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The Effect of Aqueous Crude Extract of *Hypericum triquetrifolium* on the (Number, Sex, Generation and their interactions) of Wild Fruit Flies

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ABSTRACT

This study was designed to evaluate the effect of three concentrations of crude aqueous extracts of *Hypericum triquetrifolium* on (number, sex, generation and their combinations) of wild fruit flies. The concentrations (100, 50 and 25 µg/ml) were given to first generation parents in culture media and left for completing the life cycle under suitable temperature (25 Co ±2) in the modified-lightened incubator. Five replications were used for each treatment, and untreated flies were considered as control. The results were obtained in first and second generations successively. The results showed significant effect ($P<0.05$) of concentrations and concentration-generation interactions on the number of fruit flies and highly significant ($P<0.01$) of the impact of sex regarding the number of fruit flies.

Keywords: Fruit Fly, Hypericum, Mitotic Index, Hypericin

I. INTRODUCTION

Hypericum species are plants known to have medicinal properties and are widely used in phytotherapy in many countries. The genus Hypericum comprised more than 400 species, and the most abundant herbs of those are *Hypericum perforatum* and *Hypericum triquetrifolium*. *Hypericum* has been used in traditional herbal medicine in many countries (Mohammed & Kheravii, 2011). In Iraq *Hypericum triquetrifolium* is very common on the upper plains and in the Khanaqin, Mosul, Ain-Sifni, Kirkuk, Arbil, Shaqlawah, Khanzad pass, Humaidat, Tal-kaif, Zawitah, Kani-Atar (near Sinjar), Gali-ali- Beg, Jazira, Tal-Afar (Al-Rawi, 1973).

The entire plant is toxic containing (hypericin, pseudo-hypericin, protohypericin, and protopseudo-hypericin), phloroglucinols (hyperforin, adhyperforin, hyperfirin, and adhyperfirin), and a broad range of flavonoids, these toxins are secondary metabolites . They do have diverse biological activities ranging from toxicity to hormonal mimicry and may play a role in protecting plants from herbivore and disease. (Al-Rawi, 1973; Nahrstedt and Butterweck, 1997).

Modern studies have been focused on the activity of *Hypericum* species extract and many researches have been published for its antimitotic (Homann *et al*., 2015; Ahmed, 2012; Penjweini *et al*., 2012 and Fox *et al*., 1998), antimicrobial, antifungal, antioxidant, antidepressant (Eslami *et al*., 2011) and antiviral activity (Liebes *et al*., 1991). Investigation of molecular mechanisms underlying hypericin photocytotoxicity in cancer cells has revealed that hypericin stimulate both apoptosis and necrosis in a concentration and light dose-dependent manner.(Patrizia *et al*., 2002; Vantieghem *et al*., 1998 and Kessel *et al*., 1997; Esposito *et al*., 1995; Hadjur *et al*., 1996; Hadjur *et al*., 1995).

Hypericin toxin and its close relative toxins show anticancer activity in treated mice, When hypericin molecules are photoenergised they can lyse cells in the dermis and cause the photosensitization associated cellulitis (an infection of deep skin dermis) that develops in field cases of Hypericum poisoning. (Mohammed and

Kheravii, 2011). Hypericin is an active, agent in the photodynamic therapy of cancers; hypericin is a potent agent in the photodynamic therapy of cancers (Van de Putte *et al.*, 2005). Recent studies also proposed that hypericin modulate inflammation and oxidative stress in reducing paracetamol-induced hepatotoxicity. (Hohmann *et al*., 20115).

There is no enough literature about the effect of medicinal plants on fruit flies, the present study aimed to test the toxicity effects of *Hypericum triquetrifolium* on number, sex, generation and their interactions of wild fruit flies.

II. METHODS AND MATERIAL

Fruit flies were captured by putting a little amount of grape and apple in a container and left for 2-3 day in room temperatures. After few day fruit flies appeared in the container. Then left for mating and increasing their numbers for treatments.

The culture containers were prepared by dissolving 5 gm agar in 150 ml hot water then 25 gm of four was added which was previously soaked with 60 ml water for a few hours. The mixture was allowed to boil. The sugar (25 gm) was dissolved in 40 ml of water, and this was added to the agar/flour mixture, boiled, stirred until the medium was uniform in consistency. Then the media was poured in culture containers for about one inch and considered as control. Untreated control flies were put in these containers. But for treatments, three concentrations (100, 50 and 25 µm/ml) designated as C1, C2 and C3 of plant aqueous crude extract were prepared by dissolving (30.5, 15.25 and 7.62 mg) of powdered extract respectively each in a 250 ml of distilled water and used for culture preparations. For each treatment, five replications were used and in each treatment two males and three females were put in culture containers for mating. Eggs were allowed to develop, and maggots to fed on different concentrations of plant extract and control at a suitable temperature (25 Co ±2) in the incubator. After 14 days, the flies were etherized by a standard method to count and separate male and females and considered as first generation G1. Males (S1) and females (S2) of the first generation were mate for each concentration in culture containers (five replication for each concentration) without the plant extract to know the effect of the plant extract in the second generation G2. Statistical analysis of the data were done by Factorial one way ANOVA to evaluate the impact of treatments (concentrations, sex, generations and their interactions) on the number of fruit flies. Least significant differences (L.S.D.) was used to compare means (Snedecor, 1956).

III. RESULTS AND DISCUSSION

Different concentrations of *Hypericum triquetrifolium aqueous crude extract* (100 µg/ml,50 µg/ml,25 µg/ml) of Hypericum extract were given to the adult parental individuals in culture media and then fruit fly numbers and sex ratio were followed in both first filial generation and second filial generations respectively.

The results that showed in the table (1) have cleared that *Hypericum triquetrifolium* treatments have significant ($P<0.05$) effect on the number of fruit flies. Mean values were shown in Table (2); there was a significant difference between control group and the middle dose of crude aqueous extract of H. triquetrifolium regarding their effect on the number of fruit flies. mean value were (37.1 ± 3.913 and 44.55 ± 7.477) respectively, (LSD value= 8.976). As seen in Table 1, the numbers of the offspring were decreased with treated cultures by plant extract compared to control. The results of the present study indicate that plant extract could have antimitotic activity or impaed the normal physiologic growth of the flies and this result is in agreement with the previous studies that show the antimitotic activity of *Hypericum triquetrifolium*.(Ahmed, 2012; Penjweini *et al*., 2012 and Fox *et al*., 1998).

Table(1): Analysis of variance for the effect of crude aqueous extract of *Hypericum triquetrifolium* (Concentrations, sex, generation and their interactions) on the number of fruit flies.

Treatments	d.f	Mean square
Replications	4	267.6375
Concentrations	**3**	**575.2333***
Sex	1	3302.45**
C G interaction	1	1036.8*
C S interaction	3	459.816
G S interaction	3	2.566
C G S interaction	1	14.45
	3	56.2833

The significant levels: $P<0.05$)* ($P<0.01$)**

According to Alali *et al.,* (2004) hypericin toxin is found in all parts of plant and this might account for anti mitotic activity and for decreasing the number of flies depending on the effect of this toxin and other variable toxins that present in this plant on various developmental stages of the organism under the study. In another research, the crude aqueous extract of *Hypericum triquetrifolium* also show the highly significant effect on the mitotic index and significant effect on the micronuclei formation (Ahmed, 2012). Cell damage and cell death could also achieve after administration of a crude aqueous extract of *Hypericum sp)* as shown by the results of the exposure of nine different concentrations of the aqueous extract of this plant which produced 17% mortality of albino mice (Mohammed & Kheravii, 2011). In our study, the fruit flies larvae were fed on culture media containing *Hypericum* extract, so the death of fruit flies might resulted from the extract effect. Other studies indicated anticancer effects of Hypericum plant due rapid inhibition of purine synthesis, inhibition of DNA synthesis, decrease RNA synthesis and early cytotoxicity in the cell cycle phase (Bökkerink *et al*., 1993).

Table(2): mean + S.E for the effect of crude aqueous extracts of *Hypericum triquetrifolium* (concentrations, sex, generation and their interactions) on the number of fruit flies.

Treatments		Mean S.E
Concentrations	C0	37.10 ± 3.913
	C1	32.40 ± 12.183
	C2	44.55 ± 7.477
	C3	34.65 ± 3.794
		L.S.D =8.976
Generations	G1	30.625 ± 5.553
	G2	43.475 ± 6.070
		L.S.D=20
sex	S1	40.65 6.587
	S2	33.45 7.27
		L.S.D=12
Concentration-Generation interactions	C0G1	37.1 ±0 9.840
	C0G2	37.1 ± 09.840
	C1G1	22.7 ± 10.909
	C1G2	42.1 ± 2.0550
	C2G1	34.1 ± 5.2170
	C2G2	55.0 ± 5.0590
	C3G1	28.6 ± 3.7940
	C3G2	39.7 ± 7.4310
		L.S.D=12.69
Concentration-sex interactions	C0S1	40.6 ± 00.000
	C0S2	33.6 ± 00.000
	C1S1	36.5 ± 10.909
	C1S2	28.3 ± 19.764
	C2S1	47.8 ± 16.443
	C2S2	41.3 ± 16.601
	C3S1	37.7 ± 10.593
	C3S2	30.6 ± 06.957
Generation-Sex interactions	G1S1	34.65 ± 5.055
	G1S2	26.60 ± 7.571
	G2S2	46.65 ± 7.616
	G2S2	40.30 ± 8.013
Concentration-Generation-sex interactions	G1C1S1	29.60 ± 1.846
	G1C1S2	15.8 0 ± 2.613
	G1C2S1	37.40 ± 8.117
	G1C2S2	30.80 ± 7.970
	G1C3S1	31.0 0 ± 4.955
	G1C3S2	26.2 0 ± 3.303
	G1C0S1	40.6 0 ± 4.016
	G1C0S2	33.60 ± 3.353
	G2C1S1	43.40 ± 8.757
	G2C1S2	40.80 ± 9.130
	G2C2S1	58.20 ± 2.777
	G2C2S2	51.80 ± 7.361
	G2C3S1	44.40 ± 5.640
	G2C3S2	35.0 ± 6.687
	G2C0S1	40.6 ± 4.016
	G2C0S2	33.6 ± 3.353

C: concentration S:sex G:generation

The results of the present study (table1) also showed that sex have an extremely significant effect ($P<0.01$) on the number of fruit flies. The mean values cleared that females were more affected (33.45 ± 7.27) by the treatment of *Hypericum triquetrifolium than males (40.65* ± 6.587) and this may be due to physiological differences between the two sexes. According to above previous studies the plant toxins retard normal mitosis. However, studies describing the effects of Hypericum sp. plant on the sex of fruit flies are rare , our data show that the extract showed less effect on males than females, and this may be due to physiologically differences between the two sexes .

The interaction between concentrations and generation also showed significant effect ($P<0.05$) on the number of fruit flies, the table of mean indicated that the lowest value was recorded for highest concentration in the first generation (mean value = 22.7± 10.909), that's mean the effects of *Hypericum triquetrifolium* crude aqueous extract were diminished with successive generations.

IV. CONCLUSION

The conclusion is that the crude aqueous extract of *Hypericum Triquitrifolium* plant have antimitotic activity or impaed the normal physiologic grothw of the flies during larval or pupal stages of wild fruit flies.

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