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# Tribulus and Ashwagandha Diets to Combat Infertility of Cadmium Chloride Injected Male Albino Rats

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**ABSTRACT**

*This investigation was carried out to estimate the influence of Puncture vine, ashwagandha, and a mixture of both on male infertile rats. The biological experiment was designed to divide the rats into five groups. Group (1): Healthy rats (6 rats) were considered as control negative. Rats (24 rats) were treated with Cadmium Chloride to induce male infertility and they are reclassified into the groups, each group with six rats and received a basal diet. Group (2): Control positive. Group (3): received 5% Puncture vine. Group (4): fed on 5% Ashwagandha. Group (5): fed on a 5% mixture of both herbs. After 28 days, the levels of glucose, kidney functions, liver functions, lipids profile, AI, and different hormones as LH, testosterone, and FSH were determined in different rat groups and the results showed improvement in all parameters in different treated groups, especially in the case of the puncture vine diet 5% followed by a mixture of both puncture vine and ashwagandha, and finally, the group, which was fed on 5% Ashwagandha.It could be recommended that the rats fed on Puncture vine and ashwagandha showed improved functions of the organs and also, LH, testosterone, and FSH hormones in infertile rats.*

**Keywords:** *Infertility, Sexual hormones, Biochemical blood parameters, Ashwagandha*

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## INTRODUCTION

The fruits of Puncture vine (*Tribulus terrestris* L.) have been utilized in traditional Chinese medicine to treat rheumatism, general weakness vesical and renal calculi, premature ejaculation, impotency, and menorrhagia. It is a very potent diuretic and tonic drug (Kumari and Singh, 2018).

*Tribulus terrestres* extract has been included in their antioxidant, anti-inflammatory, and spermatogenesis-inducing, aphrodisiac. *Tribulus terrestres* had contained high amounts of chemical composition and nutritional compounds. These constituents are known to have target effects on the testis, and the gonads (Abarikwu *et al.,* 2020).

More natural products have antioxidant characteristics and androgenic activities on productive factors and hormones. Antioxidants are the first defense barrier against free radicals produced by oxidative stress. Also, reduced reactive oxygen stress, lowering insulin resistance, decreases cardiovascular diseases, and cancer growth (Noh *et al.,* 2020).

To control male infertility that happens, hormonal imbalance, and other infections, there are now treatments, namely called contemporary treatments, such as assisted reproductive techniques. Therefore, it found that herbal treatment gives more attention as an alternative or complementary treatment method for male infertility. Therefore, ingesting the roots of a small evergreen shrub, *Withania sominifera* (Ashwagandha) has beneficial effects on semen for infertile men. Oral intake of Ashwagandha roots has also been found to inhibit lipid peroxidation, improve sperm count and motility, and regulate reproductive hormone levels (Sengupta *et al.,* 2018).

*Withania somnifera* (Ashwagandha) also known as ginseng is a widespread and valuable herb in Ayurveda, which elevates immunity and preservation of body health (Ng *et al*., 2020).

Ashwagandha can prevent chronic heart diseases and has anticancer characteristics. Its root is considered as an aphrodisiac due to its natural antioxidants, which positively affect male infertility and also, *W. somnifera* caused testicular maturing and sperm production in immature rats (Mishra *et al.,* 2012).

Ashwagandha is a medicinal plant utilized in traditional medicine for the treatment of infertility. Therefore, it was found that it improves the function of the reproductive system by way due to the improvement of semen quality Enhance enzymatic activity in seminal plasma and reduces oxidative stress. As well as, Ashwagandha extract improves luteinizing hormone and the hormonal balance stimulates the follicle, which leads to the formation of follicles and the weight gain of the gonads (Nasimi Doost Azgomi*et al.,* 2018).

This study aimed at evaluating the beneficial effect of puncture vine and ashwagandha on male fertility, as well as the liver, kidney, and testis histology that may make the puncture vine and ashwagandha of the most important foods for the future.

## MATERIALS AND METHODS

### *Materials*

Dry Puncture vine and Ashwagandha (Indian ginseng) were purchased from a herb shop.

### *Chemicals*

Cadmium Chloride Hydrate ($CdCl_2$. $2.5H_2O$) was obtained from Merck.

*Animals*

30 adult male Sprague Dawley rats, with a mean bodyweight of (150±10g) were obtained from the Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

*Methods*

*Basal diet*

According to AIN (1993), the basal diet contained corn starch (67.5%), casein (12%), corn oil (10%), minerals (4%), vitamins (1%), cellulose (5%), methionine (0.3%), cholinechloride (0.2%), and the remained is corn starch (67.5%).

*Preparation of materials*

According to Russo and Tyler (2015), all materials were powdered and stored in dusky stoppered glass bottles in a dry and cool place till use.

*Induction of infertility in rats*

Rats were injected with $CdCl_2$, 0.1% at 0.1 ml/100g body weight to induce infertility.

*Experimental design and grouping*

Rats received basal diet for one week and kept under normal healthy conditions in wire cages at room temperature. They were divided into 5 groups as follows:

**Group (1):** Control negative; normal rats received a basal diet.
**Group (2):** Control positive group; infertile rats received basal diet.
**Group (3):** Infertile rats received *Puncture vine 5*%.
**Group (4):** Infertile rats received ashwagandha 5%.
**Group (5):** Infertile rats received a mixture of both 5%.

*Biochemical Blood Parameters*

At the end of the experimental, blood samples were collected in centrifuge tubes after 12h fasting using the abdominal aorta. After that, the blood was left to clot at room temperature and then centrifuged at 3000rpm for 10 minutes. Then, serum was aspirated and transferred into clean cuvette tubes and stored at -20°C for further analysis.

Urea, creatinine**,** and uric acid were determined according to Khozeimeh *et al.* (2017).

Total cholesterol (TC), HDL-c, LDL-c, and triglyceride were determined according to Alsoodeeri *et al.* (2020). Atherogenic index (AI) was calculated according to Kikuchi *et al*., (1998). Serum glucose was determined according to Kaplan (1984).

Testosterone hormone was determined colorimetrically according to the method of Pradelles *et al*., (1985). LH and FSH were colorimetrically determined according to Akram *et al*., (2012).

*Histological examination*

For histological studies, the testis was fixed overnight in Bouin's fluid, dehydrated in ethanol, and embedded in paraffin. Tissue sections (6 µm-thick) were cut on a microtome, mounted on a glass slide. Staining of the section with hematoxylin and eosin (HE). The Seminiferous tubular diameter and germinal mass thickness were measured and the number of spermatogonia, primary spermatocyte, many spermatozoa, and average interstitium showing Leydig cells were examined in each group under a light microscope (Rezvanfar *et al*., 2013).

*Statistical Analysis*

Data analysis was performed by one-way ANOVA using a Completely Randomized Factorial Design (SAS, 2004) when a significant mean effect was detected, the means were separated with the Duncan's Multiple Range Test. $P \leq 0.05$ was considered as the significance level. The results are presented as mean±SD.

## RESULTS AND DISCUSSION

*Effect of puncture vine and ashwagandha on lipid profile of infertile rats*

Total cholesterol and triglycerides were determined in rats fed *Puncture vine*, ashwagandha, or a mixture of both, and the results are reported in **Table 1** and illustrated that the cholesterol and triglycerides in the control positive group significantly increased by 319.0 and 231.0 mg/dl than control negative group being 99.0 and 109.0 mg/dl, respectively **(Table 1)**. Moreover, the results of the treatment groups showed that the best result was in group (3) fed on 5% *Puncture vine*, which showed reduced total cholesterol and triglycerides to 100.0 and 110 mg/dl followed by group (5) fed on 5% of both from *puncture vine* and ashwagandha, which was 109.0 and 115.0 mg/dl, and group (4) fed on 5% ashwagandha, which was reported 119.0 and 129.0 mg/dl, respectively. These results are consistent withAnwer *et al*., (2017)found that the oral administration of *Withania somnifera* significantly lowered TC and TG in diabetic rats.

As shown in **Table 1**, the amount of VLDLc, LDLc, and AI of the control positive group of infertile rats was higher by 26.2, and 32.0 mg/dl, and 2.84, compared to the control (-) group, being 21.8 and 22.2 mg/dl and 0.86, respectively. Meanwhile, HDLc was lower in the control positive group (31.0 mg/dl) than the control negative healthy group (49.0 mg/dl). The increasing level of the Atherogenic index (AI) is a great risk of cardiovascular diseases as it is responsible for the process of atherosclerosis (Ducharme and Bicke, 2008), which may be due to elevated lipid levels, and lowering blood flow. Plasma levels of cholesterol and LDL-c are responsible for atherosclerosis and the increased levels of HDL-c have a protective effect (Soehnlein, 2017) in humans.

The group fed on 5% puncture vine showed the best results for VLDLc, HDLc, HDLc, and AI (24.0, 48.0, and 17.0, and 0.85, respectively), followed by the group of a mixture of both *puncture vine*, ashwagandha (25.0, 44.0, 21.0, and 1.05), as well as, the group fed on 5% ashwagandha. This may be because the puncture vine and ashwagandha highly contain polyphenols as phenolic and flavonoid compounds, which scavenge free radicals and purify the blood. Anwer *et al*. (2017) found that the oral administration of Withania somnifera significantly decreased VLDL-C and LDL-C levels and significantly increase HDL-C levels in diabetic rats.

As shown in **Table 1**, the glucose in the control positive rats group was elevated compared to the control negative group by

178.0 and 122.0 mg/dl, respectively. The best serum glucose results were found in group (3) rats fed on a basal diet plus 5% *puncture vine* (120.0 mg/dl), followed by group (5) rats received a basal diet fortified with 5% from both *puncture vine* and ashwagandha (133.0 mg/dl) and group (4) fed on 5% ashwagandha (139.0 mg/dl). This may be due to the fact that the *puncture vine* contains high amounts of polyphenols including flavonoids and phenolic compounds with free radical scavenging activities. These results confirmed the results of the study of Anwer *et al*., (2017) found that the *Withania somnifera* extract significantly lowered glucose levels in diabetic rats.

**Table 1.** Effect of *puncture vine*, ashwagandha, and a mixture of both on lipid profile, Atherogenic index (AI) and glucose of infertile rats

| Groups | Total cholesterol (mg/dl) | Triglyceride (mg/dl) | $VLDL_C$ (mg/dl) | $HDL_C$ (mg/dl) | $LDL_C$ (mg/dl) | AI | Glucose (mg/dl) |
|---|---|---|---|---|---|---|---|
| G1: Control–ve | $99^{d}$±0.3 | $109^{d}$±0.6 | $21.8^{e}$±0.05 | $49^{a}$±0.14 | $20.2^{d}$±0.01 | $0.86^{d}$±0.007 | $122^{d}$±0.3 |
| G2: Control +ve | $319^{a}$±0.8 | $231^{a}$±0.7 | $26.2^{a}$±0.07 | $31^{e}$±0.17 | $61.8^{a}$±0.07 | $2.84^{a}$±0.003 | $178^{a}$±0.1 |
| G3: Puncture vine (5%) | $100^{d}$±0.1 | $110^{d}$±0.4 | $24^{d}$±0.16 | $48^{b}$±0.15 | $17^{e}$±0.15 | $0.85^{d}$±0.004 | $120^{d}$±0.5 |
| G4: Ashwagandha (5%) | $119^{b}$±0.2 | $129^{b}$±0.3 | $25.8^{b}$±0.09 | $41^{d}$±0.11 | $32.2^{b}$±0.02 | $1.42^{b}$±0.008 | $139^{b}$±0.4 |
| G5: Mixture of both (5%) | $109^{c}$±0.5 | $115^{c}$±0.9 | $25^{c}$±0.12 | $44^{c}$±0.13 | $21^{c}$±0.12 | $1.05^{c}$±0.009 | $133^{c}$±0.8 |
| LSD at 5% | 0.82 | 1.1 | 0.19 | 0.26 | 0.17 | 0.01 | 0.87 |

Values of the same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

*Effect of puncture vine and ashwagandha on the kidney functions and different hormons of infertile rats*

As shown in **Table 2** the creatinine, urea, and uric acid were the highest in the control positive rats group fed on a basal diet by 1.06, 53.0, and 4.59 mg/dl, respectively. Margolis, (2012) showed that urea nitrogen is produced in the liver as a protein metabolism end-product and is transferred to the kidney for excretion. Almost all kidney disorders may be due to inadequate urea excretion and increasing nitrogen levels in the blood.

Uric acid is a breakdown product of purines that are produced normally in the body. If the production of uric acid is too much in the body, it may lead to kidney diseases (Sharfuddin *et al.,* 2012; Edwards, 2016).

Furthermore, different groups indicated that the rats fed on 5% *Puncture vine* give the best results for kidney functions followed by a mixture of both *puncture vine* and ashwagandha (5%) and ashwagandha (5%), respectively. These results are confirmed by Hemlatha and Hari (2014) showed that *Puncture vine terrestr* is fruits reduced creatinine and urea in treated rats. In addition, Raut *et al.,* (2012) suggested that ashwagandha (*Withania somnifera*) reduces blood urea in healthy volunteers. Also, itis categorized as Rasayanas, which control negative group by encouraging the body in debilitated conditions. Therefore, it is used as a general activating for health.

Data of **Table 2** illustrated the effect of *puncture vine*, ashwagandha, and a mixture of both on testosterone, FSH, and LH hormones of infertile rats. From the results, LH hormones of infertile rats were the highest in the control positive rats by 8.8 mIU/ml. Also, testosterone and FSH hormones reduced to 1.17 ng/ml and 2.3 mIU/ml, respectively compared with control negative healthy rats in which testosterone, FSH and LH hormones were 2.53ng/ml, 7.4, and 4.1 mIU/ml, respectively.

The rat group fed on 5% ashwagandha gave the best results of LH (3.9 mIU/ml) followed by 5% of both of *puncture vine* and ashwagandha at 5%, (3.5 mIU/ml) and the rat group fed on 5% *puncture vine* (3.0 mIU/ml). Meanwhile, testosterone and FSH showed the best results in the group fed on puncture vine at 5% being 1.28 ng/ml and 4.1 mIU/ml followed by rat group fed on 5% ashwagandha (1.70 ng/ml and 2.40 mIU/ml) and rat group fed on both puncture vine and ashwagandha at 5% (1.52 ng/ml and 3.30 mIU/ml), respectively. These results are consistent with KarimiJashni *et al.* (2012) who found that the *puncture vine* plant raises the secretion of LH and stimulates the production of testosterone and great sperm production.

*Puncture vine* contains a dioscin compound, which elevates male sexual ability by increasing free testosterone levels (KarimiJashni *et al.,* 2012). Furthermore, saponins and protodioscin were found in puncture vine, which increases the levels of testosterone and LH. This plant helps to treat sexual dysfunctions as conventional medicines.

Rahmati *et al.* (2016) indicated that *Withania somnifera* (L.) increase testosterone level in addicted male rats.

**Table 2.** Effect of *puncture vine*, ashwagandha, and a mixture of both on testosterone, LH, and FSH of infertile rats

| Groups | Creatinine (mg/dl) | Urea (mg/dl) | Uric Acid (mg/dl) | Testosterone (ng/ml) | FSH (mIU/ml) | LH (mIU/ml) |
|---|---|---|---|---|---|---|
| G1: Control–ve | $0.81^{e}$±0.005 | $35^{e}$±0.2 | $3.05^{d}$±0.001 | $2.53^{a}$±0.006 | $7.4^{a}$±0.01 | $4.1^{b}$±0.05 |
| G2: Control +ve | $1.06^{a}$±0.009 | $53^{a}$±0.6 | $4.59^{a}$±0.002 | $1.17^{e}$±0.009 | $2.3^{d}$±0.08 | $8.8^{a}$±0.03 |
| G3: Puncture vine (5%) | $0.88^{d}$±0.008 | $42^{d}$±0.5 | $3.01^{e}$±0.003 | $1.82^{b}$±0.002 | $4.1^{b}$±0.05 | $3.0^{e}$±0.07 |
| G4: Ashwagandha (5%) | $0.92^{b}$±0.006 | $48^{b}$±0.4 | $3.82^{b}$±0.004 | $1.70^{c}$±0.003 | $2.4^{d}$±0.07 | $3.9^{c}$±0.09 |
| G5: Mixture of both (5%) | $0.90^{c}$±0.003 | $45^{c}$±0.9 | $3.42^{c}$±0.007 | $1.52^{d}$±0.009 | $3.30^{c}$±0.04 | $3.5^{d}$±0.02 |
| LSD at 5% | 0.01 | 1.03 | 0.007 | 0.01 | 0.1 | 0.1 |

Values of the same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

*Histopathological changes*

*Histopathological evaluation of testes*

Histopathological evaluation of the testes of control negative healthy rats indicated the normal testes structure of spermatogonial cells and complete spermatogenesis **(Figure 1)**. On the contrary, the Figurerevealed noticed sections to the control of group positive necrosis and degeneration of spermatogonial cells lining seminiferous tubules **(Figure 2)**. On the other hand, some examined sections of the Puncture vine diet group revealed necrosis and degeneration of spermatogonial cells lining seminiferous tubules **(Figure 3)**. However, testes of rats from the Ashwagandha diet group showed necrosis and degeneration of spermatogonial cells lining seminiferous tubules **(Figure 4)**. Meanwhile, sections from the mixed diet group showed very rare necrosis and degeneration of spermatogonial cells lining seminiferous tubules **(Figure 5)**.

It is evident that histopathological changes were in line with that of biochemical and biological parameters, indicating that the best group recorded for the Puncture vine diet, followed by the mixed, then came the ashwagandha diet, provided that all three herbal diets were of pronounced value for correcting the damage in (liver, kidney, and testis) observed in control (+) group.

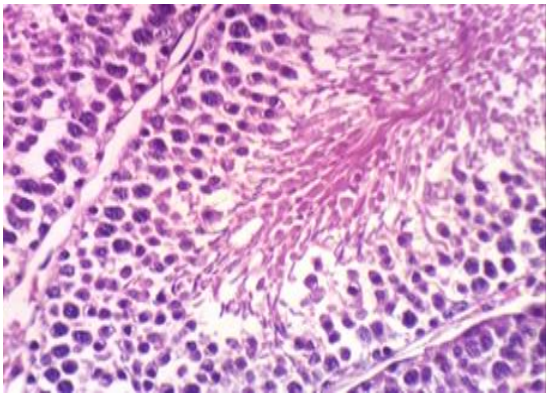

**Figure 1.** Testis of rat in control negative showed complete spermatogenesis and normal structure of seminiferous tubule (H&E X400).

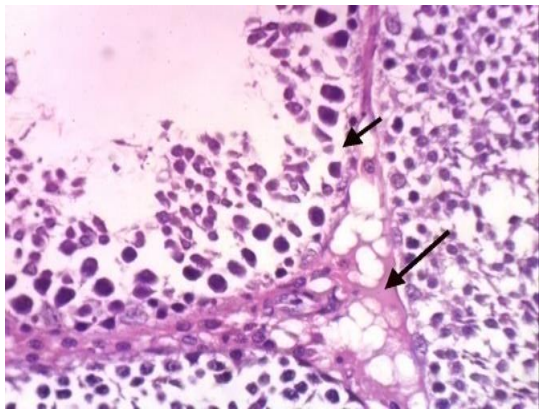

**Figure 2.**Testis of rat in control positive marked necrosis and degeneration spermatogonial cells lining seminiferous tubules with interstitial edema (H&E X400).

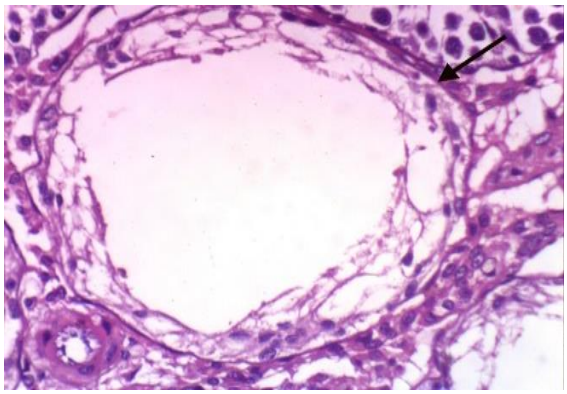

**Figure 3.** Testis of rat fed on Puncture vine showed degeneration and necrosis of spermatogonial cells lining seminiferous tubules (H&E X400).

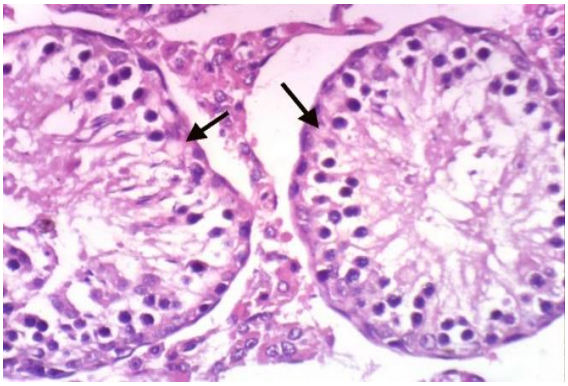

**Figure 4.** Testis of rat fed on Ashwagandha marked degeneration and necrosis spermatogonial cells lining seminiferous tubules (H&E X400).

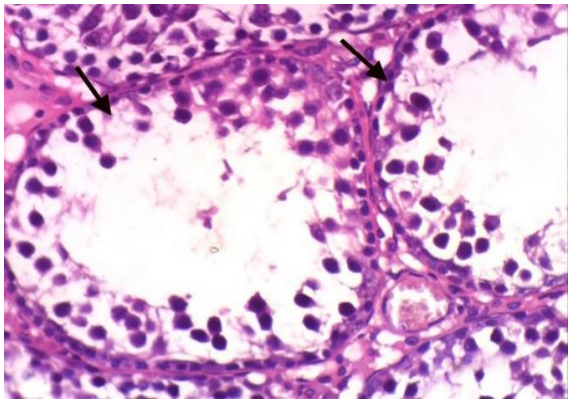

**Figure 5.** Testis of rat fed on Mix diet showed degeneration and necrosis spermatogonial cells lining seminiferous tubules (H&E X400).

## CONCLUSION

From the obvious results, it could be concluded that the *puncture vine* and ashwagandha contain rich amounts of polyphenol like phenol and flavonoid compounds, which scavenge free radicals and purify the blood. Meanwhile, the rats separately fed on 5% from *puncture vine*, ashwagandha, and a mixture of both improved the lipid profile, kidney

functions, and liver functions in male infertile rats. Thus, it could be proved that the herbs improve the testosterone, luteinizing, and follicle-stimulating hormone in male infertile rats.

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**CONFLICT OF INTEREST:** None

**FINANCIAL SUPPORT:** None

**ETHICS STATEMENT:** This study is cleared by the Nutrition and Food Science ethical committee, (Applied Nutrition), Faculty of Education, Department of Family Education. University of Umm Al-Qura, Makka Al-Mukarama, Kingdom of Saudi Arabi.

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