JOURNAL OF ZANKOY SULAIMANI

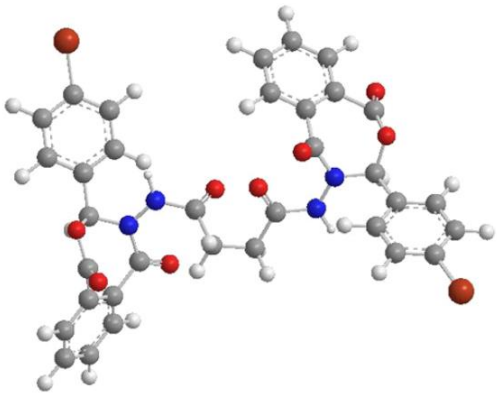
AUTHOR'S COPY

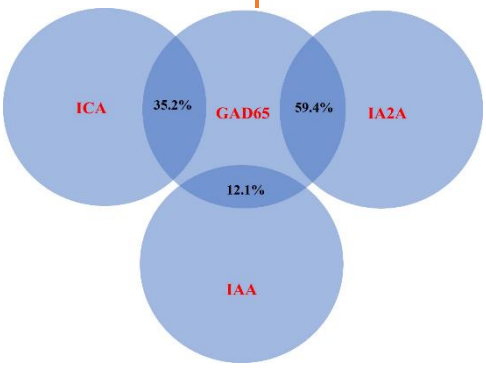
Part -A- (Pure and Applied Sciences)

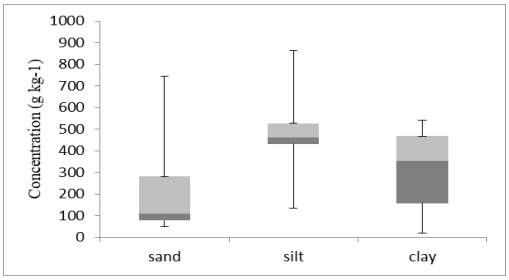
VOLUME 25 ISSUE 2 December 2023

ISSN: 1812-4100

www.jzs.univsul.edu.iq







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Journal of Zankoy Sulaimani
Part-A- (Pure and Applied Sciences)
ISSN:1812-4100

Effect of *Ficus carica* leaves Aqueous Crud Extract on the Number of Wild Fruit Flies and Sperm Morphology of Laboratory Albino Male Mice

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Article info

Original: 25/12/2022
Revised: 10/04/2023
Accepted: 16/04/2023
Published online:

Keywords:
Fig leaves extract, Drosophila melanogaster, Sex ratio, sperm abnormalities

Abstract

The purpose of this study was to compare the effects of two concentrations of aqueous crude extract from *Ficus carica* leaves on fruit fly numbers, sex ratios, and generations, as well as laboratory albino mouse sperm abnormalities. Fruit flies were given the concentrations of 1% marked as C1 and 2% marked as C2 in culture media and allowed to complete their life cycle at an acceptable temperature (25° C±2) in an incubator. Same concentrations were given to the tested mice in drinking water. Each treatment consisted of five replications, with untreated mice and flies serving as the control groups. Fruit fly findings were seen in the first and second generations, and mouse sperm morphology was examined one month following treatment. The results revealed that tested concentrations had no significant impact (P≤0.05) on the quantity of fruit flies mean values were (40.650 ± 2.40 5and 39.250 ± 2.624) froe first and second concentrations compared to 34.550 ± 2.12 in control group, but they did have a significant impact (P≤0.05) on the sex ratio (shifted toward females (40.733 ± 1.969) in comparison to males (35.566 ± 1.838), least significant differences(L.S.D.) were = 5.148, and generation-concentration interactions, the highest number were recorded was females in second generation (42.900 ± 2.203). The treated mice showed Significant effect of concentrations on sperm without tail and highly significant effect (P≤0.01) on sperm without hook (mean values were 5.6 ± 0.37 and 2.0 ± 0.44 in high concentration while in control group the values were 2.0 ± 0.44 and1.0 ± 0.44) for both aberrations respectively. Our results clearly indicated change in sex ratio among treated fruit flies and induction of some morphologic changes in mice sperms after treatment of fig leaves aqueous crud extract.

Introduction

Medicinal plants are frequently used by people how have different diseases, especially in rural people's lives, and some not aware about the toxic materials that found in these plants specially for whom that live in places with low health facilities, the dependence on medicinal plants clearly is to cure various diseases (1). In the present study, we tried to bring fig leaves extract (sp. *Ficus carica*) as a traditional medicine into a medical genetics laboratory for recognizing the potential effects on fruit fly numbers, sex ratio and sperm abnormalities as important cytotoxic assays to evaluate adverse effects of the natural compounds that present in plants in this widespread world. The aim of our study is to show adverse effect of toxic compounds that might be present particularly if consumed in high ratios without concerning the side effects by whom who use them. Infertility is one of the grate health problems of reproductive-aged males due to low sperm quality in 20th century consequently many people turned to natural products that might have potential to improve

sperm quality (2) in most of these natural herbs and fruits, the antioxidant role of these products have benefit effects on many aspects of sperm production like Sertoli cells, testosterone, sperm number and sperm quality in this respect the good effect of *ficus carica* directly or indirectly through reducing the negative effects of hazardous chemicals such as anticancer drugs were shown in may studies (3) and (4). Ficus carica leaves are has been frequently used by folk medicine for its diverse biological activity like metabolic, anti-inflammatory, respiratory cardiovascular, and also for treatment of reproductive disfunctions (5). Many chemicals and compounds have been extracted from ficus carica like; phenolic compounds, phenolic acid, chlorogenic acid, flavones, and flavonols, benzyl aldehyde, furanoid, cinnamic aldehyde, benzyl alcohol, trans- pyranoid, cinnamic alcohol, angelicin, bergapten and many others (6) that may have deleterious affect on normal sex ratio in fruit flies and normal sperm morphology of treated mice (7) and (8). Conduction of this research is aimed to know the impact of crude leave extract of ficus carica on number and sex ratio on wild fruit flies and on inducing sperm abnormalities in laboratory male mice Mus musculus.

Materials and methods

Fruit fly's treatments, etherizing and sorting

The Research Ethics Committee of the University of Sulaimani's College of Science, Department of Biology, approved the study's ethical declaration (Sulaimani, Kurdistan Region, Iraq) numbered UoS-Sci-Bio 003. Five replications of each treatment were employed, with untreated fruit flies serving as the control group. After obtaining the wild flies, the culture containers were prepared according to usual procedure and put into sterilized culture containers for roughly an inch. Treatments (C1= %2 and C2=%1 concentration) were prepared by adding 2 gram and 1 gram of plant leaves extract powder in 100 ml of media respectively. Two males and three females were placed in culture containers for mating for each treatment. Maggots were fed on different concentrations of plant extract as they grew in an incubator at an appropriate temperature (25º C ±2). After 14 days, the flies were etherized (9) in order to count and distinguish first generation G1 male and female flies. For each experiment, males (S1) and females (S2) of the first generation were mated again in culture containers to determine the impact of the plant extract on the second generation G2 (five replicates for each concentration as well).

Mice treatments

Albino Mice *Mus musculus* were obtained and kept under standard conditions in animal house of biology department-college of science- university of Sulaimani. Untreated mice considered as control group, but treated mice were grouped as C1=%2 and C2=%1 concentration by adding 2.0 gm and 1.0 gm of plant leave extract powder in 100 mL drinking water respectively. For each treatment, five replications were used.

Mice dissection and slide preparation

After one month the treated mice were killed by cervical dislocation, sperms were aspirated by (Hirofumi *et al.,* 2004) methods with few modifications, then dissected to remove the vas deference in which the sperms were extracted, then with the aid of a drop of normal saline the sperms were diluted and smeared over clean microscopic slides. After air drying the slides were stained with eosin and hematoxylin 15 minutes for each. The prepared slide examined under light microscope and the data of sperm abnormalities were recorded from 500 sperms for each treatment (10).

Eosin preparation

one gram of eosin powder was dissolved in 100 m1 of ethanol 95% as a stock solution. The stain gives yellowish orange color.

Statistical analysis

Graphpad prism software version 9 as a statistic program used to analyze experimental data to figure out the effect of treatments (concentrations, sex, generations, and their interactions) on the number of fruit flies and the impact of concentrations on the morphology of treated mice's sperm, using factorial one-way ANOVA for the first experiment and factorial two-way ANOVA for the second experiment. To compare means, least significant differences (L.S.D.) were employed.

Results and discussion

Fruit Flies

In treated fruit flies, sex and concentration-generation interactions had significant impacts ($P \leq 0.05$) when compared to control groups (Table 1). Female ratio was higher than male mean ± SD were 40.733 ± 1.969 and 35.566 ± 1.838, respectively and L.S.D =5.148.The highest number of fruit flies was recorded in low concentration in second generation (mean ± SD of 42.900 ± 2.203) while the lowest number was shown in control group second generation (mean ± SD of 29.600 ± 1.275) (Table 2).These results indicate that the crud fig leave extract could have good effect (but not adverse effect) in increasing the number of fruit flies and changed the sex ratio toward females. The other factors didn't show significant effect on treated fruit flies, although there an increase in the number of treated fruit flies but the differences were not significant**.** Fig leaves extract contains many good phytochemical substances e.g., Alkaloids, phenolic compounds, carotenoids and vitamins (11) in addition to important minerals which may accounts to such increase in number of treated fruit flies compared to control groups. Increasing the ratio of females in treated flies could be related to adaptive changes in sex ratio due to nutritional factors. In 2004, Cheryl and R. Michael wrote a review article that focused on maternal diet and sex ratio in ruminants and other mammalian species and they described many other factors to explain variations in sex ratio among different species (12). Some factors are frequently found among insects for example selective male killing (13). There could be many explanations for these results. One is that the in vitro research' culture conditions had an impact on the outcomes. For instance, in some species, the presence of glucose sugar in the medium may encourage the development or growth of males over females, and vice versa (14, 15 and 16). The ancient development scientist Darwin explained that some animal species can exhibit statistically significant changes in the ratio of sons to daughters, albeit the circumstances and underlying mechanisms that cause these changes were and are still largely unknown. The sex ratio is adjusted in birds, insects, and reptiles in response to food availability and other environmental conditions (17, 18).

Table1: Analysis of variance for the effect of *Ficus Carica* leaf extract (concentrations, sexes, generations, and their interactions) on the quantity of wild fruit flies.

Sources of variation	d.f.	Mean squares
R	4	177.5
Concentrations (C)	2	204.2
Sexes (S)	1	400.416*
Generations (G)	1	46.816
CS	2	1.667
CG	2	373.768*
SG	1	219.768
CSG	2	7.591
Error	44	97.334

*: Significant effect $P \leq 0.05$

Table 2: The mean ± standard error (S.E.) for the concentrations, generations, and interactions of the effects of *Ficus Carica* leaf aqueous crud extract on the number of wild fruit flies.

Sources of variation		**Mean ±S.E.**	**Sources of variation**		**Mean ± S.E.**
Concentrations (C)	**C0**	34.550 ± 2.120	Sex-Generation interactions (SG)	S1G1	43.533 ± 4.907
	C1	40.650 ± 2.405		S1G2	37.930 ± 4.737
	C2	39.250 ± 2.624		S2G1	34.533 ± 4.591
LSD				S2G2	36.600 ± 4.680
Sexes (S)	**S1**	35.566 ± 1.838	LSD		
	S2	40.733 ± 1.969	Concentration-Sex-Generation Interactions (CSG)	C0S1G1	44.800 ± 4.841
LSD		5.148		C0S1G2	29.800 ± 0.969
Generations(G)	**G1**	38.366 ± 2.299		C0S2G1	34.200 ± 4.017
	G2	37.226 ± 1.861		C0S2G2	29.400 ± 2.521
LSD				C1S1G1	45.600 ± 2.785
Concentration sex interactions (CS)	**C0S1**	37.300 ± 3.416		C1S1G2	40.200 ± 6.422
	C0S2	31.800 ± 2.275		C1S2G1	38.400 ± 3.820
	C1S1	42.900 ± 3.420		C1S2G2	38.400 ± 6.145
	C1S2	38.400 ± 3.406		C2S1G1	40.200 ± 6.988
	C2S1	42.000 ± 3.663		C2S1G2	43.800 ± 3.152
	C2S2	36.500 ± 3.739		C2S2G1	31.000 ± 6.025
LSD				C2S2G2	42.000 ± 3.391
Concentration-Generation interactions (CG)	**C0G1**	39.500 ± 3.452			
	C0G2	29.600 ± 1.275			
	C1G1	42.000 ± 2.525			
	C1G2	39.300 ± 4.201			
	C2G1	35.600 ± 4.612			
	C2G2	42.900 ± 2.203			
LSD		8.916			

LSD= Least significant differences

Mice Sperm abnormalities

It's clear from Table 3 that crude fig leaves extract observed significant effect (P≤0.05) on sperm without tail and highly significant effect (P≤0.01) on sperm without hook. Table 4 showed that the higher concentration (2%) produced more sperm without tail 5.6 ± 0.37) and sperm without hook (3.0 ± 0.63) compared with untreated control groups (mean ± SD of 2.0 ± 0.44 and 1.0 ± 0.44, L.S.D. values of 3.167 and 1.26, respectively for the two mentioned abnormalities. The differences between control groups and treated mice for other studied sperm abnormalities were not significant. Our results are conflicted with the results showed by Umarqayum *et al.*,2020 and Umarqayum *et al.*,2016 (19,20) in which they showed beneficial effect of *ficus carica* on fertility problems in male diabetic patients and the results obtained by Boukhalfa *et al.*, 2018, Odo *et al.*, 2016 and Nebedum *et al.*, 2010 (21,22,23). In our study, no significant effects were founds on sperm without head, defective head sperms and defective hook sperms between the control groups and the treated groups (P≤0.01), mean ± S.E were C0= 1.0 ± 0.447, 1.4 ± 0.509, 0.4 ± 0.244; C1=3.0 ± 0.894, 1.6 ±0.509, 1.2 ± 0.583; C2=2.0 ± 0.4, 1.4 ± 04, and 1.4 ± 0.244, respectively. These results are compatible with the results that observed by Ramezani *et al.*, 2014 (24) who evaluated some herbal extracts among them was *ficus carica* on 62 infertile men and they showed no significant effect on sperm morphology despite elevation of spermatozoa account by 8,14%. The present results also agree with the results obtained by Lalu *et al.* 2014 (25) because they indicated no significant differences from percentages of life sperms after fig extract treatments. Some chemicals present in *ficus caica* may have deleterious impact on normal sperm morphology specially if consumed in high quantities like benzyl aldehyde (26), while others like chlorogenic acid proved to have beneficial and protective effect and improve sperm quality (27). This is may be the reason that the ratio of some sperm abnormalities increased and the ratio of some others decreased in this study.

Table 3: Analysis of variance for the effect of *Ficus Carica* leave aqueous Crud Extract (Concentrations, sexes generations and their interaction) on sperm morphology of laboratory albino male mice *Mus musculus*.

Sources of variation	**d.f.**	**Mean squares**				
		Sperm without tail	Sperm without head	Sperm without hook	Defective head	Defective hook
R	**4**	5.066	2.933	1.5	0.333	0.332
Within treatments	**2**	19.467*	2.867	11.667**	0.467	1.4
Between treatments	**8**	4.716	1.783	0.75	1.383	0.988

*significant effect P≤0.05, **highly significant effect P≤0.01

Table 4: Mean ± S.E. for the effect of *Ficus Carica* leaf aqueous crude extract (concentrations, sexes generations and their interactions) on sperm of albino male mice *Mus musculus*.

Treatments		Mean ± S.E.				
		Sperm without tail	Sperm without head	Sperm without hook	Defective head	Defective hook
Concentrations	**C0**	2.0 ± 0.44	1.4 ± 0.509	1.0 ± 0.44	1.0 ± 0.447	0.4 ± 0.244
	C1	5.6 ± 0.37	3.0 ± 0.894	3.0 ± 0.63	1.6 ± 0.509	1.2 ± 0.583
	C2	5.2 ± 1.60	2.0 ± 0.400	1.0 ± 0.33	1.4 ± 0400	1.4 ± 0.244
LSD		3.167		1.26		

LSD = least significant differences

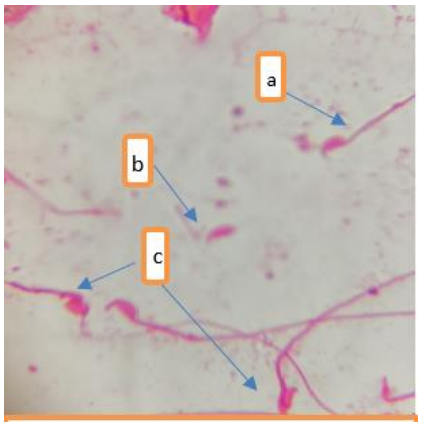


Figure (1): Normal sperm (a), Sperm without tail (b), and defective head sperms (c)

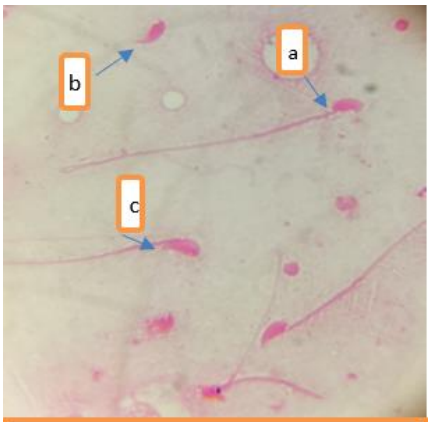
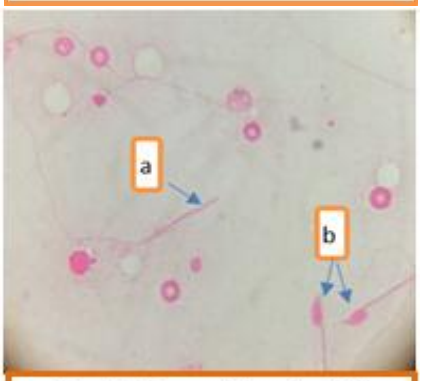
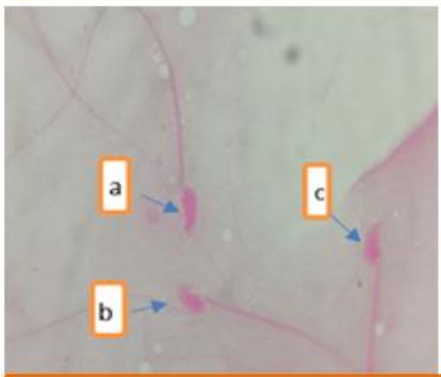


Figure (2): Sperm without hook (a), Sperm without head (b), and defective hook sperm (c)



Figure(3):Sperm without head (a), two normal sperms (b)



Figure(4): Defective hook sperm (a), defective head sperm (b), Normal sperm (c)

Conclusions

According to the study's current findings, treating wild fruit flies with fig leaves crud extract results in a change in the ratio of male to female sex. Additionally, when given to experimental albino mice, it results in two types of sperm abnormalities, including sperm without a tail and sperm without a hook.

Acknowledgement

I am grateful that the biology department at the university of Sulaimani approved my research and provided the animal house and assistance for my effort.

Conflict of interest

The authors confirm that they are not affiliated with or involved in any organization or entity with financial interests.

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