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Hepatoprotective effects of *Allium sativum* and *Withania somnifera* on ochratoxin A-induced toxicity in rats

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Abstract
Withania somnifera and *Allium sativum* are the traditional medicinal plants used to improve the liver functions. Since, oxidative stress promotes the ochratoxin A (OTA)-induced hepatotoxicity, we evaluated the hepatoprotective effect of the ethanolic extracts of *A. sativum* (20 mg/kg, p.o) bulb and *W. somnifera* (100 mg/kg, p.o) root on OTA (2 mg/kg feed)-induced toxicity in rats. A total of 120 rats were randomly divided into eight groups of 15 animals each and 5 animals each were sacrificed at 3, 6 and 9 weeks intervals. The liver was isolated, blotted free of blood and processed for biochemical analysis. OTA time-dependently increased lipid peroxidation (LPO), serum biomarkers (ALT, AST and LDH) with histopathological alteration in OTA treated rats. Further, OTA depleted thiol pool and decreased the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR). *W. somnifera* root alone reduced -LPO level, -ALT activity, -histopathological changes and increased antioxidants in liver. *A. sativum* alone significantly reduced LPO and elevated SOD without any alterations in other biochemical parameters. Combination of extracts more effectively reduced OTA-induced alterations in liver. It is concluded that ethanolic extract of *A. sativum* and *W. somnifera* can prevent OTA-induced hepatotoxicity in rats through its antioxidant activities.

Keywords: ochratoxin a, *Withania somnifera*; *Allium sativum*; oxidative stress; liver; rats

Introduction
Ochratoxin A (OTA) is a naturally occurring mycotoxin produced by several species of *Aspergillus* and *Penicillium*. Its widespread occurrence has been reported in a variety of food commodities such as cereals, green coffee, cocoa, dried fruits, and meat products. OTA has been shown to be hepatonephrotoxic [1] and several workers observed oxidative stress as important mechanism of OTA-induced toxicity in liver of rats [2-4]. OTA has been reported to increase malondialdehyde (MDA) formation *in vitro* upon incubation with rat liver microsomes in the presence of NADPH [5]. OTA stimulates lipid peroxidation (LPO) by complexing Fe^{3+} and facilitating its reduction [6]. Based on these facts, it is conceivable that natural antioxidants may alter the OTA-induced toxicity in liver.
Phytochemicals are potent free radical scavengers and the root extract of *W. somnifera* (Ashwagandha) tends to reverse the changes in LPO and damage to cells [7-8]. The roots of *W. somnifera* contain several alkaloids and withanolides, a few flavonoids and reducing sugars in which, the major active compounds of the roots are believed to be withanolides [9]. Garlic (*Allium sativum* L.) contains two main classes of antioxidant components, namely flavonoids [10] and sulfur-containing compounds [11]. Organosulfur compounds of garlic have been reported to modulate mutagenesis, metabolism and DNA binding potential of aflatoxin B_1 [12-13]. Utilization of mycotoxin-binding adsorbents such as, activated charcoal (AC) is the most common way of protecting animals from its toxicity. However, the activity of AC was differentially reported by several workers [14-15]. Despite widespread prevalence of OTA, no effective ameliorative measures are available to counteract adverse effects of OTA affected animals and poultry. In the present study, we evaluated the ameliorative efficacy of ethanolic extracts of *A. sativum* and *W. somnifera* individually and in combination, on OTA-induced hepatic damage in Wistar rats. We also investigated the protective role of AC with combination of alcoholic extracts in liver of rats.

Materials and Methods
Experiment Animals
The study was conducted in apparently healthy adult male Wistar rats (21 days) procured from the Laboratory Animals Resources Section of the Institute.

All animals were housed in polypropylene cages with chopped wheat straw as the bedding material. All the procedures, conducted on the experimental animals were duly approved by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Rats were given formulated feed (tested to be free of aflatoxin B_1 and ochratoxin-A) obtained from Feed Technology Unit of IVRI, and distilled water *ad libitum* throughout the experiment. Prior to experiment, all the animals were kept in the laboratory conditions for a period of 7 days or more for acclimatization.

OTA production, Purification and Analysis

Freeze dried culture of *Aspergillus ochraceus* NRRL-3174, procured from National Centre for Agricultural Utilization and Research, Peoria, Illinois, (USA) was grown and maintained on potato dextrose agar medium (M/s Hi Media) and sub-cultured at regular intervals. OTA production was done as per the method described by Trenk *et al.* [16], with some modifications. To prepare substrate, ingredients like maize grains (40%), soybean seeds (20%) and groundnut seeds (20%) were partially ground. Approximately 100 g quantity of the above mixture was taken in 500 ml sterilized flasks, soaked with distilled water (11%) for 2 h. A loop full inoculum containing freshly grown mycelia was added to each flask under proper sterilisation conditions and kept in B.O.D. incubator at 26±1ºC. To facilitate uniform growth of fungus, the flasks were shaken thrice a day, regularly. After the adequate quantity of fungal growth (approximately 3 weeks time of incubation), flasks were autoclaved at 15 lb pressure for 30 min. to destroy the mycelia and spores, followed by drying the culture at 80ºC for 8 h and grinding to a fine powder for the toxin extraction and purification purposes.

Toxin was extracted and purified from oven dried culture by the procedure of AOAC [17] with appropriate modifications. Approximately 100 g finely ground cultured sample was shaken with 500 ml of chloroform for 30 min and then contents were filtered to obtain clear filtrate containing OTA which was concentrated to 25-30 ml in a vacuum evaporator. The resultant extract (15 ml) was analysed by thin layer chromatography and spectrophotometry. Further, the quantification of toxin was confirmed at Animal Feed Analytical and Quality Control Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu (India) and made into aliquots in clean and dry amber coloured vials and stored at 4^0C until used.

Preparation of experimental diets

Cultured maize powder containing known amount of OTA was added to the basal rations (which were tested negative for the presence of OTA) in such a proportion that the concentration of OTA in diet was 2 ppm. Aliquots were taken from the mixed diet and toxin was quantified by above procedures to ensure the proper mixing of cultured maize.

Preparation of alcoholic extract of the plant materials

Authenticated plant materials (bulbs of *A. sativum* and roots of *W. somnifera*) were procured from authorized local Ayurvedic medical shop, Bareilly and processed plant materials (freshly crushed bulbs of *A. sativum*, shade dried and powdered roots of *W. somnifera*) were then subjected to estimation of OTA to rule out OTA contamination, if any, and were extracted with 70% ethanol. The prepared ethanolic extracts were stored at 4^0C, and before administration, extracts were dissolved in Tween 80 in such a way that the final concentration of the later should not exceeds 1% and then were administered to rats by oral gavage at the dose of 20 mg/kg bw for *A. sativum* bulb extract (ABE) and 100 mg/kg bw for *W. somnifera* root extract (WRE).

Experimental design

Male Wistar rats were divided randomly into eight groups, 15 animals each. The rats were housed in an animal facility at 22±1°C with 12-h light-dark cycle, controlled humidity and circulation of air. Then these groups were treated as follows: group I served as control; group II were given OTA @ 2 ppm in diet ad lib for a maximum period of 9 weeks.; group III, ABE; group IV, WRE; group V, and VI were given OTA along with ABE and WRE alone respectively; group VII was given both extract simultaneously with OTA; group VIII, like group VII along with AC (0.5%). OTA and AC were administered through feed while, extracts given through oral gavage. Animals in different groups were sacrificed (5 rats) under light chloroform anesthesia at each interval (3 wks) and liver was removed and homogenized in ice cold phosphate buffer solution (PBS; pH 7.4). Blood samples were collected by cardiac puncture during the sacrifice of the animals.

Assessment of hepatotoxicity

To assess hepatic function and cellular injury, activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenous (LDH) were measured using commercially available kits (Span Diagnostics Ltd., India).

Assessment of oxidative stress and antioxidants

A 200 mg of liver sample was taken in 2 ml of ice-cold PBS. Another 200 mg of sample was separately taken in 2 ml of 0.02 M EDTA for GSH estimation. The homogenate (10%) prepared with homogenizer (IKA, Germany) under ice-cold condition was centrifuged for 10 min at 3000 rpm and the supernatant was stored at -20 ºC until assay. LPO was evaluated in terms of malondialdehyde (MDA) production [18]. Total (T-SH), protein bound (PB-SH) thiol groups and reduced glutathione (GSH) in the tissue homogenate were determined as per the method of Sedlak and Lindsay [19]. The molar extinction coefficient of 13100 at 412 nm was used for the determination of thiol contents and the values were expressed in mmol/g of wet tissue. The PB-SH was calculated by subtracting the NP-SH (GSH) from the T-SH [19].

Superoxide dismutase (SOD) activity was measured following the method of Madesh and Balasubramanian [20]. One unit of SOD activity was defined as the quantity of enzyme that inhibited pyrogallol autooxidation by 50% under the given experimental conditions. Catalase activity was estimated by measuring the breakdown of hydrogen peroxide in the reaction mixture using a spectrophotometer at the wave length of 240 nm and the values were expressed in mmol H_2O_2 utilized/mg of protein [20]. The enzyme *Glutathione reductase (GR) activity* was assayed by the method of Goldberg and Spooner [22]. The activity has been expressed as µmol NADPH utilized/min/mg protein. Protein concentrations were determined by the method of Lowry *et al*. [23] calibrated with bovine serum albumin.

Histological examination

Tissues for histological examination were taken from liver and fixed in 10% neutral buffered formalin. The fixed tissues

were processed for paraffin embedding, sectioned at 4-5 µ thickness and stained with haematoxylin-eosin.

Statistical analysis

Data have been expressed as mean ± SEM. The OTA-mediated changes were compared with the control group, while ameliorative effects of alcoholic extracts were compared with OTA exposed group. Statistical analysis of data was performed using SPSS 11.0.1 software. Data were analyzed by ANOVA and means were compared with Duncan's multiple comparison *post-hoc* test. A value of $p<0.05$ was considered statistically significant.

Results

Assessment of hepatotoxicity

OTA exposure significantly increased ALT, AST and LDH activities indicated liver injury (Tab. 1). The enzyme levels were significantly reduced in animals of amelioration groups when compared with OTA group. Total protein content was decreased in toxicated group at 3, 6 and 9 weeks, while it was significantly increased among the amelioration groups.

Assessment of oxidative stress

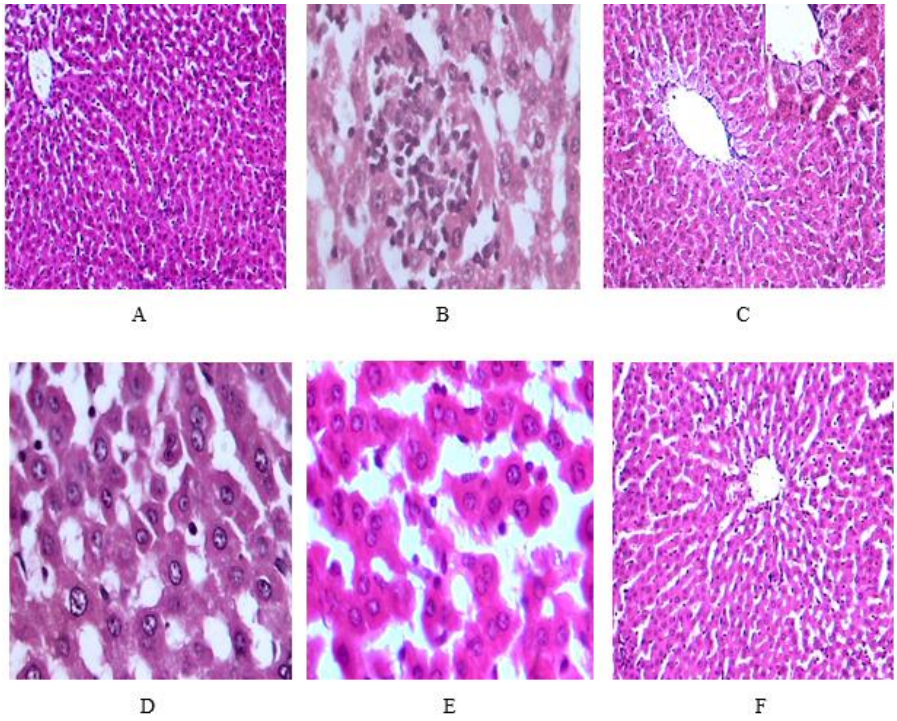
The levels of MDA concentration, an end product indicative of the degree of lipid peroxidation and thiol status in liver of rats of different groups are presented in Table 2. MDA levels in liver showed increasing trend from 3 weeks onwards in the OTA fed groups. Amelioration groups revealed significantly lower MDA levels. Liver thiol (T-SH, GSH and PB-SH) levels were significantly decreased at 6 and 9 weeks in OTA fed rats while, WRE alone or combination with ABE and AC significantly elevated thiol status.

The activities of antioxidants enzymes in liver of different groups are presented in Table 3. The activities of SOD, CAT and GR were significantly diminished in the OTA-intoxicated group over the time course. Treatment with ABE did not show any significant difference in CAT and GR activities as compared to OTA-treated rats. However, the activities of SOD augmented significantly in ABE treated rats. WRE alone or combination of WRE with ABE and AC significantly increased the activities of antioxidant enzymes (SOD, CAT and GR) than OTA treated group.

Table 1: Preventive effect alcoholic extract of ABE and WRE on serum biochemical variables indicative of hepatic injury during OTA exposure in male rats for 9 weeks

Parameters	Interval	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
ALT (KA units/ml)	3wk	41.68±4.59^{b}	75.66±10.25^{a}	44.18±3.94^{b}	42.58±4.52^{b}	64.68±7.31	55.68±7.59	51.94±8.71^{b}	48.78±7.582^{b}
	6wk	39.60±4.27^{c}	83.46±12.12^{a}	40.76±4.43^{c}	40.72±4.94^{c}	72.66±8.67^{ab}	61.62±7.47	62.86±8.17	53.72±7.002^{bc}
	9wk	40.58±5.09^{c}	81.34±11.20^{a}	43.08±4.92^{c}	43.18±4.13^{c}	69.64±7.65^{ab}	54.14±18.78^{bc}	53.24±7.19^{bc}	49.72±8.75^{bc}
AST (KA units/ml)	3wk	48.28±3.10^{c}	83.54±8.99^{a}	46.22±4.10^{c}	48.84±4.54^{bc}	70.28±7.81^{ab}	60.68±7.85^{bc}	59.60±6.92^{bc}	54.76±7.93^{bc}
	6wk	46.38±4.19^{c}	91.46±9.43^{a}	45.30±4.27^{c}	45.00±3.73^{c}	79.38±8.58^{ab}	71.44±7.68^{ab}	69.24±9.26^{ab}	60.70±7.06^{bc}
	9wk	48.00±3.57^{cd}	88.54±8.32^{a}	45.72±3.80^{d}	46.74±3.66^{d}	75.34±9.21^{ab}	69.34±7.88^{abc}	64.34±9.31^{bcd}	57.84±6.90^{bcd}
LDH (IU/L)	3wk	56.36±3.74^{b}	84.42±8.19^{a}	58.68±4.04^{b}	56.96±4.55^{b}	72.72±10.46	64.20±10.09	63.80±7.79	59.72±8.12
	6wk	53.04±3.61^{c}	98.46±10.66^{a}	53.74±5.10^{c}	54.10±4.93^{c}	85.08±10.55^{ab}	75.38±10.18	70.52±8.60^{bc}	65.74±6.92^{bc}
	9wk	54.64±4.54^{b}	90.94±9.89^{a}	56.58±3.57^{b}	56.04±4.57^{b}	76.70±10.05	70.34±7.04	65.14±8.63^{b}	61.74±7.61^{b}
TP (g/dl)	3wk	7.24±0.26^{a}	6.28±0.36^{bA}	7.14±0.16^{a}	7.32±0.10^{a}	6.84±0.11^{A}	7.00±0.18^{a}	7.08±0.20^{aA}	7.16±0.133^{aA}
	6wk	7.12±0.10^{a}	5.56±0.21^{c}	7.12±0.26^{a}	7.12±0.13^{a}	5.88±0.26^{bcB}	6.30±0.33^{b}	6.36±0.27^{b}	6.52±0.25^{abB}
	9wk	7.20±0.27^{a}	5.12±0.19^{cB}	7.20±0.14^{a}	7.26±0.15^{a}	5.82±0.24^{bB}	6.18±0.24^{b}	6.20±0.26^{bB}	6.40±0.19^{bB}

ALT: Alanine aminotransferase (KA units/ml); AST: Aspartate aminotransferase (KA units/ml); LDH: Lactate dehydrogenous (IU/L). Means bearing at least one common superscript small letters do not differ significantly between groups and capital letters do not differ significantly within groups (P<0.05). Group I (Control), Group II (OTA), Group III (ABE: *A. sativum* bulb extract), Group IV (WRE: *W. somnifera* root extract), Group V (ABE+OTA), Group VI (WRE+OTA), Group VII (ABE+ WRE+OTA), Group VIII (ABE+ WRE+AC+OTA).



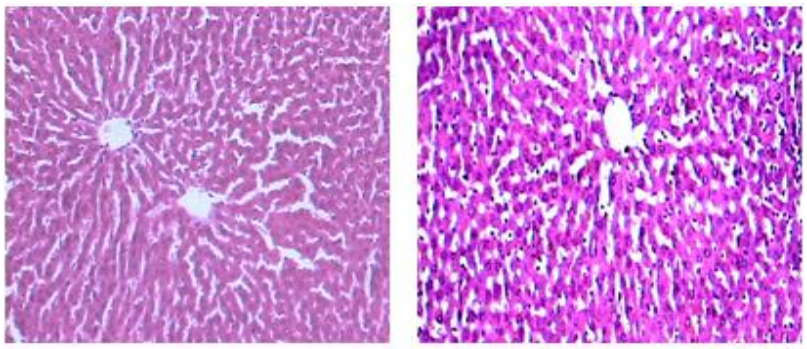


Fig 1: Hepatoprotective effect of alcoholic extract of ABE and WRE on OTA-induced histopathological changes in rats. (A) Normal liver architechture; (B, C, D & E) OTA-induced changes at 3, 6 & 9 weeks, respectively; WRE alone or combination with ABE administered rats showed milder histological alterations (F, G) while, normal histological details were observed at all intervals in rats received extracts with activated charcoal (H)

Histopathology

Liver in rats of control and extracts administered group did not show any significant microscopic alterations and revealed normal histologic details (Fig. 1A). In OTA fed rats**,** at 3 weeks interval revealed hepatocytic degeneration and necrosis with indistinct cytoplasmic boundaries and focal area of MNC infiltration (Fig. 1B). At 6 weeks, severe degeneration of hepatocytes surrounding the central vein revealing rarefied to coarsely granulated cytoplasm with occasional binucleation were observed in three cases (Fig. 1C). Distorted hepatic cords with individualization of hepatocytes with hepatocytomegaly, karyomegaly and binucleations were also observed at 6 weeks (Fig. 1D). At 9 weeks, the changes were similar to those observed at 6 weeks but with increase in severity (Fig. 1E). Rats exposed to OTA and ABE revealed, moderate degree of disruption of hepatic cords and focal infiltration of mononuclear cells (three out of five cases) in the portal region as well as in hepatic parenchyma at 9 weeks. However, in WRE alone or combination with ABE administered rats showed milder distortion of hepatic cords with prominence of kupffer cells (Fig. 1F and 1G) while, normal histological details were observed at all intervals in rats of group VIII (Fig. 1H).

Discussion

We evaluated the ameliorative efficacy of ethanolic extracts of *W. somnifera* and *A. sativum* individually and in combination, on OTA-induced hepatic damage in Wistar rats. The major findings are i) OTA increased hepatocyte integrity markers *viz*. ALT, AST and LDH activities while, WRE alone or its combination with ABE reduced its activities ii) the magnitude of OTA-induced hepatopathies were duration-dependent while, milder histopathological lesions were observed in ABE or WRE treated rats at all intervals iii) OTA time-dependently increased LPO in liver whereas, alcoholic extracts were decreased OTA-induced LPO when they administered alone or in combinations iv) OTA-mediated reduction in antioxidants status were augmented by WRE alone or its combination with ABE v) administration of lower concentration of adsorbent along with alcoholic extracts effectively counteracted the OTA-induced LPO, antioxidants and histological alterations.

Levels of ALT and AST are most frequently used biomarkers of liver injury in rats and enhanced or diminished severity of hepatic lesions can be assessed by measurement of these enzyme activities. In our study, significant increase of serum ALT, AST and LDH activities reflect OTA-induced liver injury and alteration of cell membrane integrity. Observed results are coinciding with findings of other investigators [24-25]. Serum ALT, AST and LDH activities were reduced in ameliorative group suggests hepatoprotective effect of WRE [8-26] and ABE [27], as evidenced by lesser magnitude of histological changes in these group. The observed histopathological changes including swollen hepatocytes with granular or vacuolated cytoplasm, karyomegaly, double nuclei and anisokaryosis, proliferation of bile ductules and mononuclear cell infiltration in the portal areas were accordance with OTA-induced hepatopathies in earlier studies in rats [28-30] and poultry [31]. The observed degenerative changes in OTA treated rats might also have caused decreased synthesis of proteins [32] as reflected in the form of hypoproteinemia in these rats. The milder histopathological lesions observed in plant extracts treated rats exposed to OTA would be attributed to the efficacy of ABE, WRE and AC as ameliorating agents in ochratoxicosis.

In the present study OTA exposure increased LPO accompanied with reduction of GSH levels and activities of antioxidant enzymes viz, SOD, CAT and GR indicating OTA-induced oxidative damage in liver and corroborated well with the findings of other researchers [1, 33-34]. The observed inverse relationship between LPO and antioxidants and positive correlation between thiols and antioxidant enzymes suggest that these antioxidant systems play an important role in OTA-induced toxicity. The increase in LPO could be due to OTA-mediated increase of iron release, which is involved in fenton reaction [6]. The observed GSH depletion and higher LPO levels with excess level of free iron could have enhanced $OH^{\bullet}$ formation via fenton reaction [35] in these rats. The structural and functional significance of OTA-induced LPO can be substantiated by tissue damage revealed by higher ALT, AST and LDH activities and histopathological changes. This also supported the hypothesis that oxystress associated with LPO was an important mechanism of OTA toxicity [36-37].

In this study, ethanolic extracts enhanced the activities of antioxidant enzymes (SOD, CAT, and GR), non enzymatic antioxidants (TSH, GSH and PBSH) and diminished LPO level in OTA-intoxicated rats, suggesting that the reduction of oxidative stress plays a role in the mechanism of its hepatoprotective effects. Baudrimont *et al*. [38] reported that in the presence of SOD and CAT, the MDA production induced by OTA was decreased. Quantification of ameliorative efficacy revealed, *A. sativum* had lesser efficacy than *W. somnifera* when they administered alone with reference to ALT (15% Vs 33%), LPO (20% Vs 32%) and GSH (10% Vs

62%) which was well corroborated with histopathological findings. The *W. somnifera*-mediated higher efficacy was found to be mostly through improvement in thiol status. Chong and Rahimtula, [39] reported that hepatoprotection afforded by antioxidant against OTA was mediated by enhancing hepatic thiol status rather than by inhibiting OTA metabolism. Therefore, the observed beneficial effects of *W. somnifera* could be due to its antioxidative, antiperoxidative and free radical quenching posterities [7, 40-41] In our study, *A. sativum* alone decreased LPO without any appreciable alteration in antioxidants and liver injury markers levels. This indicates that the partial protection of hepatic LPO in ABE treated rats and suggested LPO cascade could be independent with alteration of antioxidant systems. For instance, Manimaran *et al.* [42] reported that decrease of LPO were due to reduction in the excessive production of free radicals rather than the alteration in the antioxidants in rat liver. Scavenging of free radicals is an important mechanism for the inhibitory activity of garlic towards LPO [43]. They also suggested that differential effect of garlic, could be due to differences in the method of sample extraction, potential antioxidant compounds, chemical structures, experimental conditions used in different assays. Hepatoprotective effect of ethanolic extracts of *A. sativum* could be due antioxidant, antiperoxidative and free radical quenching posterities [44-46].

In this study, 0.5% of AC reduced the OTA absorption from the intestine when administered along with extracts suggested its efficacy, which was also evidenced by milder histological lesions and improved antioxidant status in liver of these rats. Although, beneficial effects of AC was confirmed in earlier studies [14, 47], it was included at higher levels (5-10 % in feed) in these studies and 0.5 % of AC or super-activated charcoal was found to be ineffective against aflatoxicosis [24] and trichothecenes [48]. It suggests that administration of lower concentration of adsorbent along with alcoholic extracts may effectively counteract the OTA-induced hepatotoxicity.

Conclusion

Hepatoprotective effect of alcoholic extracts of *A. sativum* bulb and *W. somnifera* roots could be attributed to enhancement of antioxidative enzymes activities, thiols status and lipid peroxidation. Since, high concentration of adsorbents may impair essential nutrient utilization; incorporation of plant extracts with adsorbent will be an effective alternative for amelioration of ochratoxicosis.

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References

1. Abdel-Wahhab MA, Abdel-Galil MM, El-Lithey M. Melatonin counteracts oxidative stress in rats fed an ochratoxin A contaminated diet. J Pineal Res. 2005; 38(2):130-135.
2. Gagliano N, Donne ID, Torri C, Migliori M, Grizzi F, Milzani A *et al*. Early cytotoxic effects of ochratoxin A in rat liver: a morphological, biochemical and molecular study. Toxicol. 2006; 225:214-224.
3. Kamp HG, Eisenbrand G, Janzowski C, Kiossev J, Latendresse JR, Schlatter J, *et al*. Ochratoxin A induces oxidative DNA damage in liver and kidney after oral dosing to rats. Mol Nutri Food Res. 2005; 49:1160-1167.
4. Mally A, Völkel W, Amberg A, Kurz M, Wanek P, Eder E *et al*. Functional, biochemical, and pathological effects of repeated oral administration of ochratoxin A to rats. Chem Res Toxicol. 2005; 18:1242-1252.
5. Rahimtula AD, Bereziat JC, Bussacchini GV, Bartsch H. Lipid peroxidation as a possible cause of ochratoxin A toxicity. Biochem Pharmacol. 1988; 37:4469-4477.
6. Omar RF, Hasinoff BB, Mejilla F, Rahimatula AD. Mechanism of ochratoxin A stimulated lipid peroxidation. Biochem Pharmacol. 1990; 40:1183.
7. Dhuley JN. Adaptogenic and cardioprotective action of ashwagandha in rats and frogs. J Ethnopharmacol 2000; 70:57-63.
8. Bhattacharya A, Ramanathan M, Ghosal, Bhattacharya SK. Effect of *Withania somnifera* glycowithanolides on iron induced heopatotoxicity in rats. Phytotherapy Res. 2000; 14:568-70.
9. Ganzera M, Choudhary MI, Khan IA. Quantitative HPLC analysis of withanolides in Withania somnifera. Fitoterapia. 2003; 74(9):68-76.
10. Bozin B, Dukic NM, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (*Allium sativum L.*, Alliaceae). Food Chem. 2008; 111:925-929.
11. Kodera Y, Suzuki A, Imada O, Kasuga S, Sumioka I, Kazenawa A, *et al*. Physical, chemical, and biological properties of S-allylcysteine, an amino acid derived from garlic. J Agri and Food Chem. 2002; 50:622-632.
12. Yamasaki T, Teel RW, Lau BHS. Effect of allixin, a phytoallexin produced by garlic, on mutagenesis, DNA-binding and metabolism of aflatoxin B1. Cancer. Lett. 1991; 59:89-94.
13. Sheen L, Wu C, Li C, Tsai S. Effect of diallyl sulfide and diallyl disulfide, the active principles of garlic, on the aflatoxin B(1)-induced DNA damage in primary rat hepatocytes. Toxicol Lett. 2001; 122:45-52.
14. Bauer J. Moglichkeiten zur Entgiftung mykotoxinhaltiger Futtermittel. Monatsh Vet Med. 1994; 49:175-181.
15. Kubena LF, Huff WE, Harvey RB, Corrier DE. Influence of ochratoxin A and deoxynivalenol on growing broiler chicks. Poultry Sci. 1988; 67:253-260.
16. Trenk HL, Butz ME, Chu FS. Production of ochratoxin in different cereal products by *Aspergillus ochraceus.* Applied Microbiol. 1971; 21:1032-1035.
17. AOAC. Official Method of Analysis. 16th (ed.), Association of Official Analytical Chemistry, Washington, DC, 1995.
18. Shafiq-Ur-Rehman. Lead-induced regional lipid peroxidation in brain. Toxicol Lett. 1984; 21:333-337.
19. Sedlak J, Lindsay RH. Estimation of total, protein-bound and nonprotein bound sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25(1):192-205.
20. Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J Biochem and Biophy. 1998; 35(3):184-188.
21. Aebi HE. Catalase, in: Bergmeyer, H.U., Bergmeyer, J., Grabi, M. (Eds.), Methods of Enzymatic Analysis, 3rd ed., vol. III, Verlag Chemie, Weinheim, 1983, 273-286.
22. Goldberg DM, Spooner RJ. Glutathione reductase, in: Bergmeyer HU, Bergmeyer J, Grabi M (Eds.), Methods of Enzymatic Analysis, 3rd ed, Weinheim, Germany, 1983; III:258-265.

23. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951; 193(1):265-275.
24. Kubena LF, Huff WE, Harvey RB, Corrier DE. Influence of ochratoxin A and deoxynivalenol on growing broiler chicks. Poultry Sci. 1988; 67:253-260.
25. Kumar M, Dwivedi P, Sharma AK, Telang AG, Singh ND, Patil RD, *et al*. Ochratoxin A and citrinin induced biochemical changes in New Zealand White rabbits. Indian J Vet Pathol. 2007; 31(2):135-139.
26. Saxena M, Faridi U, Srivastava SK, Darokar MP, Mishra R, Pal A *et al*. A cytotoxic and hepatoprotective agent from *Withania somnifera* and biological evaluation of its ester derivatives. Natural Product Communications. 2007; 2:775-778.
27. Ezeala CC, Nweke IN, Unekwe PC, El-Safty IA, Nwaegerue E. Fresh garlic extract protects the liver against acetaminophen-induced toxicity. J Nutr Wellness. 2009; 7(1).
28. Albassam MA, Yong SI, Bhatnagar R, Sharma AK, Priog MG. Histopathologic and electron microscopic studies on the acute toxicity of ochratoxin a in rats. Vet Pathol. 1987; 24(5):427-435.
29. Satheesh CC, Sharma AK, Dwivedi P, Prasanna K, Rahul S. Immunosuppressive effect of ochratoxin A in Wistar rats. J Ani Vet Advan. 2005; 4(6):603-609.
30. Prasanna K, Sharma AK, Dwivedi P, Satheesh CC. Pathomorphology of subchronic effect of low doses of ochratoxin A on, liver, lymphoid organs and testes in Wistar rats. Indian J Vet Pathol. 2006; 30(1):21-25.
31. Dwivedi P, Burns RB. Pathology of ochratoxicosis A in young broiler chicks. Res Vet Sci. 1984; 36:92-103.
32. Rahimtula AD, Bereziat JC, Bussacchini GV, Bartsch H. Lipid peroxidation as a possible cause of ochratoxin A toxicity. Biochem Pharmacol. 1988; 37:4469-4477.
33. Abdel-Wahhab MA. Antioxidant and radical scavenging effects of garlic and cabbage extracts in rats fed ochratoxin-contaminated diet J Egypt Med Assoc. 2000; 83(1-6):1-19.
34. Meki AR, Hussein AA. Melatonin reduces oxidative stress induced by ochratoxin a in rat liver and kidney. Comparative Biochemistry and Physiology Part C: Toxicol Pharmaco. 2001; 130(3):305-13.
35. Hoehler D, Marquard RR, McIntosh AR, Hatch G. Induction of free radicals in hepatocytes, mitochondria and microsomes of rats by ochratoxin A and its analogues. Biochem and Biophy Acta. 1997; 1357:225-233.
36. Gautier JC, Holzhaeuser D, Markovic J, Gremaud E, Schilter B, Turesky RJ. Oxidative damage and stress response from ochratoxin A exposure in rats. Free Radical and Biol Med. 2001; 30:1089-1098.
37. Baldi A, Losio MN, Cheli F, Rebucci R, Sangalli L, Fusi E. Evaluation of the protective effects of alpha-tocopherol and retinol against ochratoxin A cytotoxicity. Brit J Nutri. 2004; 91:507-512.
38. Baudrimont I, Ahouandjivo R, Creppy EE. Prevention of lipid peroxidation induced by ochratoxin A in vero cells in culture by several agents. Chem Biol Inter. 1997; 104(1):29-40.
39. Chong X, Rahimtula AD. Alterations in ATP-dependent calcium uptake by rat renal cortex microsomes following ochratoxin A administration *in vivo* or addition *in vitro*. Biochem Pharmacol. 1995; 44:1401-1409.
40. Ahmad M, Saleem S, Ahmad AS, Ansari MA, Yousuf S. Neuroprotective effects of Withania somnifera on 6-hydroxydopamine induced Parkinsonism in rats. Hum Exp Toxicol. 2005; 24(3):137-147.
41. Panda S, Kar A. Evidence for free radical scavenging activity of Ashwagandha root powder in mice. Indian J Physiol Pharmacol. 1997; 41(4):424-426.
42. Manimaran A, Sarkar SN, Sankar P. Toxicodynamics of subacute co-exposure to groundwater contaminant arsenic and analgesic-antipyretic drug acetaminophen in rats. Ecotoxicol Environ Safety. 2010; 73(1):94-100.
43. Nuutila AM, Puupponen-Pimia R, Aarni M, Oksman-Caldentey KM. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chem. 2003; 81:485-493.
44. Banerjee SK, Mauli M, Mancahanda SC, Gupta SK, Maulik SK. Dose dependent induction of endogenous antioxidants in rats heart by chronic administration of garlic. Life Sci. 2002; 70:1509-1518.
45. Dillon SA, Burmi RS, Lowe GM, Billigton D, Rahman K. Antioxidant properties of aged garlic extract: an *in vitro* study incorporating human low density lipoprotein. Life Sci. 2003; 72:1583-1594.
46. Gedik N, Kabasakal L, Sehirli O, Ercan F, Sirvanci S, Keyer-Uysal M, *et al*. Long-term administration of aqueous garlic extract (AGE) alleviates liver fibrosis and oxidative damage induced by biliary obstruction in rats. Life Sci. 2005; 76:2593-2606.
47. Plank G, Bauer J, Grunkemeier A, Fischer S, Gedek B, Berker H. The protective effect of adsorbent against OTA in swine. Tierarztliche Praxis. 1990; 18:483-489.
48. Edrington TS, Kubena LF, Harvey RB, Rottinghaus GE. Influence of a super activated charcoal on the toxic effects of aflatoxin or T-2 toxin in growing broilers. Poult Sci. 1997; 76(9):1205-1211.