Therapeutic Potentials of ashwagandha extract on the toxic effect of benzo(a) pyrene in male rats

By

Nanees Youssef Elmetwally Elsayed Awad

Home Economics Department, Faculty of Specific Education, Mansoura University, Egypt

Research Journal Specific Education

Faculty of Specific Education

Mansoura University

ISSUE NO. 69, JULY , 2022

Therapeutic Potentials of ashwagandha extract on the toxic effect of benzo(a) pyrene in male rats

*Nanees Youssef Elmetwally Elsayed Awad**

Abstract:

Benzo(a) pyrene the first form of PAHs is genotoxic carcinogenic. This study was conducted to examine the potential therapeutic effects of levels of the extract of ashwagandha against testicular toxicity induced by BaP in rats. 30 mature rats were divided into 5 groups, (-) control group, BaP (10 mg/kg body weight), as (+) control BaP + treated with vit. E oral at a dose of 200 mg/kg body weight/day, BaP + AHE curative (1 ml /kg/day) and BaP + AHE curative (2 ml/kg /day) groups for 6 weeks. Many side effects were observed in animals injected with BaP such as loss of body weight, weakness activity and yellowish body hair. The results recorded a significant increase in body and testes weights, sperm count, sperm motility, serum levels of testosterone, luteinizing (LH), Follicle stimulating hormone (FSH), total antioxidants capacity (TAC), and (SOD) in the control-positive groups. And the groups curative with vitamin E and the levels of ashwagandha extract when compared to the negative control group, while a significant increase serum level MDA and luteinizing (LH) and in the BaP curative groups with vitamin E and the levels of ashwagandha extract compared to negative control group. Conclusions, administration at levels of ashwagandha extract caused ameliorative effect against BaP-induced testicular toxicity considered a powerful plant with health-promoting properties.

Key words: Withania somnifera, Curative, testosterone, testicular toxicity

* Home Economics Department, Faculty of Specific Education, Mansoura University

INTRODUCTION

Ashwagandha or Indian ginseng belonging to *Solanaceae family* which is also known as *Withania somnifera*. For almost 3000 years, it's leaves and roots has been utilised in Ayurvedic medicine (**Verma & Kumar, 2011**). The plant is small woody herb, thick, straight that normally reaches a height of 30 to 50 cm. Ashwagandha considered as adoptogenic herb used to improve overall health and promote endurance (**Winters, 2006**). The leaves can have about 0.1m of length and the florent can be green or vivid and glaring yellow in shade; its pod appears tangerine in the complex when it is full-grown and the favorable month for implanting the seeds in between June and July (**Tiwari *et al.*, 2014**). *Withania somnifera* is indigenous to Mediterranean countries of Africa, Southern Europe, and Western Asia and it is extensively scattered in parts of India, Pakistan, and Sri Lanka (**Shrivastava & Sahu, 2013**). Since time immemorial, the countless curative and medicative peculiar characteristics of this herbal plant is very well known to mankind. This plant renders aid in opposition to many afflictions and sicknesses like seizures, dullness, diabetes, and many more (**Dutta *et al.*, 2019**). The plants roots and leaves are usually used for medicinal purposes. Ashwagandha belonging to the family Solanaceae is an herb with a height of 30 to 150 cm maximum (**Sapra *et al*., 2020**). Ashwagandha herbs are "Queen of herbs" because of its numerous beneficial effects. It's used in ayurvedic medicines to promote enhance muscle strength and endurance, and improve overall health (**Kulkarni** & **Dhir 2008 and Mishra *et al*., 2000**).

Pyrene is a polycyclic aromatic hydrocarbon that burns at temperatures ranging from 300 to 600 degrees Celsius (**Ranjit *et al.*, 2018)**. Many ingredients can be found in foods, particularly grilled foods. The formula C20H12 is a benzopyrene, formed from a benzene ring fused with a pyrene. Epoxidediol metabolites known as BPDE interact. It is included in the first group of cancers before IARC (**Zhu *et al.*, 2014)**. Already known as the Mendelian virus. Benzpyrene (C 2 (/ 0) H 1 2) is a pentagonal aromatic hydrocarbon to which another benzene ring is added to the pyrene ring. When burning organic matter, large amounts of coal are produced.

Benzo[a]pyrene (BaP), and benzo[e]pyrene are both insoluble in water, soluble in perine in air, and have a melting point of about 180°C (**Lin *et al.*, 2018 and Gao *et al.*, 2020)**. The study aimed to investigate the effect of the oxidative properties ashwagandha herbs caused by Benzo[a]pyrene (BaP), on sperm parameters, serum level of hormones parameters and serum activities of antioxidant enzymes for experimental rats.

Material and Method

Material:

Ashwagandha (*Withania somnifera*): Ashwagandha powder was obtained from the local market, cairo, Egypt.

Rats: 30 mature albino male rats of Sprague Dawley strain weighing 210±5 g from Laboratory of Animal Colony, Helwan, Egypt. Basal diet prepared according to **(Reeves *et al.*, 1993).** The vitamin and mineral mixture prepared according to **(Campbell, 1963).**

Drugs:

Vitamin E©: Alpha tocopherol, α TF was purchased from Pharco Company for Pharmaceutical, Egypt.

Benzo(a)pyrene©: was obtained from Sigma Chemical Co. (St Louis, Mo, USA).

Method

Chemical analysis:

(a) Ashwagandha extract (AHX)

The ashwagandha powder (100 g) was dissolved in 1000 mL of ethanol (<99%) and kept at 27 °C for 24 h under continuous stirring, then filtered using Whatmann No. 1 filter paper. The filtrate was then evaporated to get concentrated filtrate. It was reconstituted distilled water to obtain the used dose of (AHX) according to (**Elhadidy, *et al.*, 2018**).

(b) Determination of Phenolic compounds of (AHX) extract

Ashwagandha for HPLC analysis was performed using a waters 2487 HPLC system consisting of a dual λ detector and a Waters 1525 binary pump, and equipped with a Waters Symmetry® C18 column (5 mm, 4.6 ×

50 mm) with Waters Sentry universalguard column (5 mm, 4.6 × 20 mm) (**Waters Corporation, Milford, MA, USA**). Phenolic compounds of ashwagandha were studied using the reference HPLC method by comparing experimental retention times with reported reference values **(Sakakibara *et al.*, 2003).**

Experimental design:

30 mature rats were divided into 5 groups of (n=6 rats) rats as followed: first group was fed on basal diet only and kept as control negative group, while groups (2, 3, 4 & 5) of rats (n=24) were injected (subcutaneous) one milliliter of toxic solution containing (10 mg/kg) benzo (a) pyrene (BaP) according to (**Kallistratos and Fasske, 1976**). Group (2) was left as positive control group, groups (3, 4 & 5) were divided into three curative groups:

Group 3: Positive control addition vitamin E of 200 mg/kg /b.wt./day by according to **(Shalaby *et al.*, 2004)**.

Group 4 and group 5: positive control addition with 1ml & 2 ml /kg /b.wt./day/rats ashwagandha extract (AHE). During the experiment period (6 weeks), the quantities of diet, which were consumed and / or wasted, were recorded every day. In addition, rat's weight was recorded weekly, to determine food intake and body weight gain percent according to **(Chapman *et al.*, 1950).**

Blood and Semen Sampling:

At the end of the experiment period, rats were anaesthetized by intraperitoneal injection of sodium thiopental (40 mg/kg). The testes and accessory sexual organs were removed and dissected out and weighed. Blood samples were withdrawn from the orbital plexs of veins of eye and the serum was separated after centrifugation. Serum samples were used for estimation of testosterone, FAH and LH levels. Semen samples were collected from cuda epididymis by cutting the tail of epididymis (cuda epididymis) and squeezing it gently in petri dish containing 0.5 ml of 2.9 % sodium citrate solution. Few droups of semen suspension were examined microscopically for epididymal sperm parameters. The testes, seminal

vesicles and prostate glands were dissected out and weighed. The left tested were quickly taken on ice till preparation of homogenate. The seminal content of epididymis was obtained by cutting of cuda epididymis using surgical baades and squeezed into a clean petri dish. The content was diluted 10 times with 2.9 % sodium citrate solution and thoroughly mixed to estimate the percentage of sperm progressive motility and sperm count as described by **(Bearden and Fluquary, 1980).** One drop of sperm suspension was withdrawn, smeared on clean glass slide and stained by eosin-nigrosin stain. The stained seminal smears were examined microscopically to determine percentage of sperm viability (ratio of alive/dead) and morphology as described by **(Amann, 1982).**

Biochemical analysis of serum:

Serum testosterone concentration was determined using Radioimmunoassay (RIA) method which is intended for the quantitative determination of total testosterone in the serum. The method is based on the competitive binding principal according to (**Wilke and Utley, 1987**). Levels of FSH and LH hormones in the serum were determined by an enzyme-linked immunosorbent assay (ELISA) using specific commercical kits as described by **(Ballester *et al.*, 2004).** The determination of the activity of tissue antioxidant enzymes activities total antioxidants capacity (TAC), malondialdehyde (MDA) and superoxide dismutase (SOD), were determined according to (**Nishikimi *et al.*, 1972, Ohkawa *et al.*, 1979 and Cao *et al.*, 1993),** respectively.

Statistics:

The findings were expressed as the mean ± SD. Statistical and correlation analyses were undertaken using independent One-way ANOVA with post-hoc tukey test and Pearson's rank correlation coefficient test, respectively. A *P*-value <0.05 was accepted statistically significant. All the previous statistical analyses of data were carried out by SPSS software ver. 17 (**SPSS Inc., 2008**).

Results and Discussion

The phenolic compounds of ashwagandha extract (AHX)

Data tabulated in Table (1) show that ashwagandha extract (AHX) contains some of phenolic compounds such as Flavonoids, Flavones and Polyphenols. The wavelength for determination each of Flavonoids, Flavones and Polyphenols were (489 nm, 612 nm and 335 nm) respectively. The experimental retention times were 56.4 min, 41.1 min and 24.2 min for Flavonoids, Flavones and Polyphenols respectively. While the standard retention times were 46.6 min, 25.3 min and 9.5 min for flavonoids, flavones and polyphenols respectively. The current study was similar to reports **Udayakumar *et al.*, (2010) and Balkrishna *et al.*, (2020)** who found that the AHX is a complex mixture of a large number of phytochemicals including phenolic compounds and flavonoids.

Table 1: Phenolic compounds of ashwagandha extract (AHX)

Phenolic compounds	**λ^a (nm)**	**EtR[b] (min)**	**RtR[c] (min)**
Flavonoids	489	56.4	46.6
Flavones	612	41.1	25.3
Polyphenols	335	24.2	09.5

[a] wavelength for determination, [b] experimental retention time, [c] standard retention time.

Effect of different levels of ashwagandha extract (AHX) on feed intake, body weight and FRE of the experimental rats groups

The results showed that there were significantly decreases in the mean body weight, food intake and food efficiency ratio in BaP group induced testicular toxicity compared with normal control group. After BaP fed with either Vit. E or AHX levels, the increase in body weight, food intake and food efficiency ratio were significantly increased when compared with BaP group ($P < 0.05$) (Table 2). It could be observed that group (5) had the high level of feed intake compared to other groups. The body weight of group (5) was 89.44±7.14 g while was 90.87± 8.62, 51.62± 5.11, 90.44± 6.61g and 85.41± 7. 31 g for (negative control group, positive group,

vitamin E group and group 4) respectively. FER in group (5) was more than group (3) and group (4). These data may be due to the biochemical properties of ashwagandha extract (AHX) which acts as antioxidant agent and its pharmacodynamics. These results are in harmony with those obtained by **(Rice-evans *et al.*, 1995 and Biswal *et al.*, 2013).**

Table (2): Effect of ashwagandha extract (AHX) on feed intake and body weight gain and FER in rats

Groups / **Parameters**	**Control (-ve)**	**Control (+ve)**	Rats received benzo(a)pyrene		
			Group 3 (Vit. E)	**Group 4 (1ml)**	**Group 5 (2ml)**
Feed intake (g/d)	15.33± 1.21 a	13.55± 1.18c	15.80± 1.61b	15.65± 1.27b	15.15± 1.41a
Body weight (g)	90.87± 8.62 a	51.62± 5.11 d	90.44± 6.61 b	85.41± 7. 31 c	89.44± 7.14 b
FER %	0.097± 0.008 a	0.063± 0.006 c	0.085± 0.004 b	0.085± 0.002b	0.091± 0.003 ab

Each means± standard deviations in the same row with different letters are significantly difference ($p \leq 0.05$).

Effect of different levels of AHX on relative weight of sexual of the experimental rats groups

Table (3) showed that the weight of testes in positive control group was significantly decreased 1.12± 7.00 g compared to negative group; groups (3), (4) and (5) were 2.62± 0.05 g, 2.29±0.01 g, 1.90±0. 02 g and 2.03± 0.04 g respectively. The weights of the prostate gland were 0.63±0.03 g, 1.05±0.01 g, 0.98±0.03 g, 0.90±0.02 g, 0.92±0.02 g in ((+) control group, (-) group, vitamin E group, group 4 and group 5) respectively. The weight of seminal vesicle was significantly decreased in positive control group in compare to negative control group. Meanwhile, treating by different levels of AHX reversed the toxicity of BaP. A significant deficiency of this vitamin in the body of men leads to atrophy of the cells of the testicles.

Getting the antioxidant vitamin E may help improve the quality of men's sperm and protect it from damage, which increases men's overall fertility and improves their sexual health **(Ulatowski and Manor 2013, Azzi 2018 and Khadangi and Azzi 2019).** The sexual organs weight and sperm parameters of the experimental rats' groups were clear that the group 5 was the best group compared to the positive group and less than control, these may be due to the effect of level of AHX which high content of phenolic and flavonoid and antioxidant activity. These results were agreed with **(Sapra *et al.*, 2020)** which investigated the effects of the beneficial effects of ashwagandha in reproductive system. Ashwagandha leaves improves sexual function and sperm parameters in male rats by activation of Nrf2/ HO-1 pathway while inhibiting the NF-κB levels (**hanaa and Shalaby 2016).**

Table (3): Effect of ashwagandha (AHX) extract on relative weight of sexual organs in rats

Groups Parameters	Control (-ve)	Control (+ve)	rats received benzo(a)pyrene		
			Group 3 (Vit. E)	Group 4 (1ml)	Group 5 (2ml)
Seminal vesicle	0.79± 0.01 a	0.35± 0.02c	0.65± 0.03a	0.50± 0.01b	0.68± 0.02a
Testes	2.62± 0.05 a	1.12± 7.00 c	2.29± 0.01 a	1.90± 0. 02 b	2.03± 0.04 a
Prostate glands	1.05± 0.01 a	0.63± 0.03 c	0.98± 0.03 a	0.90± 0.02 b	0.92± 0.02 b

Each means± standard deviations in the same row with different letters are significantly difference ($p \leq 0.05$).

Effect of different levels of AHX on sperm parameters of the experimental rats groups

The effect of AHX on sperm parameters of the experimental rats' groups was shown in Table (4). Treating with benzo(a)pyrene led to decrease in the count (10^6/ml), viability (%) and motility (%) as it was

43.54±4.66, 42.92±2.13 % and 53.76±2.18% respectively in compare to negative control group. On the other hand, treating with different level of AHX has been reversed the toxicity of BaP in all the parameters. These results are in harmony with those obtained by (**Legraverend *et al.*, 2010**) who noticed that BaP may cause reduction in fertility and decreasing in gonadal weights. Higher doses of BaP (40 mg/kg) caused almost complete sterility in both sexes of off spring (**Ramesh *et al.*, 2008).** The best results of sperm parameters were recorded by group 5 that treated by 2ml AHX. Recent studies show that AHX is effective in increasing sperm count and sperm motility (motility) in infertile men. Another study found similar results, showing that AHX can significantly improve sperm count and motility. Spermatogenesis is a high-energy process that requires an optimal nutrition of antioxidants, minerals, and nutrients **(Tripathi *et al.*, 2021**).

Table (4): Effect of ashwagandha (AHX) on sperm parameters in rats

Groups Parameters	Control (-ve)	Control (+ve)	rats received benzo(a)pyrene		
			Group 3 (Vit. E)	Group 4 (1ml)	Group 5 (2ml)
Count (10^6/ml)	75.31± 3.01 [a]	43.54± 4.66 [c]	70.30± 3.55 [a]	67.19± 2. 42 [b]	69.75± 3.74 [ab]
Viability (%)	98.15± 3.18 [a]	42.92± 2.13 [c]	94.09± 3.43 [a]	84.61± 3.15[b]	91.13± 4.19 [ab]
Motility (%)	90.25± 3.12 [a]	53.76± 2.18 [c]	89.52± 3.43[a]	79.55± 3.44[b]	85.09± 3.52[b]

Each means± standard deviations in the same row with different letters are significantly difference ($p \leq 0.05$).

Effect of different levels of AHX on serum level of sex hormones of the experimental rats

Serum levels of sex hormones in experimental rats were shown in Table (5). The testosterone, folicle stimulating and luteinizing hormones and serum levels in rats of group (5) that treated by 2ml of AHX were found to be 34.74± 1.05 nmol/L, 161.00± 7.22 and 4. 68± 0.4 ng/ml respectively. These results were significantly increased than the positive group which was 18.22± 1.30 nmol/L, 103.16± 7.12 and 2.08± 0.2 ng/ml respectively. Indeed,

group 5 group 3(vitamin E) showed better results than group 4 and compared to positive control. A study conducted on rats showed that taking different doses of vitamin E daily for a period of months led to a significant increase in testosterone levels, while another study found that vitamin E supplementation affected the level of testosterone as a result of its deficiency (**Miller *et al.*, 2009 and Nicastro and Dunn 2013)**.

These results were in agreement with those obtained by (**Smith *et al.*, 2020**) who found that course intake of ashwagandha root led to increase in serum testosterone concentration. There has been much research into the ashwagandha benefits on reproductive system, (**Lopresti *et al.*, 2019**) studied the effect of a daily intake of 240 mg ashwagandha for 60 days on serum testosterone levels in men who found that there was an increasing in Total testosterone increased from 472.9 to 526.9 ±48.0 ng/dl. Ashwagandha has been considered a source of natural antioxidants for its polyphenol content and powerful antioxidant properties that cause diseases such as STZ-induced hyperglycemia and infertility that enhance testicular capacity, sperm count and quantity, levels of sex hormones, and levels of antioxidants in the blood. It showed an improvement in sperm count and testosterone level when taking ashwagandha, and it helped the body overcome problems caused by testosterone deficiency such as osteoporosis (**Shah *et al.*, 2015 and Smith *et al.*, 2020)**.

Table (5): Effect of ashwagandha (AHX) on serum level of testosterone, luteinizing (LH), and Follicle stimulating hormone (FSH) in rats

Groups / Parameters	**Control (-ve)**	**Control (+ve)**	rats received benzo(a)pyrene		
			Group 3 (Vit. E)	**Group 4 (1ml)**	**Group 5 (2ml)**
Testosterone (nmol/L)	38.50± 1.15 [a]	18.22± 1.30 [c]	35.14± 1.05[a]	28.34± 1. 10 [b]	34.74± 1.05 [a]
FSH (ng/ml)	164.02± 7.03 [a]	103.16± 7.12 [c]	160.09± 6.04 [a]	151.76± 6.05[b]	161.00± 7.22 [a]
LH (ng/ml)	5.19± 0.4 [a]	2.08± 0.2 [c]	4.99± 0.4[a]	4.26± 0.4[b]	4. 68± 0.4[a]

Each means± standard deviations in the same row with different letters are significantly difference ($p \leq 0.05$).

Effect of different levels of AHX on serum activities of antioxidant enzymes of the experimental rats

The effect of different levels of AHX on superoxide dismutase (SOD), Total antioxidants capacity (TAC) and malondialdehyde (MDA) showed in Table (6). The positive control group treated with BaP showed significant decrease in levels of total antioxidants capacity (TAC) and superoxide dismutase (SOD) while significant increase revealed in level of malondialdehyde (MDA) in compare to negative control group. Obviously, treating with different levels of AHX reversed the toxicity of BaP. It was found that the rats treated by vit E and 2ml of AHX groups increased significantly in the levels of TAC and SOD (5.09±0.47µ/mg and 28.91 ± 3.61mmol/L) compared to positive group. Vitamin E is an antioxidant, as it eliminates free radicals in the body that attack cells and cause abnormalities in them, increasing the possibility of them turning into cancerous cells.

Table (6): Effect of ashwagandha (AHX) on serum activities of antioxidant enzymes in rats

Groups **Parameters**	**Control -ve**	**Control +ve**	Rats received benzo(a)pyrene		
			Group 3 Vit. E	**Group 4 (1ml)**	**Group 5 (2ml)**
Total antioxidants capacity **mmol/L))**	6.16± 0.33 [a]	1.23± 0.12 [c]	5.85± 0.75 [b]	4.54± 0.44 [b]	5.09± 0.47 [b]
Malondialdehyde **(µmol/g)**	6.55± 1.44 [c]	12.41± 2.77[a]	5.94± 1.42 [b]	5.56± 1.73[b]	5.60± 1.44 [b]
Superoxide dismutase **(µ/mg)**	36.26± 7.01[a]	18.41± 1.21[c]	30.87± 2.11[a]	25.96± 3.21[b]	28.91± 3.61[b]

Each means± standard deviations in the same row with different letters are significantly difference ($p \leq 0.05$).

These results were agreement with (**Ruann and Hélia 2015**). These data may be due to Ashwagandha root contains withaferin A, essential amino acid called tyrosine and sitoindosides VIIX. Those compounds show a high antioxidant activity via enhance and increase the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase which play a vital role in scavenging the free radical and decrease the oxidative stress.

Ashwagandha root contains phenolic compounds and flavonoids (**Bhat *et al.*,2015**) that act as vital compounds in reversing and delaying the occurrence and development of tumors Withaferin A is the major constituent of AHX which has been used for active action against cancerous cells **(Rai *et al.*, 2016).** The phenolic compounds and total antioxidant capacity found in ashwagandha plays a great role as an anti-inflammatory agent by decreasing the pro-inflammatory markers TNF-α. The most therapeutically important chemicals are Withanolides, particularly withaferin A and withanolide D, are the most therapeutically relevant compounds (**Kalra and Kaushik 2017**). Ashwagandha contributes to the recovery of sperm, its absorption of free radicals, with its very high antioxidant properties, it increases oxidation energies by BaP-infected sperms evidenced by the parameters of the restored sperm MDA level BaP reactivation due to H2O expulsion, reactivates the military oxidation defense level MDA. BaP reactivation due to H2O expulsion, reactivates the military oxidation defense (**Smith *et al.*, 2020)**. Vitamin E appears to strengthen lymphocytes, reduce production of the immunosuppressive prostaglandin E2, and reduce immunosuppressive serum lipid peroxides. Vitamin E has an anti-platelet effect, so it reduces their sticking to the walls of blood vessels and increases the efficiency of fertilization (**Nicastro and Dunn 2013)**.

CONCLUSION

Feeding rats at concentrations (1 and 2 ml) and vit. E may significantly reduce oxidative stress and enhance sperm analysis, so this study recommends the use of AHX for male hormones, could be a potential bioactive therapeutic agent in control and management of in men.

References

- **Amann, R.P. (1982).** Use of animal models for detecting specific alterations in reproduction. Fundam Appl Toxicol., 2(1):13-26.
- **Azzi, A. Many (2018).** Tocopherols, one Vitamin E. Mol. Asp. Med. 61, 92–103.
- **Balkrishna, A., Pokhrel, S., Singh, J., and Varshney, A. (2020).** Withanone from *Withania somnifera* may inhibit novel coronavirus (COVID-19) entry by disrupting interactions between viral S-protein receptor binding domain and host ACE2 receptor. Virology Journal, 17806-17829.
- **Ballester, J., Munoz, M. c., Dominguez, J., Rigau, T., Guinovart, J. J. and Rodriguez, J. E. (2004).** Insulin-dependent diabetes affects testicular function by FSH and LH linked mechanisms. J. Androl., 25 (5): 706-719.
- **Bearden, H.J. and Fluquary, J. (1980).** Applied animal reproduction. Restore Publishing Co. Inc., Reston, USA, page, 158-160.
- **Bhat, H. P., Jakribettu, R. P., Boloor, R., Fayad, R., and Baliga, M. S. (2015).** Use of ayurvedic medicinal plants as immunomodulators in Geriatrics: Preclinical Studies. In Foods and Dietary Supplements in the Prevention and Treatment of Disease in Older Adults. Academic Press, Philadelphia, pp., 143-149.
- **Biswal, B. M., Sulaiman, S. A. and Ismail, H. C. (2013).** Effect of *withania somnifera* (Ashwagandha) on the development of chemotherapy-induced fatigue and quality of life in breast cancer patients. Integr Cancer Ther., 12: 312–322.
- **Campbell, J. A. (1963).** Methodology of protein evaluation, PAG. Nutr. Document R. 101 Add 37, June, Meeting, New York.
- **Cao, G., Alessio, H. and Cutler, R. (1993).** Oxygen radical absorbance capacity assay for antioxidants. Free Radic Biol Med.; 14:303-311.
- **Chapman, D. G., Gastilla, R. and Campbell, T. A**. **(1950).** Evaluation of protein in food. I. A. Method for the determination of protein efficiency ratio. Can. J. Bioehem. Physio. I (37) 679-686.
- **Dutta, R., Khalil, R., Green, R., Mohapatra, S. S. and Mohapatra, S. (2019).** *Withania Somnifera* (Ashwagandha) and Withaferin A: Potential in Integrative Oncology. Int. J. Mol. Sci., *20*: 5310.

- **Elhadidy, M. E., Sawie, H. G., Meguid, N. A. and Khadrawy, Y. A. (2018).** Protective effect of ashwagandha (*Withania somnifera*) against neurotoxicity induced by aluminum chloride in rats. Basic Research, 8(1): 59-66.
- **Gao, M., Zheng, A., Chen, L., Dang, F., Liu, X. and Gao, J. (2020).** Benzo(a)pyrene affects proliferation with reference to metabolic genes and ROS/HIF-1α/HO-1 signaling in A549 and MCF-7 cancer cells. Drug Chem. Toxicol., 45, 1–9.
- **Hanaa F. El-Mehiry and Shalaby, A. O. (2016).** Effect of additive maple syrup to croissant on hormonal and enzymatic status in experimental rats Egyptian J. of Nutrition (4): 3, 81-107
- **Kallistratos, G. and Fasske, E. (1976).** Prevention of 3,4-benzopyrene carcinogenesis in presence of putrescine. Z Krebsforsch Klin Onkol Cancer Res Clin Oncol., 87: (1) 81-92.
- **Kalra, R. and Kaushik, N. (2017).** *Withania somnifera (Linn.)* Dunal: a review of chemical and pharmacological diversity. Phytochem. Rev., 16:953-987.
- **Khadangi, F. and Azzi, A. (2019).** Vitamin E the Next 100 Years. IUBMB Life, 71, 411–415.
- **Kulkarni, S. K. and Dhir, A. (2008).** *Withania somnifera*: An Indian ginseng. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 32: (5) 1093-1105.
- **Legraverend, C., Guenthner, T. M. and Nebert, D. W. (2010).** Importance of the route of administration for genetic differences in benzo[a]pyrene-induced in utero toxicity and teratogenicity. Teratology, 29: 35-47.
- **Lin, S., Ren, A., Wang, L., Huang, Y., Wang, Y., Wang, C. and Greene, N. D. (2018).** Oxidative stress and apoptosis in benzo[a]pyrene-Induced Neural Tube Defects. Free Radic. Biol. Med., 116, 149–158.
- **Lopresti, A. L., Smith, S. J., Malvi, H. and Kodgule, R. (2019).** An Investigation into the Stress-relieving and pharmacological actions of an ashwagandha (*Withania Somnifera*) extract: A Randomized, Double-Blind, Placebo Controlled Study. Medicine, 98, e17186
- **Miller, E. R., Pastor-Barriuso, R., Dalal, D., Riemersma, R.A., Appel, L. J. and Guallar, E. (2009).** Meta-Analysis: High-Dosage Vitamin E

Supplementation May Increase All-Cause Mortality. Ann. Intern. Med., 142, 37–46.

- **Mishra, L. C., Singh, B. B. and Dagenais, S. (2000).** Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha) a review. Altern Med Rev., 5: 334–346.
- **Nicastro, H. and Dunn, B. (2013).** Selenium and Prostate Cancer Prevention: Insights from the Selenium and Vitamin E Cancer Prevention Trial (SELECT). Nutrients, 5(4): 1122–1148.
- **Nishikimi, M., Rao, N. and Yogi, K. (1972).** Colorimetric determination of superoxide dismutase. Biochem. Biophys. Res. Common., 46: 849-854.
- **Ohkawa, H., Ohishi, N. and Yagi, K. (1979).** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Bio., 95: 351-358.
- **Rai, M., Jogee, P. S., Agarkar, G. and Santos, C. A. D. (2016).** Anticancer activities of *Withania somnifera*: Current research, formulations, and future perspectives. Pharmaceutical biology, 54(2), 189-197.
- **Ramesh, A., Inyang, F., Lunstra, D. D., Niaz, M. S., Kopsombut, P., Jones, K. M., Hood, D. B., Hills, E. R. and Archibong, A. E. (2008).** Alteration of fertility endpoints in adult male F-344 rats by subchronic exposure to inhaled benzo(a)pyrene". Exp Toxicol Pathol., 60 (4–5): 269–80.
- **Ranjit, S., Sinha, N., Kodidela, S. and Kumar, S. (2018).** Benzo(a)pyrene in Cigarette Smoke Enhances HIV-1 Replication through NF-κB Activation via CYP-Mediated Oxidative Stress Pathway. Sci. Rep., 8: 10394.
- **Rasgon, N. L., Altshuler, L. L. and Fairbanks, L. (2005)**. Reproductive function and risk for PCOS in women treated for bipolar disorder. Bipolar Disord; 7(3): 246-259.
- **Reeves, P., Nielsen, F. and Fahey, G. (1993)**. AIN-93. Purified diets for laboratory rodents: Final report of the American Institute of Nutrition adhoc writing committee on the reformulation of the AIN-76 A Rodent diet. J. Nutrition, 123: 1939-151.
- **Rice-evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M. and Pridham, J.B. (1995).** The relative antioxidant activities of plantderived polyphenolic flavonoids, Free radical research, 22 (4): 375-383.

- **Ruann Janser Soares de Castro and Hélia Harumi Sato (2015).** Synergistic actions of proteolytic enzymes for production of soy protein hydrolysates with antioxidant activities: An approach based on enzymes specificities. Biocatalysis and Agricultural Biotechnology, 4: 694–702.
- **Sakakibara H., Honda Y., Nakagawa S., Ashida H. and Kanazawa K. (2003).** Simultaneous Determination of All Polyphenols in Vegetables, Fruits, and Teas. J. Agric. Food Chem. 2003, 51, 3, 571–581.
- **Sapra, NC.; Kalyanrao, P.; Sasidharan, N.; Arna Das and Susmitha, P. (2020).** Effect of mechanical, chemical, growth hormone and biofertilizer treatments on seed quality enhancement in Ashwagandha (*Withania somnifera Dunal*), Med. Aromat Plants, Los Angeles, (9): 35-40.
- **Shah, N. Singh, R.Sarangi, N. Saxena, A. Chaudhary, G. Kaur, S.C. Kaul, R. and Wadhwa, E. (2015).** Combinations of Ashwagandha leaf extracts protect brain-derived cells against oxidative stress and induce differentiation. PLoS One., 10(3): e0120554.
- **Shalaby M.A., El- Zorba, H.Y and Kamel, G.H (2004).** Effect of alpha-tocopherland simvastatin on male fertility in hypercholesterolemic rats. Pharmacol. Res., 50(2):137-142.
- **Shrivastava1, A. K., and Sahu, P. K. (2013).** Economics of Yield and Production of Alkaloid of *Withania Somnifera (L.)* Dunal. American Journal of Plant Sciences, 4: 2023-2030
- **Smith, S.J., Lopresti, A.L., Teo, S.Y.M. and Fairchild, T.J. (2020).** Examining the Effects of Herbs on Testosterone Concentrations in Men: A Systematic Review. Adv. Nutr. 22-47.
- **SPSS Inc. (2008).** SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.
- **Tiwari R., Chakraborty S., Saminathan M., Dhama K. and Singh S. V. (2014).** Ashwagandha (*Withania somnifera*): Role in Safeguarding Health, Immunomodulatory Effects, Combating Infections and Therapeutic Applications: A Review. Journal of Biological Sciences, 14(2):77-94
- **Tripathi, M.KP., Singh, P., Sharma, S., Singh, T.P., Ethayathulla, A. and Kaur, S. P. (2021).** Identification of bioactive molecule from *Withania*

somnifera (Ashwagandha) as SARS-CoV-2 main protease inhibitor J. Biomol. Struct. Dyn., 39, 5668-5681.

- **Udayakumar, R., Kasthurirengan, S., Vasudevan, A., Mariashibu, TS., Rayan, J. J. S., Choi, C. W., Ganapathi, A. and Kim S. C. (2010).** Antioxidant effect of dietary supplement *Withania somnifera* L. reduce blood glucose levels in alloxan-induced diabetic rats, Plant foods for human nutrition, 65(2): 91-98.
- **Ulatowski, L. and Manor, D. (2013).** Vitamin E trafficking in neurologic health and disease. Annu. Rev. Nutr., 33, 87–103.
- **VERMA, S. K. and KUMAR, A. (2011).** Therapeutic uses of *Withania somnifera* (Ashwagandha) with a note on withanolides and its pharmacological actions. Asian Journal of Pharmaceutical and Clinical Research, 4(1):1-4.
- **Wilke, T.J. and Utley, D.J. (1987).** Total testosterone free androgenic index and calculated free testosterone by analog RIA method. Clinc. Chem., 33: 1372-1375.
- **Winters, M. (2006).** Ancient medicine, modern use: *Withania somnifera* and its potential role in integrative oncology. Altern. Med. Rev.,11(4):269-77.
- **Zhu, W., Cromie, M.M., Cai, Q., Lv, T., Singh, K., and Gao, W. (2014).** Curcumin and Vitamin E Protect against Adverse Effects of Benzo[a]pyrene in Lung Epithelial Cells. PLoS ONE 9, e92992.

الاحتمالية العلاجية لمستخلص الأشواجاندا على التأثير السام للبنزو (أ) بيرين في ذكور الفئران

نانيس يوسف المتولي السيد عوض *

الملخص العربي:

بنزو (أ) بيرين الشكل الأول من الهيدروكربونات العطرية متعددة الحلقات هو مادة مسرطنة سامة جينيا. أجريت هذه الدراسة لفحص التأثيرات العلاجية المحتملة لمستويات مستخلص الأشواجاندا ضد سمية الخصية التي يسببها بنزو (أ) بيرين في الفئران. تم تقسيم ٣٠ فأر ناضج إلى ٥ مجموعات ، كنترول سالبة، بنزو (أ) بيرين كنترول موجبة (١٠ مجم / كجم من وزن الجسم) ، مجموعة بنزو (أ) بيرين + عولجت بفيتامين هـ عن طريق الفم بجرعة ٢٠٠ مجم / كجم من وزن الجسم / يوم ، مجموعة بنزو (أ) بيرين + مستخلص الأشواجندا المعالج (١ مل / كجم/ يوم) ومجموعة بنزو (أ) بيرين + مستخلص الأشواجندا المعالج (٢ مل / كجم/ يوم) لمدة ٦ أسابيع. لوحظت العديد من الآثار الجانبية في الحيوانات المحقونة بالبنزو (أ) بيرين مثل فقدان وزن الجسم وضعف النشاط وشعر الجسم المصفر. سجلت النتائج زيادة معنوية في أوزان الجسم والخصيتين ، وعدد الحيوانات المنوية ، وحركة الحيوانات المنوية ، ومستويات التستوستيرون في سيرم الدم، واللوتين ، والهرمون المنبه للجريب (FSH)، والكثافة الإجمالية لمضادات الأكسدة ، وفوق اكسيد الديسميوتيز. في مجموعات الكنترول الموجبة. والمجموعات العلاجية بفيتامين E ومستويات مستخلص اشوجاندا عند مقارنتها بالمجموعة الضابطة السالبة، بينما هناك زيادة معنوية في مستوى المالونالدهيد واللوتين في سيرم الدم وفي المجموعات العلاجية بنزو (أ) بيرين + فيتامين E ومستويات مستخلص الأشواجاندا مقارنة بالمجموعة الضابطة السلبية. الاستنتاجات، الحصول على مستويات من مستخلص الأشواجاندا يسبب تأثيرًا محسنًا ضد سمية الخصية التي يسببها بنزو (أ) بيرين والتي تعتبر نباتًا قويًا له خصائص تعزز الصحة.

الكلمات المفتاحية: أشواجندا، علاجي، هرمون تستوستيرون، سمية الخصية.

* قسم الاقتصاد المنزلي، كلية التربية النوعية، جامعة المنصورة