galluzzo M, Miceli N. *Opuntia ficus indica* (L.) Mill. fruit juice protects liver from carbon tetrachloride-induced injury. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivative, (9), 2005, 796-800.

[31] Zou DM, Brewer M, Garcia F, Feugang JM, Wang J, Zang R, Liu H, Zou C. Cactus pear: a natural product in cancer chemoprevention. Nutrition journal, 4(1), 2005, 25-36.

[32] Smida A, Ncibi S, Taleb J, Saad AB, Ncib S, Zourgui L. Immunoprotective activity and antioxidant properties of cactus (*Opuntia ficus indica*) extract against chlorpyrifos toxicity in rats. Biomedicine & Pharmacotherapy, 88, 2017, 844-851.