http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2017.403.409

Research Article
Protective Role of *Achillea biebersteinii* Pretreatment on Dimethoate Induced Oxidative Stress in Guinea Pigs Liver

[1]Yahya Saleh Al-Awthan, [2,3]Mohamed Ali Al-Duais, [1]Amany Abdulfatah Hazeb and [1]Waleed Ahmed Alril

[1]Department of Biology, Faculty of Science, Ibb University, 70270 Ibb, Yemen
[2]Department of Chemistry, Faculty of Science, Ibb University, 70270 Ibb, Yemen
[3]Department of Biochemistry, Faculty of Science, University of Tabuk, 71491 Tabuk, Saudi Arabia

Abstract

Objective: The present study investigated the influence of *Achillea biebersteinii* (*Ab*), a medicinal herb used widely in Yemeni's folk medicine as analgesic, antipyretic, against diarrhea and flatulence and for liver diseases, on the liver antioxidant potential of guinea pigs acutely intoxicated with dimethoate pesticide (DM). **Materials and Methods:** Animals were administered with *Ab* aqueous extract (50 and 100 mg kg^{-1} b.wt., orally) or silymarin (100 mg kg^{-1} b.wt., orally) for 2 weeks followed by single acute DM administration (80 mg kg^{-1} b.wt., 1/4 of the LD_{50} orally). Data were analyzed by one-way ANOVA using SPSS. **Results:** The results showed that the treatment with *Ab* extract significantly lowered the DM induced serum levels of hepatic marker enzymes (AST, ALT and ALP). Liver histopathology also showed that *Ab* extract reduced the incidence of lesions including the swelling of cells, lymphocytes infiltration, nucleus fragmentation and condensation and necrosis induced by DM treatment in guinea pigs. **Conclusion:** The results of this study suggest that *Ab* aqueous extract could protect the liver against DM-induced oxidative damage.

Key words: *Achillea biebersteinii*, dimethoate, hepatotoxicity, oxidative stress, aqueous extract, silymarin

Citation: Yahya Saleh Al-Awthan, Mohamed Ali Al-Duais, Amany Abdulfatah Hazeb and Waleed Ahmed Alril, 2017. Protective role of *Achillea biebersteinii* pretreatment on dimethoate induced oxidative stress in guinea pigs liver. Pak. J. Biol. Sci., 20: 403-409.

Corresponding Author: Yahya Saleh Al-Awthan, Department of Biology, Faculty of Science, Ibb University, 70270 Ibb, Yemen Tel:+967777376908

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Organophosphate (OP) compounds are used worldwide in agriculture for crop protection and pest control and include the most toxic chemical agents. Thousands of different OP compounds have been synthesized and are used in agriculture for crop protection and pest control[1]. Some have also been used in the medical treatment as ectoparasiticides, in public health programs as insecticides, acaricides and nematicides and in commerce as lubricants, plasticizers and flame-retardants[2,3]. Exposure to low level of these OP pesticides is known to produce a variety of adverse biological effects reported in human and experimental studies which can be used as markers of exposure or effect[1]. The inhibition of acetylcholinesterase (AChE) activity is one of the primary effects of OP pesticides which accumulates acetylcholine and prevents the normal transmission of nerve impulses leading to continuous stimulation of the muscles, glands and the central nervous system which finally cause death[4]. Previous report indicated that the toxicity of OP insecticides caused devastating effects on many organs and systems[4,5]. Dimethoate (DM), which is considered as one of the most important OP insecticide, is frequently used in agriculture against a wide range of insects especially in qat farms in Yemen. Recent study showed that DM was one of the more contamination sources[6] and was detected at high concentration in water samples in Saudi Arabia[7]. Main groups at risk of high rates of DM exposure are pesticide producers, pesticide workers and farm owners[8]. Previous studies showed that DM intoxication cause oxidative stress by generation of free radicals and induce hepatic lipid peroxidation in rat[9], guinea pig[10], rabbit[11] and mice[12]. The DM, similar to other OPs, inhibits AChE which is present in mammals, fish, birds and insects. The inhibition creates a buildup of acetylcholine at the nerve synapses which results in continuous stimulation of the muscles eventually leading to seizures, exhaustion and possibly death[13]. In addition, previous reports showed that OP pesticides caused oxidative stress involved by generation of free radicals and lipid peroxidation[14]. The liver is the primary organ involved in xenobiotic metabolism and is a major target organ for chemicals and drugs. Therefore, the toxicity of liver is an important endpoint in the evaluation of a particular xenobiotic effect. Natural antioxidants from plants origin are reported to provide a good protection that slows down the process of oxidative damage caused by reactive oxygen species (ROS) that involved in the toxicity of various OP pesticides[15]. *Achillea* L. (*Asteraceae*) is represented by 140 species in the world[16]. *Achillea biebersteinii* (*Ab*), one of the medicinal plants of Yemen, is an erect herbaceous perennial plant upto 1 m high. The leaves are densely hairy with 2-3 pinnatisect and contain golden yellow flowers which are ray-florets 4-5[17]. There are different *Achillea* species used worldwide for their pharmaceutical properties. Their extracts exhibit pharmacological activities such as anti-inflammatory, analgesic, antipyretic, diuretic, in wound healing, against diarrhea, hypoglycemic, anthelmintic and anti-bacterial remedies[18-21]. Antioxidant activity of different species of *Achillea* including *Achillea distans*[22], *Achillea ligustica*[23], *Achillea wilhelmsii*[24], *Achillea millefolium*[25] and *Ab*[26,27] have been previously investigated. These studies reported that infusions prepared from *Achillea* species had antioxidant capacity and protective effect, which is consistent with their total flavonoid and phenol contents. In addition, three *Achillea* species extract showed protective effects against lipid peroxidation and DNA damage *in vitro*[28]. Recently, Mais *et al.*[29] reported that *Ab* showed hypolipidemic effect. Also, Abd-Alla *et al.*[30] reported that *Ab* showed antiulcer agent with hyperglycaemia lowering effect. More recently, Varasteh-Kojourian *et al.*[31] showed that two *Achillea* extracts including *Ab* possess remarkable antioxidant activity. Accordingly, it is more interested to study the influence of plant extracts on oxidative stress induced by pesticide intoxication. This has encouraged us to investigate the effect of acute DM exposure on oxidative stress by assessing liver marker enzymes and other histological parameters, as well as the protective action of *Ab* aqueous extract on DM-intoxicated guinea pigs. So, the aim of the present study was to investigate some of the biochemical and histological alterations which might occur as a result of DM intoxication in guinea pigs. In addition, to study the protective effects of the aqueous extract of *Ab* against DM -induced acute liver injury in guinea pigs.

MATERIALS AND METHODS

Plant material: The whole plant of *Ab* was collected in Feb-March, 2010 from Ibb, Yemen. The plant was authenticated by Mr. Esam Aqlan, Biology Department, Ibb University, Yemen. Voucher specimens were kept in the herbarium for future references.

Preparation of the aqueous extracts: The plants were washed, cut into small pieces, shade dried for 5 days and then dried overnight in an oven. The dried plants (200 g) were boiled for 30 min with distilled water (2000 mL). The resulting water extract was filtered and subsequently concentrated with a water bath (90°C) until it became creamy and was then dried in an oven (60°C) that finally gave 20 g (10% of initial amount) of powder. The dried extracts were dissolved in saline and administrated orally when experiments were performed.

Chemicals: Dimethoate 40 EC was applied as a commercial emulsifiable concentrate formulation containing 40% active ingredient. It was diluted in corn oil for the final concentration.

Animals and treatments: Adult male guinea pigs (350±50 g) were obtained from the animal house of Biology department, Faculty of Science, Ibb University, Yemen and kept for 1 week on a commercial diet in environmentally controlled conditions with free access to diet and water *ad libitum*. Animals were divided into 5 groups of 5 each. Animals of group I received oral administration of corn oil 24 h prior to decapitation. The animals of group II were given single administration of DM (80 mg kg^{-1}) orally dissolved in corn oil 24 h prior to decapitation. The animals of group III and IV were given the pretreatment of aqueous extracts of *Ab* (50 and 100 mg kg^{-1}, respectively) orally dissolved in normal saline once daily for 2 weeks in succession followed by single administration of DM (80 mg kg^{-1}, 1/4 of the LD_{50}) orally. The animals of group V were given silymarin pretreatment (100 mg kg^{-1} p.o.) suspended in normal saline once daily for 2 weeks followed by single administration of DM (80 mg kg^{-1}, 1/4 of the LD_{50}) orally. Twenty four hours after the toxin administration, the animals of each group were anaesthetized with ether and blood was collected directly from the portal vein. The blood sample of each animal was divided in two tubes, one of them mixed with heparin to prevent coagulation and the other was allowed to clot at room temperature for 1 h and then centrifuged (Beckman, UK) at 3000 rpm and 4°C for 15 min to obtain sera. The separated serum was sampled into clean tubes and kept in a deep-freezer (Labotech, India) at -24°C for further analysis.

Biochemical indicators of liver function: Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were determined using Medichem Middle East Diagnostics kits (Syria) according to the method of Tietz[32]. The activity of serum alkaline phosphatase (ALP) was determined using Reactivos GPL Diagnostics kits (Spain) according to the method of King[33]. The enzyme activity was expressed as U L^{-1}.

Histopathological examination: Control and experimental animals were put under light ether anaesthesia, dissected as quickly as possible and then livers were removed. Small pieces were fixed in 10% neutral formalin for 24 h, then washed by the running tap water and stored in 70% ethyl alcohol (Analytical grade) until further processing. Blocks of about 5×5 mm size were dehydrated, cleared and embedded in paraffin wax. Paraffin sections of 5 microns thickness were cut using rotary microtome (Leica, Germany) and stained with haematoxylin and eosin.

Statistical analysis: Data were analyzed using IBM SPSS statistics 20 software and the results of the biochemical estimations were reported as mean±standard deviation (SD). Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA) and follow up test (LSD). Differences with a $p<0.05$ were considered as statistically significant.

RESULTS

Results of liver enzymes (AST, ALT, ALP): Single acute DM treatment (80 mg kg^{-1}) for 24 h in guinea pigs caused a statistically significant increase ($p<0.001$) in the level of AST, ALT, ALP in serum when compared to control animals, whereas, *Ab* (100 mg kg^{-1}) and silymarin (100 mg kg^{-1}) pre-treatment to the DM-intoxicated animals resulted in a significant normalization ($p<0.001$) of the enzymes activities (Table 1).

Results of liver morphological changes: The liver sections of the guinea pigs treated with DM showed many histological changes. The parenchymatous cells showed cytoplasmic vacuolization and degeneration in nuclei. Moreover, an increase in the number of Kupffer cells in the liver parenchyma was observed. Severe congestion, leucocytes infiltration in the parenchymatous tissue and portal area, enlargement of the hepatic sinusoids and enlargement of the central and the portal veins, clear size of hemorrhage, remarkable dilated

Table 1: Effects of *Achillea biebersteinii* (*Ab*) on serum marker enzymes in DM-intoxicated guinea pigs

Groups	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})
Control	36.40±7.66[a]	71.80±5.76[a]	53.80±8.46[a]
DM (80 mg kg^{-1})	111.00±9.61[b]***	147.20±8.16[b]***	114.40±13.35[b]***
DM+50 mg kg^{-1} *Ab*	95.00±8.00[b]**	116.75±9.61[b]**	97.60±9.23[b]
DM+100 mg kg^{-1} *Ab*	63.20±7.39[c]***	99.00±10.38[c]***	85.60±8.44[c]**
DM+100 mg kg^{-1} Silymarin	53.00±6.76[c]***	94.80±9.54[c]***	73.20±7.19[c]***

Values are expressed as Mean±SD, n= 5 for each treatment group. Means assigned with the same letter show insignificant differences between these values. [a]Significantly different from controls. [b,c]Significantly different from DM-treated animals. Significance *$p<0.05$, high significance **$p<0.01$ and very high significance ***$p<0.001$ compared with control respectively. Significant differences between groups were calculated by ANOVA and follow up test (LSD)

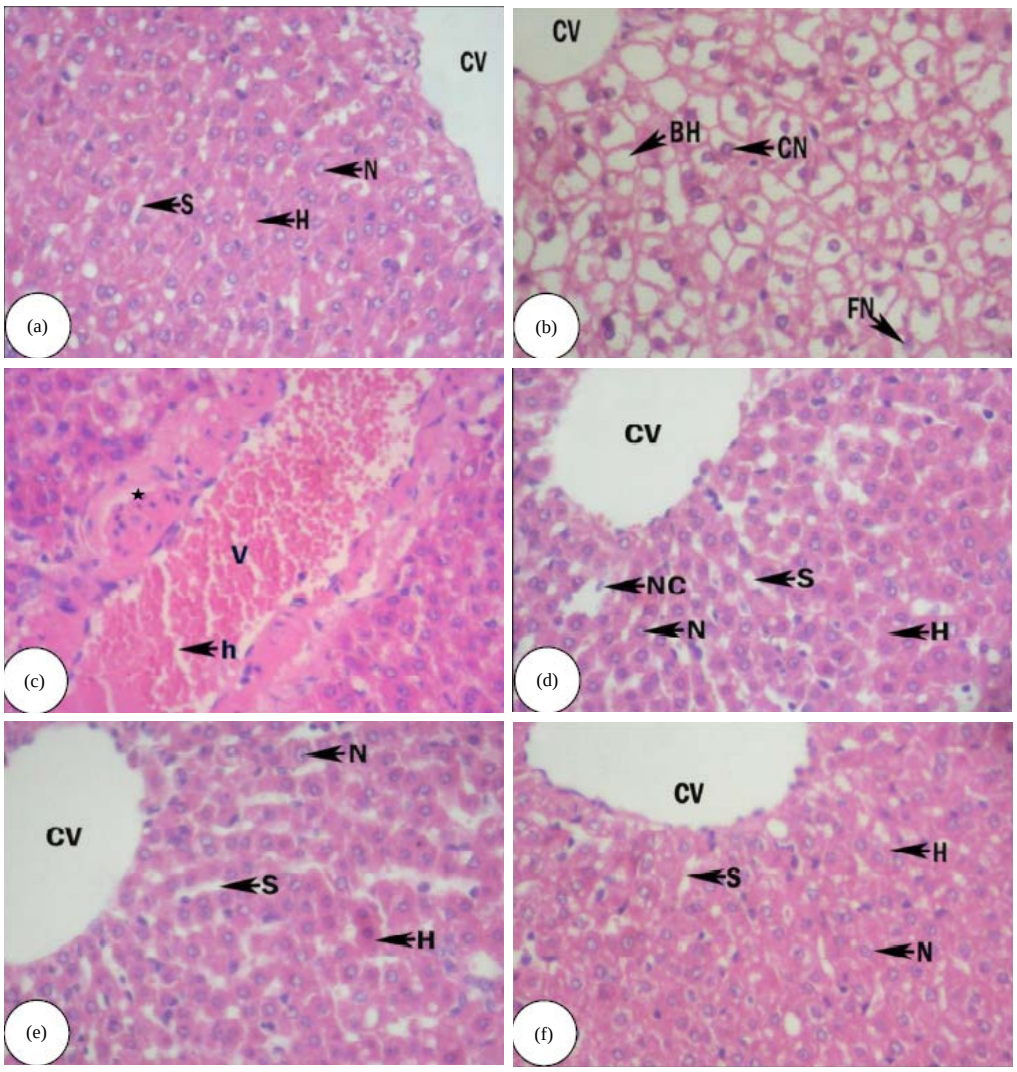

Fig. 1(a-f): Hepatoprotective effect of *Achillea biebersteinii* (*Ab*) against DM-induced hepatotoxicity in guinea pigs. Liver sections were stained with H and E: (a) Normal, (b, c) DM-treated animals, (d) *Ab* (50 mg kg^{-1} b.wt.)+DM, (e) *Ab* (100 mg kg^{-1} b.wt.)+DM and (f) Silymarin (100 mg kg^{-1} b.wt.)+DM, magnification 400X

H: Hepatocytes, CV: Central vein, N: Nucleus, S: Sinusoidal space, BH: Ballooning hepatocytes, V: Dialated vessel, h: Hemorrhage, NC: Necrosis, FN: Fragmented nucleus, CN: Condensed nucleus, *Hepatic degeneration

veins and disruption of hepatic architecture. Changes in the nucleus appeared in the form of condensed chromatin, binucleated cells and fragmented nuclei. The protective effect of 50 and 100 mg kg^{-1} p.o., of aqueous extracts of *Ab* extract produced 30 and 60% inhibition in inflammatory changes against 80% inhibition observed in silymarin treated group (Fig. 1).

DISCUSSION

For several decades, the extensive use of pesticides in most developing countries is becoming an increasingly serious environmental problem due to factors such as water contamination, ecosystem disruption and habitat contamination[34]. It is an established fact that pesticides and some of their metabolites can cause harm to human and the environment[35]. The current study was performed to investigate the hepatotoxicity of a commonly used OP insecticide, DM, in guinea pigs following single acute exposure. In the present study, single acute DM treatment (80 mg kg^{-1}) for 24 h in guinea pigs caused a statistically significant increase in the level of AST, ALT and ALP in serum when compared to control animals. In fact, available data on the hepatotoxicity action of DM were limited for adult

mice[12,36], rat[37-39] and rabbit[11] but there is little data available on the effect of DM on guinea pigs performed previously in our lab[10]. DM also caused a significant hepatic damage, as observed from the elevation of hepatospecific enzyme activities, as well as severe alterations in different liver parameters. The liver seemed to be mostly affected by DM treatment alone, as the liver is the most active mammalian organ in xenobiotic metabolism and contains a larger variety of enzymes for this action. Accordingly, its role in metabolic conversions is its susceptibility to the toxicity from these agents[40]. The co-administration of *Ab* extract and silymarin with DM to guinea pigs resulted in marked improvement of the liver enzymes activities when compared to that which received DM alone. One of the possible explanations for the observed recovery of various enzyme activities involved in the detoxification following *Ab* or silymarin treatment could be because these materials exert their hepatoprotective influence by acting as antioxidants which mediated by its free radical scavenging and modulation of the xenobiotic detoxification process[41]. These findings were in agreement with the results reported by Al-Said *et al.*[42]. There is no information concerning the protective action of *Achillea* species on oxidative injury induced by DM. However, there are several reports concerning with the protective effects of different *Achillea* species against chemical intoxication. For example, Konyalioglu and Karamenderes, (2005) demonstrated that 15 *Achillea* L. species showed protective effects against H_2O_2-induced oxidative damage in human erythrocytes and leucocytes[43]. Also, Yaeesh *et al.*[44] reported that the aqueous-methanol extract of *Achillea millefolium* exhibeted a hepatoprotective, antispasmodic and calcium antagonist activities[44]. In addition, three *Achillea* species extracts showed protective activity against lipid peroxidation, protein oxidation and DNA damage *in vitro*[28]. Whereas, other results obtained by Baris *et al.*[45] showed that the methanol extract of *Ab* showed weaker antioxidative capacity.

Recently, Babelly *et al.*[29] reported that *Ab* showed hypolipidemic effect suggesting the potential protective role in coronary heart disease. Finally, Varasteh-Kojourian *et al.*[31]. showed that two *Achillea* extracts including *Ab* possess remarkable antioxidant activity and could be good natural alternatives to synthetic antioxidants in pharmaceutical and food industries[31]. The increases in plasma transaminase (AST and ALT) were observed in all doses of *Ab* extract on carbon tetrachloride-induced liver damage in rats[46]. The co-administration of *Ab* extract and silymarin with DM to guinea pigs resulted in marked improvement of the liver histology when compared to that which received DM alone. There are several reports supported the role of antioxidant in attenuating the histopathology of some pesticides and toxins in experimental animals. For example, Sutcu *et al.*[47]. revealed a histopathological changes in liver tissue of rats treated with methidathion and the severity of these lesions was reduced by administration of a combination of vitamin C and E. Also, Zama *et al.*[48] concluded that chlorpyrifos can lead to an increase in LPO production in adult and fetal tissues of rat, while treatment with the *Paronychia argentea* extract leads to protection against chlorpyrifos toxicity. In addition, recent published articles demonstrated the hepatoprotective role of vitamins (C and E) and *Withania somnifera* aqueous extract in attenuating DM-induced histopathology in liver of guinea pigs[10,49] were in consistence with the results of the present study. Therefore, a study of some commonly used plant products as antioxidants against xenobiotic-induced oxidative stress, appeared to be of interest. The present study demonstrated that administration of *Ab* extract to guinea pigs modulates the oxidative stress induced by DM insecticide and suggests a possible adaptive mechanism to counteract oxidative stress situation. Hence, such studies on oxidative/antioxidant status during a free radical exposure can be used as an index of protection against xenobiotic challenges. Finally, the exact chemical ingredient that could introduce the protective effect in this study, should be separated and characterized from the plant extract and studied alone. Therefore, these steps were difficult to be done in our lab. So, it is of importance to recommend such study research in the future.

CONCLUSION

The extensive use of pesticides in developing countries by farmers without consideration to their harmful effects and the increasing use of medicinal plants encourage us to design the research. The presented study concluded that guinea pigs administered with *Ab* aqueous extract protect the hepatocytes against an acute dose of DM insecticide, but further studies are required to explain the exact mode of protection. In addition, the characterization of the chemical ingredients that are responsible for the hepatoprotective effect is highly recommended.

SIGNIFICANCE STATEMENTS

This study discovers the positive effect of *Achillea biebersteinii* aqueous extract against dimethoate induced oxidative stress in the liver of guinea pigs. Oral administration of plant extract in different concentration provided protective effect when compared to silymarin group suggesting the

antioxidant capacity of *Achillea biebersteinii*. This study will help the researcher to reveal the critical areas of antioxidants-oxidative stress relationship especially in pesticide toxicity. Thus, a new idea on the use of *Achillea* plant extract to manage pesticide toxicity may be achieved.

ACKNOWLEDGMENTS

The authors are grateful to Afaf A. Obad, Hend Y. Alqade, Salah M. Aboesba, Esam M. Aqlan and Samira Hezabr, Department of Biology, Faculty of Science, Ibb University, Yemen, for their assistance in this study.

REFERENCES

1. Kumar, S.V., M. Fareedullah, Y. Sudhakar, B. Venkateswarlu and E.A. Kumar, 2010. Current review on organophosphorus poisoning. Arch. Applied Sci. Res., 2: 199-215.
2. Balali, M. and K. Balali, 2008. Neurotoxic disorders of Organophosphorus compounds and their managements. Arch. Iran. Med., 11: 65-89.
3. Buyukokuroglu, M.E., M. Cemek, M. Tosun, Y. Yurumez, O. Bas and Y. Yavuz, 2008. Dantrolene may prevent organophosphate-induced oxidative stress and muscle injury. Pestic. Biochem. Physiol., 92: 156-163.
4. Karami-Mohajeri, S. and M. Abdollahi, 2011. Toxic influence of organophosphate, carbamate and organochlorine pesticides on cellular metabolism of lipids, proteins and carbohydrates: A systematic review. Hum. Exp. Toxicol., 30: 1119-1140.
5. Sayim, F., 2007. Histopathological effects of dimethoate on testes of rats. Bull. Environ. Contam. Toxicol., 78: 479-484.
6. Al-Wabel, M.I., M.H. El-Saeid, A.M. Al-Turki and G. Abdel-Nasser, 2011. Monitoring of pesticide residues in Saudi Arabia agricultural soils. Res. J. Environ. Sci., 5: 269-278.
7. El-Saeid, M.H., A.M. Al-Turki, M.I. Al-Wable and G. Abdel-Nasser, 2011. Evaluation of pesticide residues in Saudi Arabia ground water. Res. J. Environ. Sci., 5: 171-178.
8. Sanborn, M., J.K. Kerr, L.H. Sanin, D.C. Cole, K.L. Bassil and C. Vakil, 2007. Non-cancer health effects of pesticides: Systematic review and implications for family doctors. Family Phys., 53: 1712-1720.
9. Attia, A.A. and H.M. Nasr, 2009. Dimethoate-induced changes in biochemical parameters of experimental rat serum and its neutralization by black seed (*Nigella sativa* L.) oil. Slovak J. Anim. Sci., 42: 87-94.
10. Al-Awthan, Y.S., M.A. Al-Douis, G.H. El-Sokkary and E.M. Aqlan, 2012. Dimethoate-induced oxidative stress and morphological changes in the liver of guinea pig and the protective effect of vitamin C and E. Asian J. Biol. Sci., 5: 9-19.
11. Salih, E.M.A., 2010. Toxic effect of dimethoate and diazinon on the biochemical and hematological parameters in male rabbits. Jordan J. Biol. Sci., 3: 77-82.
12. Alarami, A.M.J., 2015. Histopathological changes in the liver and kidney of albino mice on exposure to insecticide, dimethoate. Int. J. Curr. Microbiol. Applied Sci., 4: 287-300.
13. Van Scoy, A., A. Pennell and X. Zhang, 2016. Environmental fate and toxicology of dimethoate. Rev. Environ. Contamin. Toxicol., 237: 53-70.
14. Yarsan, E. and O. Cakir, 2006. Effects of dichlorvos on lipid peroxidation in mice on subacute and subchronic periods. Pest. Biochem. Physiol., 86: 106-109.
15. Mansour, S.A. and A.T.H. Mossa, 2010. Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pestic. Biochem. Physiol., 96: 14-23.
16. Nemeth, E. and J. Bernath, 2008. Biological activities of yarrow species (*Achillea* spp.). Curr. Pharm. Des., 14: 3151-3167.
17. Aqlan, E.M.K., 2008. Studies on the flora of Ibb governorate, Republic of Yemen. M.Sc. Thesis, Department of Biology, Faculty of Science, Sanaa University.
18. Pirbalouti, A. and A. Golparvar, 2007. Evaluation of ethnobotany in the region of Chaharmahal and Bakhtyari, West Central Iran. Proceedings of the 48th Annual Meeting of the Society for Economic Botany, June 4-7, 2007, Chicago, USA.
19. Akkol, E.K., U. Koca, I. Pesin and D. Yilmazer, 2011. Evaluation of the wound healing potential of *Achillea biebersteinii* Afan. (Asteraceae) by *in vivo* excision and incision models. Evidence-Based Complement. Altern. Med., 10.1093/ecam/nep039.
20. Alikhanzade, M., M. Tehranipour and J. Khayyatzade, 2013. The protective effects of alcoholic extracts of *Achillea biebersteinii* leave on density neuroglia cell spinal cord after the sciatic nerve compression in rats. Q. J. Anim. Physiol. Dev., 1: 11-18.
21. Amiri, M.S. and M.R. Joharchi, 2013. Ethnobotanical investigation of traditional medicinal plants commercialized in the markets of Mashhad, Iran. Avicenna J. Phytomed., 3: 254-271.
22. Benedec, D., L. Vlase, I. Oniga, A. Mot and G. Damian *et al*., 2013. Polyphenolic composition, antioxidant and antibacterial activities for two Romanian subspecies of *Achillea distans* Waldst. et Kit. ex Willd. Molecules, 18: 8725-8739.
23. Tuberoso, C.I.G., P. Montoro, S. Piacente, G. Corona and M. Deiana *et al*., 2009. Flavonoid characterization and antioxidant activity of hydroalcoholic extracts from *Achillea ligustica* All. J. Pharm. Biomed. Anal., 50: 440-448.

24. Hosseini, M., F. Harandizadeh, S. Niazamand, M. Soukhtanloo and M. Mahmoudabady, 2013. Antioxidant effect of *Achillea wilhelmsii* extract on pentylenetetrazole (seizure model)-induced oxidative brain damage in Wistar rats. Indian J. Physiol. Pharmacol., 57: 418-424.
25. Trumbeckaite, S., R. Benetis, L. Bumblauskiene, D. Burdulis and V. Janulis *et al*., 2011. *Achillea millefolium* L. s.l. herb extract: Antioxidant activity and effect on the rat heart mitochondrial functions. Food Chem., 127: 1540-1548.
26. Tawaha, K., F.Q. Alali, M. Gharaibeh, M. Mohammad and T. El-Elimat, 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem., 104: 1372-1378.
27. Alfatemi, S.M.H., J.S. Rad, M.S. Rad, S. Mohsenzadeh and J.A.T. da Silva, 2015. Chemical composition, antioxidant activity and *in vitro* antibacterial activity of *Achillea wilhelmsii* C. Koch essential oil on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* spp. Biotech, 5: 39-44.
28. Kizil, M., G. Kizil, M. Yavuz and B. CeKen, 2010. Protective activity of ethanol extract of three *Achillea* species against lipid peroxidation, protein oxidation and DNA damage *in vitro*. Acta Aliment., 39: 457-470.
29. Mais, B., A.M. Yaser and N. Ream, 2016. Hypolipidemic effect of *Achillea biebersteinii* ethanolic extract in hamsters with diet-induced hypercholesterolemia. Int. J. Pharmacogn. Phytochem. Res., 8: 1555-1559.
30. Abd-Alla, H.I., N.M.M. Shalaby, M.A. Hamed, N.S. El-Rigal, S.N. Al-Ghamdi and J. Bouajila, 2016. Phytochemical composition, protective and therapeutic effect on gastric ulcer and α-amylase inhibitory activity of *Achillea biebersteinii* Afan. Arch. Pharm. Res., 39: 10-20.
31. Varasteh-Kojourian, M., P. Abrishamchi, M.M. Matin, J. Asili, H. Ejtehadi and F. Khosravitabar, 2017. Antioxidant, cytotoxic and DNA protective properties of *Achillea eriophora* DC. and *Achillea biebersteinii* Afan. extracts: A comparative study. Avicenna J. Phytomed., 7: 157-168.
32. Tietz, N.W., 1982. Fundamental of Clinical Chemistry. W.B. Saunders Co., Philadelphia, pp: 674.
33. King, J., 1965. The Hydrolases-Acid and Alkaline Phosphatases. In: Practical Clinical Enzymology, King, J. (Ed.). Van Nostrand Company Limited, London, UK., pp: 191-208.
34. Marquis, S., 2013. Agrochemical use in South Africa: The toxicological and sociological impacts of agrochemicals on susceptible populations in South Africa's agricultural sector. Ph.D. Thesis, Cape Town University, South Africa.
35. UNEP., 2010. UNEP emerging issues: Global honey bee colony disorder and other threats to insect pollinators. United Nations Environment Programme, Nairobi, Kenya.
36. Sivapiriya, V., Jayanthisakthisekaran and S. Venkatraman, 2006. Effects of dimethoate (O,O-dimethyl S-methyl carbamoyl methyl phosphorodithioate) and Ethanol in antioxidant status of liver and kidney of experimental mice. Pestic. Biochem. Physiol., 85: 115-121.
37. Abu El-Saad, A.M. and M.S.A. Elgerbed, 2010. Dimethoate-induced hepatotoxicity in rats and the protective roles of vitamin e and n-acetylcysteine. Egypt. J. Exp. Biol. (Zool.), 6: 219-230.
38. Ben Amara, I., N. Soudani, A. Troudi, H. Bouaziz, T. Boudawara and N. Zeghal, 2011. Antioxidant effect of vitamin E and selenium on hepatotoxicity induced by dimethoate in female adult rats. Ecotoxicol. Environ. Saf., 74: 811-819.
39. Salim, A.B., A.A.K. Abou-Arab, S.R. Mohamed and T.A. Eldesouky, 2014. Influence of pomegranate (*Punica granatum* L.) on dimethoate induced hepatotoxicity in rats. World Acad. Sci. Eng. Technol., 8: 925-930.
40. Seif, H.S.A., 2016. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. Beni-Suef Univ. J. Basic Applied Sci., 5: 134-146.
41. Thilakchand, K.R., R.T. Mathai, P. Simon, R.T. Ravi, M.P. Baliga-Rao and M.S. Baliga, 2013. Hepatoprotective properties of the Indian gooseberry (*Emblica officinalis* Gaertn): A review. Food Funct., 4: 1431-1441.
42. Al-Said, M.S., R.A. Mothana, M.M. Al-Yahya, S. Rafatullah and M.O. Al-Sohaibani *et al*., 2016. GC-MS analysis: *In vivo* hepatoprotective and antioxidant activities of the essential oil of *Achillea biebersteinii* Afan. growing in Saudi Arabia. Evid. Complement. Altern. Med., 10.1155/2016/1867048.
43. Konyalioglu, S. and C. Karamenderes, 2005. The protective effects of *Achillea* L. species native in Turkey against H_2O_2-induced oxidative damage in human erythrocytes and leucocytes. J. Ethnopharmacol., 102: 221-227.
44. Yaeesh, S., Q. Jamal, A.U. Khan and A.H. Gilani, 2006. Studies on hepatoprotective, antispasmodic and calcium antagonist activities of the aqueous-methanol extract of *Achillea millefolium*. Phytother. Res., 20: 546-551.
45. Baris, O., M. Gulluce, F. Sahin, H. Ozer and H. Kilic *et al*., 2006. Biological activities of the essential oil and methanol extract of *Achillea biebersteinii* Afan. (Asteraceae). Turk. J. Biol., 30: 65-73.
46. Hosbas, S., A. Hartevioglu, M. Pekcan and D.D. Orhan, 2011. Assessment of hepatoprotective activity of *Achillea biebersteinii* ethanol extract on carbon tetrachloride-induced liver damage in rats. FABAD J. Pharm. Sci., 36: 33-39.
47. Sutcu, R., I. Altuntas, B. Yildirim, N. Karahan, H. Demirin and N. Delibas, 2006. The effects of subchronic methidathion toxicity on rat liver: Role of antioxidant vitamins C and E. Cell Bio.Toxi., 22: 221-227.
48. Zama, D., Z. Meraihi, S. Tebibel, W. Benayssa, F. Benayache, S. Benayache and A.J. Vlietinck, 2007. Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: The protective role of the butanolic extract of *Paronychia argentea* L. Indian J. Pharm., 39: 145-150.
49. Al-Awthan, Y.S., S.M. Hezabr, A.M. Al-Zubairi and F.A. Al-Hemiri, 2014. Effects of aqueous extract of *Withania somnifera* on some liver biochemical and histopathological parameters in male Guinea pigs. Pak. J. Biol. Sci., 17: 504-510.