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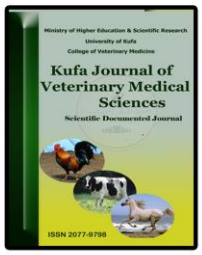

Study of in vitro and in vivo cytotoxicity effect of some medicinal plants

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Abstract:

The medicinal plants contain many ingredients which enhance the development and synthesis of many drugs the ingredients content of medicinal plants that have therapeutic activity naturally synthesized and accumulated in plants. The presence study was conducted to investigate in vitro and in vivo cytotoxicity effect of some medicinal plants, extracted by soxhlet with methanol alcohol and fractionation of active constituents from *Curcuma longa* L. rhizomes , *Commiphora myrrha* L. gums and *Ginkgo biloba* L. leaves with water , chloroform , ethyl acetate and hexane. The methanolic alcohol extract and fractions of *Curcuma longa* L. rhizomes , *Commiphora myrrha* L. gums and *Ginkgo biloba* L. leaves show (in vitro) relatively significant ($p \leq 0.05$) low cytotoxicity against RBC , while (in vivo) the cytotoxicity at 2000 mg / ml did not occur which indicated the study plants safety.

دراسة التأثير السام للخلايا لبعض النباتات الطبية في داخل وخارج الجسم الحي

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الخلاصة:

تحتوي النباتات الطبية على العديد من المكونات التي ساهمت في صناعة الأدوية والعلاجات لكثير من الأمراض وهنا في هذه الدراسة تم التحري عن السمية الخلوية للمستخلص الكحولي المستحصل بواسطة جهاز السكسوليت وأجزائه (الكلورفورم ، الاثيل اسيتيت ، الماء ، والهكسان) لنباتات الكركم والمر والجنكة داخل الجسم الحي ($p \leq 0.05$) ، إذ بينت النتائج ولجميع النباتات ان المستخلص الكحولي وأجزائه له تأثير سمي منخفض معنويا وخارجه عند الاختبار خارج الجسم الحي بينما داخل الجسم الحي فان جميع النباتات لم تظهر أي تأثير سمي مميت أو قاتل للحيوانات المختبرة.

1. Introduction

The plants have been used by human for many purposes as they have been used for the treatment of many diseases and health disorder since ancient times that ancient civilizations occupy the home flora plants, leaves, herbs and roots with fauna for nutrition and health related purposes including different ailments such as fever, aches, infections, infertility and others[1].

The medicinal plants contain many ingredients which enhance the development and synthesis of many drugs ,the ingredients content of

medicinal plants that have therapeutic activity naturally synthesized and accumulated in plants body since many plants activity is called primary and secondary metabolites[2] and modern ethno-pharmacological surveys revealed that about 250 plants are used in Arab habitual medicine for the management cure of different diseases[3]. Many compounds have the ability of cytotoxic effects which compromise cell membrane integrity, thus the present study efforts have been made to study in vivo and out vivo cytotoxicity effect of alcoholic extracts and their (chloroform , hexane , ethyl acetate and water) fractions of *Curcuma longa* L. rhizomes , *Commiphora myrrha* L. gums and *Gingko biloba* L. leaves.

2. **Methods:**

2.1 The plant preparing:

This employment was passed in the Department of Biology , Faculty of Science , Kufa University (January 2016 – April 2016). *Gingko biloba* L. plants obtained from pharmacies as 500 mg food supplement tablets manufacturing in the United Kingdom by FSC Food Supplement Company, while Plants *Commiphora myrrha* L. gum and *Curcuma longa* L. rhizome were collected from Najaf city markets .

The plant parts powders were extracted by Soxhlet by putting twenty five grams of desiccated plants powder in filter paper and dissolved with 250 ml of solvent (methanol 95%) for 24 hours, the resultant dried and then mixed in separatory funnel with 1:1 rate of water and chloroform solution and shaking well. The result show two layers , the lower layer (chloroform layer) mixed immediately in separatory funnel with 1:1 rate of chloroform and hexane solution , the result show two layers , the upper layer(hexane layer) and the lower layer(chloroform layer).The upper layer (water layer) mixed immediately in separatory funnel with 1:1 rate of water and ethyl acetate solution , the result show two layers , the upper layer(ethyl acetate layer) and the lower layer(water layer). All the layers were dried and stored until used. Then , the methanolic extract and four fractions (chloroform layer , hexane layer , water layer and ethyl acetate layer) were used for investigation about biological activity of plants. Chemical detection of the active components in alcoholic plant extracts of studied plants were chemically tested for the presence or absence of the following active compounds by treatment with precipitation reagents[4].

2.2 Evaluation of the cytotoxic effects of plants extracts:

A. In vitro cellular toxicity (blood hemolysis):

The hemolysis of blood was observed (in vitro) approbate to Bouma , 2002[5] that about 30 ml of plants solutions (extract and fractions) was collected with (0.2 ml) of donor blood (from healthy non – smoker volunteer). For (5 seconds), the plant solutions (extract and fractions) were jumbled kindly then about (20 ml) of normal saline were appended to beat any hemolytic reaction supposed that would be occurred. Later and for (10) minutes the combination solutions centrifuged by the side of 3000 round / minute and finally the absorbency of the solutions top layer was distinct at 540 nm. DMSO at (30 ml) with combination to normal saline and blood on the same ratio that used for plant solutions represented the positive control of this experiment while the

100% hemolysis occurred by reducing the blood concentration with reduplicate amount of distilled water as an alternative of normal saline. The percentage hemolysis was evaluated by equation : Hemolysis % = (test solution absorbency – normal saline absorbency) / (100% hemolysis absorbency – normal saline absorbency) x 100%

B. In vivo cellular toxicity (Lethal dose 50 (LD_{50})):

Albino rats took the plants solutions (extracts and fractions) intraperitoneally at one time for different concentrations levels (500 , 1000 and 2000 mg / ml) to 3 sections (6 female rats per section. The test extracts were dissolved in Dimethylsulfoxide (DMSO). All doses were intraperitoneal injection in rats at rate 0.1 ml / 10 gm.

Similarly, one group of rats (6 female rats) was given the same size of (DMSO) intraperitoneally (intraperitoneal controls). The injected rats were placed separately for 72 hours to obtain sure examination especially the relative hours after injection. Alteration in the rat behavior , death-rate and toxicity signs were observed and the weight after 72 hours were recorded[6].

Results and discussion:

Phytochemical screening of methanol alcoholic extracts of *Curcuma longa* L. rhizomes , *Commiphora myrrha* L. gums and *Ginko biloba* L. leaves by using precipitation reagents reveal that many of active compounds are found in the extracts as tannins , alkaloids , glycosides and phenols and this is in agreement with many previous studies[7,8,9].

The positive control shows about 100% lysis, whereas the phosphate buffer saline (PBS) shows no lysis of RBCs. The results show that all study plants extracts have significant relatively low cytotoxicity.The cytotoxicity of methanolic plants extract and fractions are assayed using in vitro haemolytic activity against blood erythrocytes (RBCs) and it is observed that the plants shows minor cytotoxicity as compared to the positive control. The red blood cells (RBCs) membrane mechanical stability is a good display to estimate in vitro cytotoxicity because the cells when lysis with a cytotoxic compound can cause different hitch to health and many disorders[10]. It is identified that in the transferable diseases haemolysis took place due to the action of the microbes[11] , so in the present study, the effect of plant extract and fractions for hemolytic activity was evaluated.

Phenolic compounds , glycosides and flavonols have large variety of biological activities that they may play an important role in the antioxidant and cytotoxic activity[12].

In vivo cytotoxicity and LD_{50} estimation results for the acute toxicity studies show no visible signs of toxicity and no mortality are observed in the treated groups and no death of rats is recorded in the control and they do not expressed changes in their physio - pathological activities. Although there was an increase in the weights of rats in the treated groups and this is agreement with the studies which indicated that *Curcuma longa* , *Commiphora myrrha* and *Gingko biloba* is nontoxic[13 , 14 , 15].

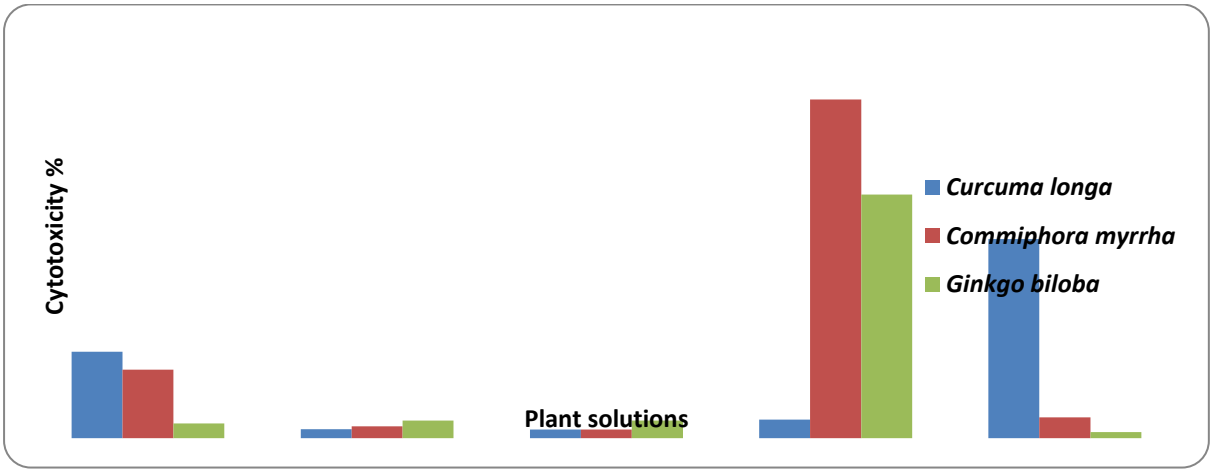

Figure (1): The study plants solutions cytotoxic effects on erythrocyte with the low concentration (100 mg / ml).

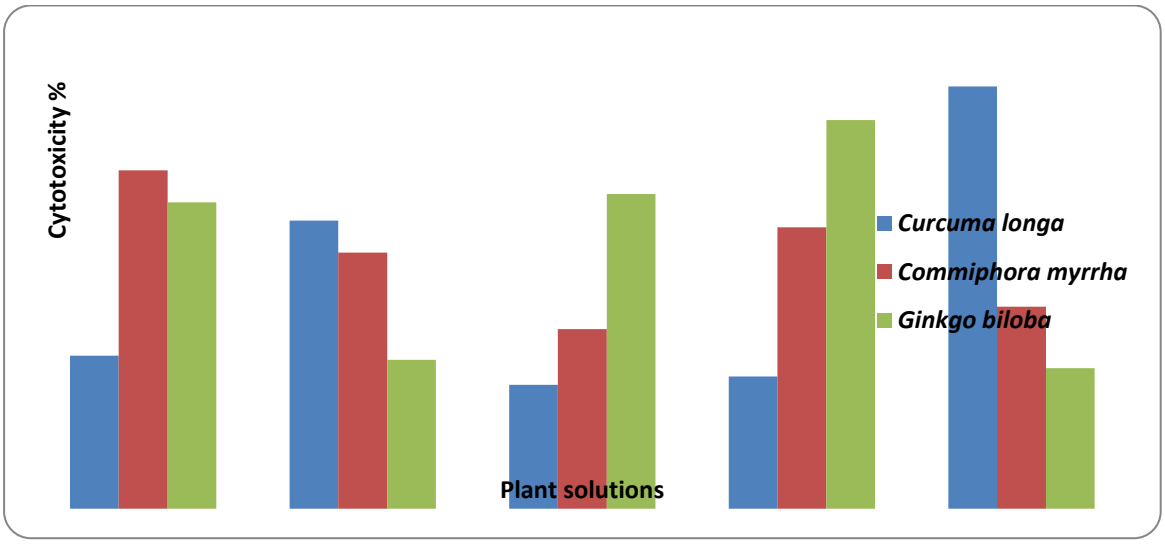

Figure (2): The study plants solutions cytotoxic effects on erythrocyte with the high concentration (1000 mg / ml).

Table(1): Cytotoxicity effect of *Curcuma longa* L. rhizomes, *Commiphora myrrha* L. gums and *Gingko biloba* L. leaves solutions on RBC.

	Conc.	*C. longa*	*C. myrrha*	*G. biloba*
Methanol extract	**100**	**2.56 ± 0.40**	**2.03± 0.40**	**0.44 ± 0.55**
	250	**4.74 ± 0.53**	**9.26 ± 0.53**	**5.82 ± 0.95**
	500	**7.14± 0.53**	**15.52 ± 0.66**	**16.22 ± 0.5508**
	1000	**9.700± 0.669**	**21.42 ± 0.795**	**19.40 ± 0.402**
Hexane fraction	**100**	**0.2650 ± 2.6467**	**0.3500 ± 0.4026**	**0.5267 ± 0.6992**
	250	**6.1733 ± 0.8095**	**10.493 ± 0.4069**	**4.5867 ± 0.4069**
	500	**9.4333 ± 0.6642**	**11.110 ± 0.5300**	**5.6433 ± 0.5468**
	1000	**18.253 ± 1.3965**	**16.226 ± 0.9330**	**9.4333 ± 0.4069**
Water fraction	**100**	**0.26 ± 0.26**	**0.26 ± 0.26**	**0.52 ± 0.26**
	250	**6.43 ± 0.66**	**0.61 ± 0.55**	**1.23 ± 0.85**
	500	**6.436± 0.804**	**10.050 ± 0.5300**	**3.8800 ± 0.669**
	1000	**7.850± 0.669**	**11.373 ± 0.699**	**19.930 ± 0.402**
Ethyl acetate fraction	**100**	**0.5508± 0.616**	**10.053 ± 0.2650**	**7.2300 ± 0.5524**
	250	**3.2633 ± 0.6649**	**12.786 ± 0.5508**	**9.8767 ± 0.5508**
	500	**7.2333 ± 1.5923**	**16.226 ± 0.5508**	**12.786 ± 0.4069**
	1000	**8.3767 ± 0.6649**	**17.810 ± 0.4026**	**24.603 ± 5.8043**
Chloroform fraction	**100**	**5.9067± 0.8041**	**0.6167 ± 0.5508**	**0.1767 ± 0.3060**
	250	**7.9400 ± 0.5300**	**3.5267 ± 0.5508**	**0.3500 ± 0.4026**
	500	**19.576 ± 0.7950**	**4.6733 ± 0.4069**	**8.2033 ± 0.6992**
	1000	**26.736 ± 0.7072**	**12.786 ± 0.4069**	**8.9067 ± 0.8095**

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