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Toxicity, antioxidative, antibacterial and biological activities of poisonous plants of Al-Hassa

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ABSTRACT

Poisonous plants are widely distributed allover the world. The diagnoses of toxicity by these plants are very difficult. We choose five poisonous plants from Al- Hassa in the Eastern region of Saudi Arabian, *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia, Sisymbrium irio* and *Withania somnifera.* LD50 of these plants were 10.718, 500.0, 1211.7, 4.133, 522.27 mg/kg.bwt i.p. in rats, respectively. Rats poisoned by *A. arvensis* suffered from anorexia, restlessness, diarrhea, thirst (the animal goes toward drinking water), difficult breathing, and tremors and ended by coma and death of some individuals. Rats poisoned by *C. arvensis* showed mild toxic, it causes diarrhea, diuretic, laxative. Rats poisoned by *E. helioscopia* included increase activity and irritability, salivation, itching the nose and mouth on the cage floor, and diarrhea. The animals were tried to make tunnels under the bed and they were reluctant to stand at the cage corners. The animals closed its eyes and become calm. The animal poisoned by *S. irio* showed close eyes, extended head and neck, tend to sleep on the sternum, often that tremors, tend to hop, convulsion with elevated tail, increased respiration, dyspnea, cyanosis of the mouth and legs, very obvious cyanosis with elapsed time, gasping and coma followed by death with the appearance of luster of eye cornea. Poisoning of *W. somnifera* increased heart and respiratory rates, unconsciousness, closed eyes, stupor and paralysis of the hind legs, after which the animals became drowsy and finally died. On the other hand, various signs appeared such as shaking of the head, licking of the legs, petecheal hemorrhages from the eye canthus, lying on the sternum, creeping on the abdomen. Diarrhea occurring on the 2nd day and lastly irregular gasping fits ending by coma and death. In the present study, the aqueous extracts of the *Withania somnifera, Convolvulus arvensis* and *Sisymbrium irio* inhibited superoxide radical generation. The aqueous plant extracts exhibited different degradation effect on both DNA and bovine serum albumin. There are diverse antibacterial effects of the aqueous extracts on both Grams' positive and negative bacterial strains.

INTRODUCTION

The diagnosis of plant poisonings is a difficult process. Ingestion of several plants produces non-specific clinical signs that must be differentiated from other disease conditions. In addition, death due to toxic plant ingestion frequently does not result in characteristic post-mortem lesions. Unfortunately, relatively a small number of laboratory tests are available to detect plant toxins in either ante-mortem or post-mortem samples. In most cases, the most excellent way to support a diagnosis of plant poisoning is to confirm the presence of a

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toxic plant in the animal's environment (this will require positive identification of the suspect plant), confirm that the plant has been ingested (noting that the candidate plants have been chewed and/or finding plant fragments in vomits or gastrointestinal tract samples), and correlate clinical findings, where possible, with those known to be associated with the suspect plant.

Janzen[1] described a rapid and accurate method for the quick identification of plant species based on extracting DNA from a tiny tissue sample of a leaf, flower or fruit. Appropriately called 'DNA barcoding', referring to the coded labels on numerous products, DNA barcodes are a short sequence of DNA between 400 and 800 base pairs long that can be easily extracted and characterized for all species on the planet. These genetic barcodes possibly will then be accessed through a digital library and used to identify unknown plants in the field, garden or market[2]. Once fully developed, DNA barcoding has the potential to completely revolutionize our knowledge of plant diversity and our relationship to Nature. People will be able to quickly and cheaply recognize known species and retrieve information about them[3]. Barcoding could be a vital new tool for appreciating and managing the Earth's immense and changing biodiversity.

Superoxide dismutases (SOD) are an essential enzyme in both prokaryotic and eukaryotic cells. The superoxide is formed *in vivo* as a result of both enzymatic and spontaneous oxidation reaction[4]. The superoxide radical is toxic to the living cells as it oxidizes and degrades the biological macromolecules as DNA, protein and lipids. The SOD (EC 1.15.1.1) are metalloenzymes that catalyze the dismutation of super oxide radical ($O2^{-}$) to H_2O_2 and O_2. The SODs are classified into four groups according to their metal cofactor[5]. Eukaryotic cells are generally served by cytosolic CuZn-SOD containing Copper and zinc, mitochondrial Mn-SOD containing manganese, Fe-SOD containing iron and Ni-SOD containing nickel[6]. The mitochondrion is an imperative site for the single-electron reduction of O_2 to $O_2^{\bullet}$. Therefore, mitMn-SOD is consideration to be a major scavenger of damaging ROS metabolites in the mitochondrial matrix[7].

In the present study we achieved a survey and definition of poisonous plants of Al- Hassa in the eastern region in King Saudi Arabia. We determined the acute toxicity of some popular plants in this district and measuring their antibacterial effects. Moreover, the effect of the aqueous extracts of the studied plant on both DNA-barcode and BSA were demonstrated.

MATERIAL AND METHODS

Plant extraction

The plants examined in this investigation were collected from Saudi Arabia environment particularly the eastern district. These plants included *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia, Sisymbrium irio* and *Withania somnifera.* The dried herbs were cut into small pieces, washed thoroughly with distilled water and soaked in ethanol (80%) at room temperature for 36 hours according to[8]. The extract was decanted and centrifuged at 5000 rpm for 10 min. The supernatant was lyophilized, and the resulting powder was used in the present study.

Determination of the LD_{50} in rats

According to the method of[9] for determination of the dose of LD_{50}, exploratory trials were performed in five groups each of 4 rats of both sexes for every five poisonous plants. Alcoholic extract was administered i.p. in different successive doses in the five groups for each extract to find the smallest toxic dose to start with. The 1st dose which was the least dose to cause signs of toxicity was multiplied by a constant factor (1.5) for each succeeding group of rats or mice. Five groups of rats or mice were used, (10 each). 1st, 2nd, 3rd and 4th were given four successive doses alcoholic extract respectively. The fifth group was kept as a control. Mortality rate was recorded after 24 hours.

Agarose gel electrophoresis

The plant extracts (50 µg) of *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia, Sisymbrium irio and Withania somnifera* were added individually to 1µg of the DNA isolated from *E. coli* strain W3110[10]. The samples were incubated for 1 h at 37 °C. The DNA was analyzed by using horizontal agarose gels electrophoresis. The electrophoresis was performed using 0.7% (w/v) agarose gels in TAE buffer (5 mM sodium acetate, 1 mM EDTA and 0.04 M Tris-HCl pH 7.9). The agarose gels were stained with ethidium bromide (0.5 µg/ml) and the DNA was visualized on a UV transilluminator[11].

Polyacrylamide gel electrophoresis

Bovine Serum Albumin (BSA) (1mg) was treated with the plant extract of the following plants *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia, Sisymbrium irio and Withania somnifera* (50 μg) individually. The reaction mixtures were incubated for 1 h at 37 °C. The protein samples were analyzed by using vertical one dimensional SDS-polyacrylamide gel electrophoresis[12]. The molecular weight standard (Sigma MWND500) containing (α-lactaolbumin from bovine milk, 14.2 kDa), (Carbonic anhydrase from bovine erythrocytes, 29 kDa),) Albumin from chicken egg white, 45kDa), (Albumin from bovine serum, 66 kDa) and (Urease from Jack bean, 272 kDa) was utilized as protein marker.

Determination of superoxide dismutase (SOD) like activity

The plant extracts of *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia, Sisymbrium irio and Withania somnifera* were assayed for super oxide dismutase enzyme like activity according to the method of Bridges and Salin[13]. SOD like activity of the aqueous plant extracts were assayed by using phenazen methosulphate (PMS) to generate a superoxide anion radicals at pH = 8.3 (phosphate buffer). Reduction of nitroblue tetrazolium (NBT) to form blue formazan was used as an indicator of superoxide production and followed spectrophotometrically at 560 nm. The addition of PMS (9.3×10^{-5} M) to a solution of NBT (3×10^{-5} M), NADH (4.7×10^{-4} M) and phosphate buffer (final volume of 1 ml) caused a change of OD (Δ1) 560 nm per 5 min. The reactions in blank samples and in the presence of complexes were measured for 5 min.

Antimicrobial effect

The antimicrobial investigation of the plant extracts of *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia, Sisymbrium irio* and *Withania somnifera* was carried out using cup diffusion technique[14]. The test was done against the Gram- negative bacterial strains *Pesudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) and the Gram- positive bacterial strains *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*). The tested aqueous plant extracts were dissolved in sterilized H_2O at concentration I mg/ml. The Luria-Bertani Agar (LBA) Medium (10 g bacto-tryptone, 5 g yeast extract, 20 g agar, and 10 g NaCl in 1Liter de-ionized water) was made for inoculation and bacterial growth. An aliquot of the solution of the tested complexes equivalent to 100 μg was placed separately in cups, cut in the agar. The LBA plates were incubated for 24 hours at 37 °C and the resulting inhibition zones were measured. From the inhibition zone diameter data analysis, the antimicrobial activity against the Gram- negative and Gram- positive bacteria was determined.

RESULTS AND DISCUSSION

LD_{50} of *A. arvensis* alcoholic extract was 10.718mg/kg.b.wt. i.p. in rats. *Anagallus arvensis produce* gastrointestinal symptoms. Rats suffered from anorexia, restlessness, diarrhea, thirst (the animal goes toward drinking water), difficult breathing, tremors and ended by coma and death of some individuals. These results are parallel to that recorded by other groups[15-17].

LD50 of *C.arvensis* extract in mice was 500 mg/kg/day when injected i.p. The clinical signs are mild toxic, it causes diarrhea, diuretic, laxative. There is no available literatures concerning with the determination of the LD_{50} of this plant.

L D 50 of *Euphorbia heloscopia* extract was 1211.7 mg / kg bwt. i.p. in rats. The clinical signs were included increase activity and irritability, salivation, itching the nose and mouth on the cage floor, and diarrhea. The animals were tried to make tunnels under the bed and they were reluctant to stand at the cage corners. Finally the animals closed its eyes and become calm. The animals administered high dose were died after a latent period (3 hours) and some of the animals in other groups were died within 24 hours. There is no available literature concerning to mammals toxicity.

The LD_{50} of *Sisymbrium irio* alcoholic extract was 4.133mg/kg.b.wt. after i.p injection of albino rats. Clinical signs: the animal showed close eyes, extended head and neck, tend to sleep on the sternum, often that tremors, tend to hop, convulsion with elevated tail, increased respiration, dyspnea, cyanosis of the mouth and legs, very obvious cyanosis with elapsed time, gasping and coma followed by death with the appearance of luster of eye cornea. The large dose after 20 – 30 minutes 5.0625gm/kg.bwt caused death

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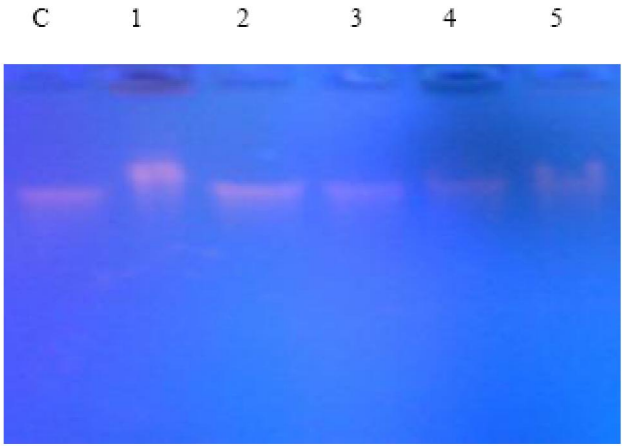
with the appearance of luster of eye cornea. There is no available literatures concerning with the determination of the LD_{50} of this plant.

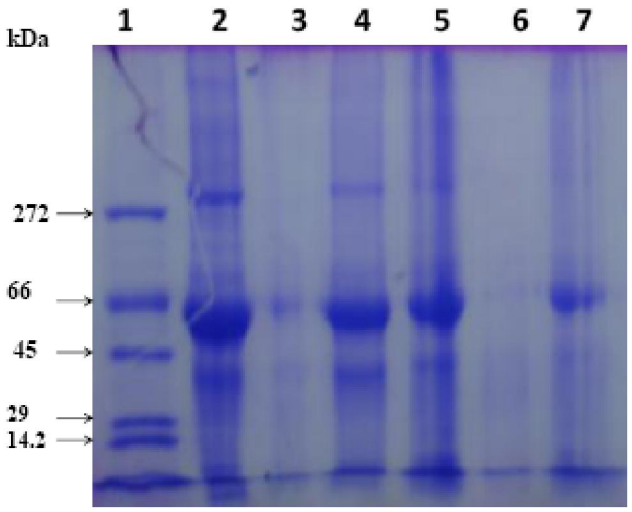
The LD_{50} of *Withania somnifera* alcoholic extract was 522.27 mg/kg bwt after i.p injection of albino rats. In these animals the most prominent signs were increased heart and respiratory rates, unconsciousness, closed eyes, stupor and paralysis of the hind legs, after which the animals became drowsy and finally died. On the other hand, various signs appeared such as shaking of the head, licking of the legs, petecheal hemorrhages from the eye canthus, lying on the sternum, creeping on the abdomen. Diarrhea occurring on the 2nd day, and lastly irregular gasping fits ending by coma and death. The symptoms of toxicity due to successive administration were similar to those of acute toxicity. This result disagrees with[18] who reported an LD_{50} of 764 mg/kg bwt. and[19] who recorded an LD50 of 309±0.32 in rats. This difference between the results may be due to the stage of plant growth, the climatic and environmental conditions under which the plants grow the species difference, and method of extraction.

The degradation effect of 50 µg of the plant extract of *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia, Sisymbrium irio and Withania somnifera* on the DNA *in vitro* is illustrated in Figure 1. The control sample (only DNA) does not exhibit any



Lane 1 : *E. coli* DNA
Lane 2 : *E. coli* DNA + *Anagallus arvensis*
Lane 3 : *E. coli* DNA + *Convolvulus arvensis*
Lane 4 : *E. coli* DNA + *Euphorbia helioscopia*
Lane 5 : *E. coli* DNA + *Sisymbrium irio*
Lane 6 : *E. coli* DNA + *Withania somnifera*

Figure 1 : A figure showing the degradation effect of the plant extracts on the DNA isolated from *E. coli* strain AB1157.



Lane 1 : protein standard molecular weight marker.
Lane 2 : BSA protein
Lane 3 : BSA protein + *Anagallus arvensis*
Lane 4 : BSA protein + *Convolvulus arvensis*
Lane 5 : BSA protein + *Euphorbia helioscopia*
Lane 6 : BSA protein + *Sisymbrium irio*
Lane 7 : BSA protein + *Withania somnifera*

Figure 2 : A figure showing the degradation effect of the plant extracts on the bovine serum protein.

DNA degradation through the incubation period as illustrated in Figure 1 lanes 1. The extract of *Convolvulus arvensis* does not degrade the DNA as illustrated in Figure 1 lane 3. However, the extract of *Anagallus arvensis* exhibits a partial degradation effect on the DNA as illustrated in Figure 2 lane 2. A considerable degradation effect were exhibited by the extracts of *Euphorbia helioscopia, Sisymbrium irio* and *Withania somnifera* as represented in Figure 1 lanes 3, 4 and 5 respectively. It is clear that both *Sisymbrium irio* and *Withania somnifera* have a strong degradation effect on the DNA *in vitro* more than the degradation effect which recorded with the rest of plant extracts.

Therefore, the extracts of *Sisymbrium irio* and *Withania somnifera* can be used as a promising anti-tumor agent *in vivo* to inhibit the DNA replication in the cancer cells and not allow the tumor for further growth. More work *in vivo* need to be carried out to elucidate their entire role and understand the exact pathway of both *Sisymbrium irio* and *Withania somnifera in vivo*.

Further biochemical studies to illustrate the exact role of the promising degradation effect by plant extracts on bovine serum albumin as a high molecular weight biological compound was carried out. The effect of the plant extracts of *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia,*

Sisymbrium irio and *Withania somnifera* on the BSA was carried out and the results illustrated in Figure 2. The BSA was utilized as a control as shown Figure 2 Lane 2. The extract of *Withania somnifera* has more degradation effect on the BSA as shown in Figure 2 lane 7 more than that observed with the extracts of *Convolvulus arvensis* and *Euphorbia helioscopia* as illustrated in Figure 2 lanes 4 and 5 respectively. A strong degradation effect on the BSA was recorded with the extract of *Convolvulus arvensis* compared to the control as represented in Figure 2 lanes 3 and 2, respectively. Additionally, the complete degradation effect on the BSA was exhibited by *Sisymbrium irio* as shown in Figure 2 lane 6.

It is clear that the plant extract of *Withania somnifera* has apparent effect on the DNA but has a weak apparent effect on the BSA. Contrary, the extract of *Convolvulus arvensis* has a considerable degradation effect on BSA but has a weak effect on the DNA. Moreover, the extract of *Sisymbrium irio* has a strong degradation effect on both DNA and BSA. While, *Euphorbia helioscopia* extract has a weak effect on both DNA and BSA.

The SOD mimics like activity of the plant extracts of *Anagallus arvensis, Convolvulus arvensis, Euphorbia helioscopia, Sisymbrium irio* and *Withania somnifera* is represented in TABLE 1. The plants extracts of *Euphorbia helioscopia* and *Anagallus arvensis* exhibit a low SOD like activity and cause an inhibition percent 39.33% and 42.53%, respectively. In addition to that the extract of *Withania somnifera* exhibited a moderate SOD like activity as well as represented in TABLE 1 inhibition percent of 54.13 %. However, the extracts of both *Convolvulas arvensis* and *Sisymbrium irio* show a strong SOD like activity as represented in TABLE 1 as inhibition percent of 65.60 % and 69.67%, respectively.

TABLE 1: Superoxide (SOD) like activity of the plant extracts as antioxidative enzyme

	Δ through 4 min	% inhibition
Control	0.689	-
Anagallus arvensis	0.396	42.53%
Euphorbia helioscopia	0.237	65.60%
Convolvulus arvensis	0.418	39.33%
Sisymbrium irio	0.209	69.67%
Withania somnifera	0.316	54.14%

% inhibition = (Δ Control – Δ Test / Δ Control) X 100

In the present study, the aqueous extracts of the *Withania somnifera*, *Convolvulus arvensis* and *Sisymbrium irio* inhibited superoxide radical generation. Maintaining the balance between the rate of radical generation and the rate of radical scavenging is an essential part of biological homeostasis. Therefore, it is suggested that the inhibition of superoxide radical generation by these plant extracts is attributable to their free radical scavenging activity. They are preventive antioxidants. It was reported that antioxidants are deficient in HIV-infected populations due to an increased utilization of antioxidants. It was suggested that an increased intake of antioxidants may delay progression of HIV infection to AIDS[20]. Therefore, it is estimated that these activities may be related to their antioxidant and free radical scavenging activities.

The ampicillin a broad-spectrum antibiotic was utilized a positive control for this test. The results of the antimicrobial test of the plant extracts against Gram-negative *P. aeruginosa* and *E. coli* and Gram- positive *B. subtilis* and *S. aureus* bacterial strains are summarized in TABLE 2. The extract of *Withania somnifera* has maximal antimicrobial activity regarding with inhibition zone diameter against *B. subtilis* 20 mm, *S. aureus* 21 mm, moderate effect against *P. aeruginosa* 18 mm and *E. coli* 17 mm as represented in TABLE 2. Also, the extract of *Sisymbrium irio* showed a wide spectrum of antimicrobial activity regarding with the inhibition zone diameter against *B. subtilis* 17 mm, *S. aureus* 14 mm, *P. aeruginosa* 21 mm and *E. coli* 15 mm. The extracts of *Convolvulus arvensis* and *Euphorbia helioscopia* have a considerable antimicrobial activity regarding with inhibition zone diameter against the tested bacterial strains as illustrated in TABLE 2. However, the extract of *Anagallus arvensis* exhibited low antimicrobial activity as the inhibition zone diameter against *B. subtilis* 10 mm, *S. aureus* 7 mm, *P. aeruginosa* 5

TABLE 2 : Effect of plant extracts on some microorganisms the results expressed as zone inhibition in mm diameter.

	E. coli	*B. subtilis*	*P. aeruginosa.*	*S. aureus*
Anagallus arvensis	8	10	5	7
Convolvulus arvensis	15	12	16	11
Euphorbia helioscopia	12	13	16	15
Sisymbrium irio	15	17	21	14
Withania somnifera	17	20	18	21
Ampicillin	23	22	25	20

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mm and *E. coli* 8 mm and that's compared with the other tested extracts antimicrobial activity.

CONCLUSION

The aqueous extracts of *Sisymbrium iris* and *Withania somnifera* have a strong degradation effect on the DNA *in vitro* and this can be utilized as an antitumor to inhibit tumor growth. The extracts of *Withania somnifera*, *Convolvules arvensis* and *Sisymbrium iris* scavenge the formation of super oxide radical. Moreover the plant extracts can be utilized as antimicrobial agents.

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