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Effect of Stick Sweet Cherry (*Prunus aviam*) on the Reproductive System of Male Mice

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ABSTRACT

Cherry is a traditional fruit grown in many places and have many types like sweet cherry and sour cherry, have a wide nutritional value which had strong activity as antioxidant, many phenolic compounds and anthocyanin. In this study, the oral uptake effect of stick alcohol extract of sweet cherry (*Prunus aviam*), for 10 days at a concentration 125, 250 and 500 mg/ml, showed no significant differences (P<0.05) as compared with the control. The effect on the male reproductive system of mice showed there is an increase in the number of abnormal and dead sperm, and the number of live, normal sperm and sperm motility decreased. The seminiferous tubules diameter, primary spermatocytes, number of leydig cells and spermatids decreases with the increase of the extract concentration.

Keywords: *Prunus aviam*, sperm, health; histology of testis.

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INTRODUCTION

Many of the herbs and spices have biological activities for long time ago, and many fruits that attracted attention including cherry, containing strong antioxidant activity and anthocyanin, which recommended as nutritional supplement and have a wide range of pharmacological and biochemical effect [1].

Cherries have a bioactive compounds in a wide range, including phenolic compounds [2]. Sweet cherries have high percentage of flavonols, anthocyanins, flavan-3-olsand hydroxy-cinnamic acids [3]. also include antioxidant, antifungal and antibacterial. Many fruits like sweet cherry (Prunus avium L.) contains beside water, saccharides in which glucose, sucrose, sorbitol and fructose [4], pectin, vitamins A, B and C, many organic acids often malic acid [5], minerals most likely potassium, calcium, phosphor, magnesium, also endogenous hormone like melatonin [6] and different protein contents [7].

sweet cherry consumption many beneficial effects [1]. Moreover, sweet cherries reported to be lesser phenolic content than sour cherries [8].

The phenolic profile is required for specific characterization, in order to improve health effects by its consumption [9].

The study aimed to determine the effects of oral administration of the stick sweet cherry (Prunus aviam) on the reproductive system of male mice which include study the effect on the sperm formation (morphology and activity) also the effect on the testes tissue.

MATERIALS AND METHODS

Preparation of extract and administration doses:

Air dried and grinding to powder, after collected, 50 gm of stick sweet cherry (Prunus avium) were extracted by soxhlet in 300 ml of 70% ethanol [10]. To obtain the final concentration 125, 250 and 500 mg/ml Re-suspend the dried stick extracts in distilled water used for mice oral administration daily for 10 days (40 mice used in four groups: 10 mice for the control group and 10 for each concentration) [11].

Sperm preparation and microscopic examination for sperm:

According to WHO Laboratory manual the sperms were assessed [12], for abnormalities, motility and dead/live sperm percentage. Sperm collected from cauda epididymis into TCM-199 medium. The sperm suspension incubated at 37°C for 1 hour under 5% CO2. The sperm parameters were assessed for viability of sperm, morphology and sperm count with 5 replicates for each mouse. For sperm morphology and viability, the slide of sperm was stained with eosin stain and observed under light microscope [13].

Histological analysis

The processed for routine paraffin embedding for testes placed overnight in Bouin fluid after perfuse-fixed. After cut into sections of about 5-µm thickness, from each teste make three sections mounted on slides, then de-paraffinization, re-hydration was made, and stained with hematoxyline - eosin stain. Under the light microscopy examined the sections of the testes, the spermatids diameters, interstitial spaces seminiferous tubules, leydig cells and assessment of primary spermatocytes in each teste using a Micrometers previously calibrated [14,15].

Statistical analysis

Was performed to compare two different groups by using Chi-square and ANOVA-test. Statistical significance was determined at P<0.05. [16].

RESULTS AND DISCUSSION

In this research we examined the effect of stick sweet cherry extract on reproductive system and histology of mice male after 10 days of extract administration. The results show no significant differences (P<0.05) in sperm parameters (live, dead, normal, abnormal and sperm motility) among the four groups (group I= control, group II= treated with extract concentration 125 mg/ml, group II= treated with 250 mg/ml and group III= treated 500 mg/ml daily) (Table: 1).

The results revealed that the percentage of live sperm, normal sperm and sperm motility will decrease with the increase of the concentration of the stick sweet cherry extract. While the Dead and abnormal sperm percentage

increase with the increase of the stick sweet cherry extract.

According to complex and multilayered structure cell wall in some organisms the extract may be inactive, and the activity may cause changes in the DNA that lead to inhibiting of cell reproduction [17]. In spite of the composition of the biological membrane like proteins and lipids may facilitate the solubility of the polyphenols. Many factors may affect the polyphenols activity like chemicals metabolic activation and cell membrane structure. There is indication on the presence of 28 compounds belongs to phenols and 13 compounds belongs to sugar after test the ethanolic extract of stick sweet cherry by GC-Mass chromatogram [11].

Table 1. Effect of Stick Cherry extract on mice sperm

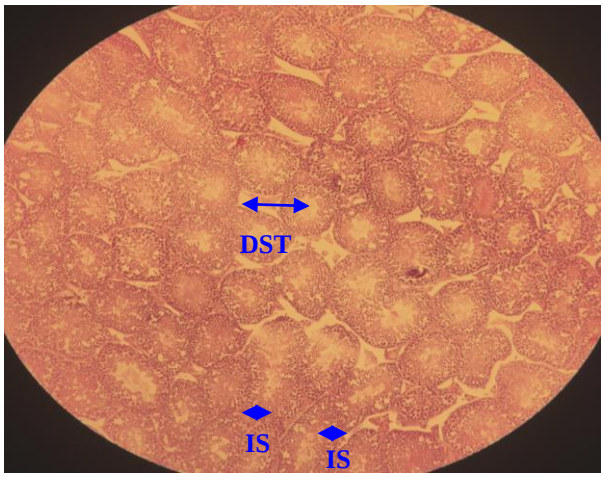
	Live sperm % (mean ± SE)	**Dead sperm % (mean ± SE)**	**Normal sperm % (mean ± SE)**	**Abnormal sperm % (mean ± SE)**	**Sperm motility % (mean + SE)**
Control	21.307 ± 5.139	3.461 ± 0.797	27.048 + 3.741	2.076 + 0.415	80.46 + 8.72
Group I (125mg/ml)	20.317 ± 3.064	3.95 ± 0.387	24.675 + 3.057	2.307 + 0.262	77.07 + 9.20
Group II (250mg/ml)	18.410 ± 2.291	7.195 ± 0.800	22.230 + 4.953	2.357 + 0.324	70.02 + 7.04
Group III (500mg/ml)	18.35 ± 2.897	7.65 ± 1.033	20.842 + 3.287	3.170 + 0.441	64.82 + 8.02

The result is not significant at $p < 0.05$.

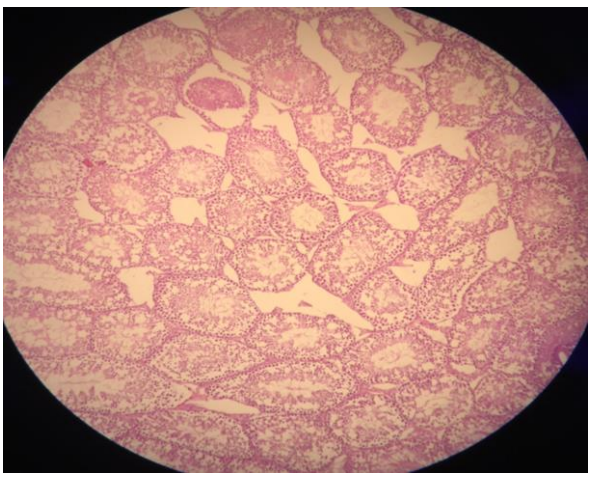
According to the histological study of reproductive system of mice male, the results showed comparison with control group there is a significant decrease in seminiferous tubules diameters and increase in the interstitial space when treated with the cherry stick extract 125, 250 and 500 mg/ml (table 2) (figure. 1).

Table 2: The interstitial space and diameter of seminiferous tubules between the treated groups and control.

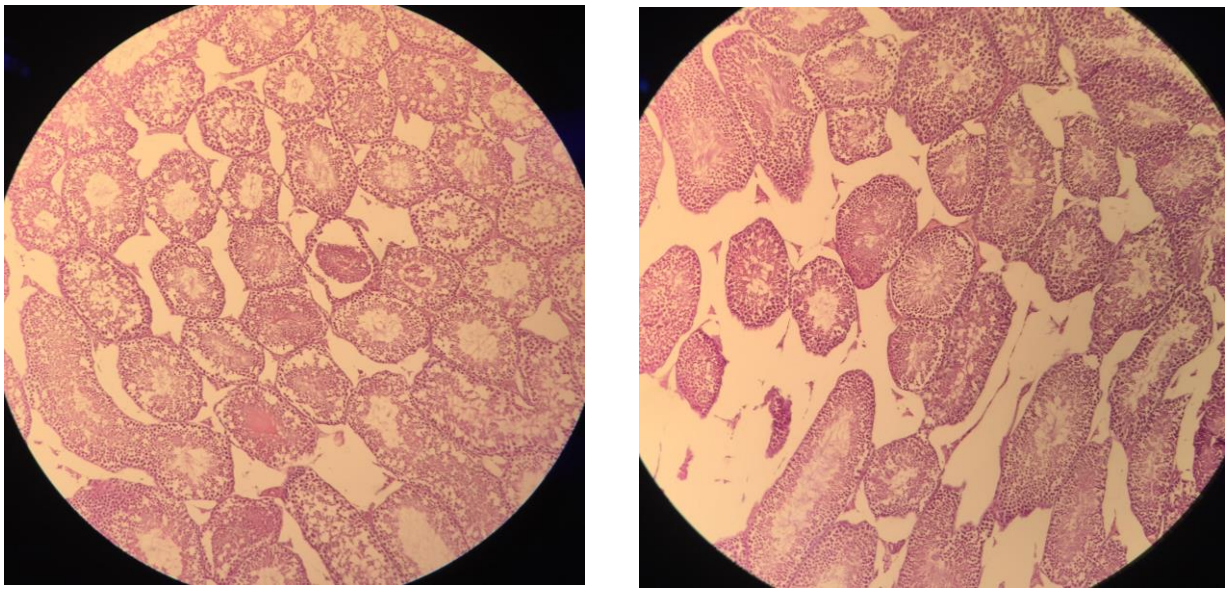
Groups	**Diameter of seminiferous tubules (μm) (mean±SE)**
Control	184.33+12.2
Group I (125mg/ml)	175.50±12.42
Group II (250 mg/ml)	168.22±11.06
Group III (500 mg/ml)	150.4±10.8



Group A (control)



Group B: (125 mg/ml)



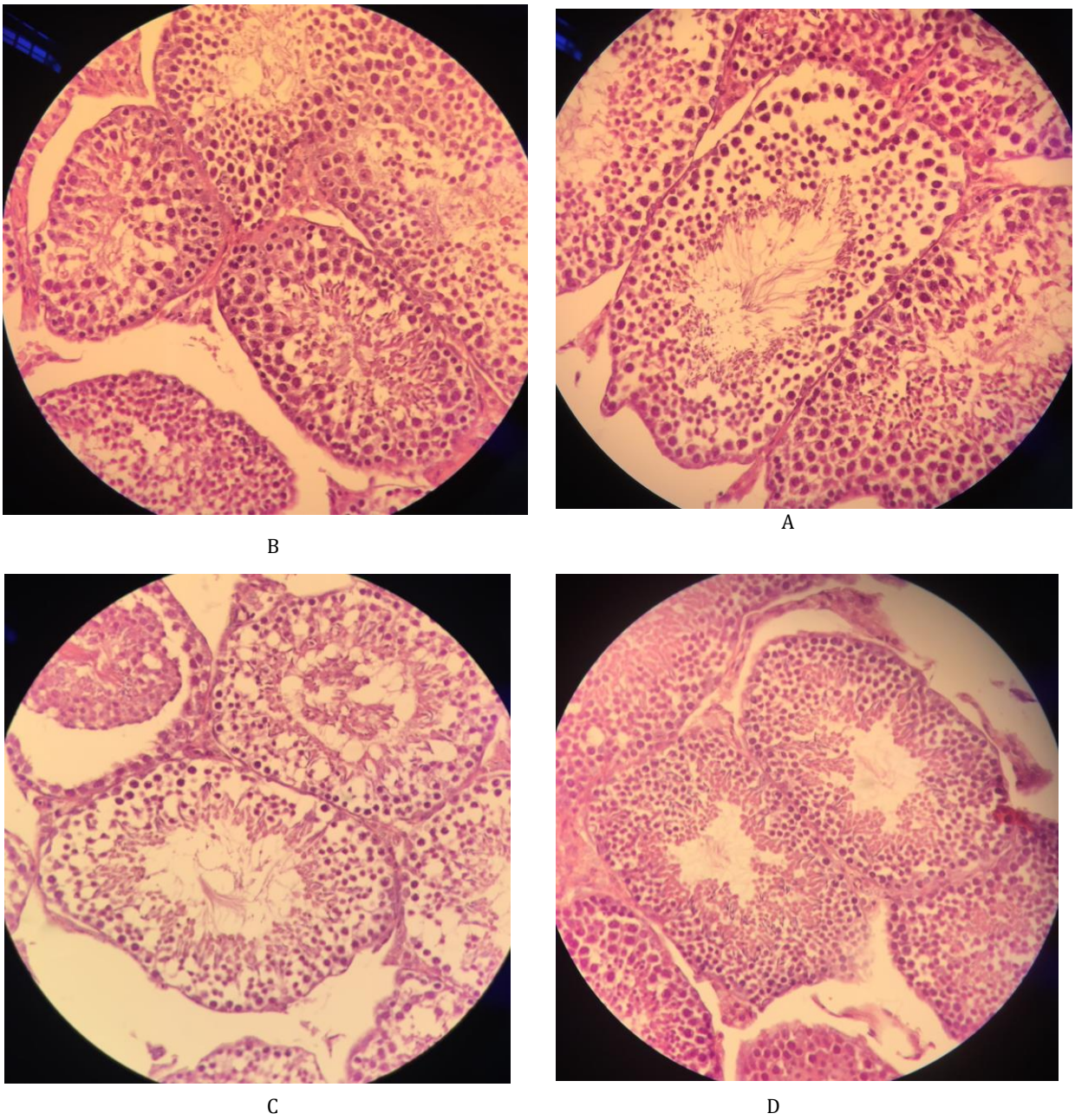
Group C: (250 mg/ml) Group D: (500 mg/ml)

Figure 1: Photomcirographs of mice testes. A: normal structure of seminiferous tubules was showed in the control group. B: treated group with 125 mg/ml of cherry extract. C: treated group with 250 mg/ml of cherry extract. And D: treated group with 500 mg/ml of cherry extract. The three groups B, C & D showing increase in interstitial space and decrease in diameter of seminiferous tubules (DST- Diameter of Seminiferous tubules, IS- Interstitial space) as compare with the control (X 10).

Also in spermatids and primary spermatocytes there is no significant differences between the groups treated with cherry stick extract and the control group (table 3) (figure 2), Spermatogenic cell layers of control showed denser packing of spermatogenic cells than the cherry stick extract treated groups. The lumen of control was more densely filled as compared to that of cherry stick extract treated groups which was filled with sperm tail this agreed with [18]. The present study shows tiny alteration in the diameter of leydig cells after the administration of cherry stick extract as compared to control. This may be related to increase in the age of leydig cells in the testes [19]. which might notice some changes in the morphology from round to oval in shape that make a non-significant result.

Table 3: Spermatids, primary spermatocytes diameter, and leydig cells number in treated and control groups.

Groups	Primary spermatocytes (μm) (mean±SE)	Spermatids (μm) (mean±SE)	Leydig cells (μm) (mean±SE)
Control	8.24±0.62	7.22±1.21	6.34±0.82
Group I (125mg/ml)	7.80±0.88	7.04±1.08	6.11±0.83
Group II (250 mg/ml)	6.92±0.81	6.84±0.88	5.64±0.75
Group III (500 mg/ml)	6.68±0.85	6.56±0.89	5.48±0.68



A B C D

Figure 2: Mice testes Photomcirograph. A: Normal structure of layers of Spermatogenic cell shown in control group. B, C and D: treated group with cherry stick extract (125, 250 and 500 mg/ml respectively) showing decrease in spermatogenic cells (X 40).

After analyzes of stick cherry extract by using GC-Mass instrument showing there are 28 phenolic compounds and 13 sugars were identified [11]. These phenolic compounds may affect the sperm formation. polyphenolic compounds are commonly present naturally in plants extracts. In many experiments on the motility of mammalian sperm in vitro there is an inhibitory effect that could cause infertility. The polyphenolic compounds action seems to be structure dependent. Their activity depends on the hydroxyl groups and methyl groups positions on the benzene ring. The double bond of carbon-carbon on the cinnamic acid side chain was essential for their activity. One of the polyphenols actions on spermatozoa is the enlarge of the superoxide radicals generated by them, which might be inhibit of the sperm motility in vitro [20].

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