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PROTECTIVE POTENTIAL OF *BUCHANANIA LANZAN* OIL IN ORGANOPHOSPHATE - INDUCED MALE REPRODUCTIVE TOXICITY: AN EXPERIMENTAL STUDY

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ABSTRACT: The purpose of this research was to examine the harmful effects of Organophosphate (OP) on reproduction as well as the putative antioxidant agent *Buchanania lanzan* Oil (BLO potential)'s protective properties. A total of 32 male Wistar rats were divided into four groups of eight each: the control group (CRL), the Organophosphate group (OP, 27 mg/kg), the *Buchanania lanzan* oil group (BLO, 0.5 ml/kg), and the combination group (OP+ BLO). For 45 consecutive days, the test animals received gavage doses (five days per week). We looked at body weight gain, weights of the reproductive organs, sperm characteristics, testosterone levels, and levels of thiobarbituric acid-reactive substances (TBARS). According to the data, OP, considerably ($p<0.001$) reduced both the rate of body weight gain and the absolute weights of reproductive organs (testes, epididymis, and seminal vesicles). Moreover, significant modifications in semen at least ($p< 0.01$) A drop in spermatid number, sperm count, sperm motility, and testosterone level, with an increase in aberrant and dead sperm and TBARS level were seen in the OP, group. BLO therapy alone has been shown to enhance spermatogenesis, increasing spermatid counts and seminal vesicle weights significantly ($p<0.001$). On the other hand, co-administration of BLO and OP, can more effectively and selectively modify the negative effects of OP on the weights of the reproductive organs, the quality of the semen, the levels of testosterone, and the TBARS (at least $p< 0.001$). This indicates the protective function of BLO against reproductive toxicity caused by OP, its antioxidant qualities, and capacity to lower TBARS levels, as demonstrated in this work, may be the cause of the induced reproductive damage.

INTRODUCTION: One of the organs in an organism that is most susceptible to occupational and environmental contaminants, particularly pesticides, is the reproductive system. In conclusion, insecticides are said to affect spermatogenesis, damage spermatozoa, harm Sertoli or Leydig cells, and disturb the endocrine function at any stage of hormonal regulation [1, 2]. To the best of our knowledge, there is not enough information to say whether or not neonicotinoids affect male reproductive function.

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In mature male rats treated with imidacloprid, [3] noted a clear shift in the quality of the semen, the quantity of testosterone, and the architecture of the testicles. Similarly, [4] showed that acetamiprid causes metabolic alterations, reproductive damage, and oxidative stress in rats. Consequently, in our opinion, more toxicological research is necessary to understand the relative risk and dangers. Herbalism is a form of complementary or traditional medicine that involves using plant tissues, oils, or extracts for therapeutic purposes.

It has grown recently and drawn a lot of study interest from scientists all over the world. 80% of the population in several Asian and African nations relies on traditional medicine for primary healthcare [5]. *Buchanania lanzan* is one of the most well-known plants among the potential medicinal plants that are thought to have medicinal and

healing capabilities. It has been used for thousands of years as seeds or oil to improve health and treat illnesses [6]. The annual plant *Buchanania lanzan* also referred to as a black seed, is a member of the Ranunculaceae family. It is an aromatic plant that has a long history of usage in traditional medicine, especially in the Middle East, Asia, and Africa, to treat a variety of ailments and symptoms such as asthma, eczema, bronchitis, back pain, fever, headache, and dysentery. (Boskabady *et al.* 2007, 2010, Mehta *et al.* 2009 [19], Ghamarnia *et al.* 2010).

Scientific testing has revealed a variety of pharmacological capabilities for *Buchanania lanzan* and its components, including antiasthmatic, hypoglycaemic, antihypertensive, anti-inflammatory, immune-stimulatory, antioxidant, anticancer, antibacterial, and antiparasitic effects [10]. Moreover, it has been noted that this spice exhibited both allelopathic potential and hepatoprotective qualities (Kanter *et al.* 2005; Abdel-Daim and Ghazy 2015). (AlCharchafchi *et al.* 2007). Further features including gastroprotective, nephroprotective, antinociceptive, anticonvulsant, spasmodic, testicular protective, antiangiogenic, antiarthritic potential, anti-oxytocic, and contraceptive actions were explored in a recent review by Ijaz *et al.* (2017). This plant has been the subject of numerous phytochemical studies; the black seeds contain a fixed oil (30–40%), an essential oil (0.5–1.5%), as well as different carbohydrates, proteins, and other active ingredients (Hosseini *et al.* 2015). Thymoquinone, alkaloids (nigellone, nigericin, and nigella dine), vitamins (including thiamin, niacin, pyridoxine, riboflavin, and folic acid), minerals, and proteins are the main active components recovered from *Buchanania lanzan* oil (Salem 2005).

The antitoxic capabilities of *Buchanania lanzan Seed*, extract, and essential oil have recently been investigated against a number of organophosphate pesticides, including dimethoate, diazinon, propoxur, malathion, and mancozeb. In these experiments, this plant demonstrated its ability to counteract the majority of hematological, biochemical, immunological, and reproductive effects brought on by these pesticides (Attia and Nasr 2009, Al-Attar and Al-Taisan 2010, Mohamadin *et al.* 2010, Barakat and El-Masry 2016).

The purpose of this study is to look at how paracetamol affects the properties of semen and whether *Buchanania lanzan* oil may have any protective benefits against paracetamol-induced reproductivity in male rats.

MATERIALS AND METHODS:

Paracetamol and *Buchanania lanzan* Oil: Organophosphate (OP,), is >97% pure and has the CAS registry number 135410-20-7. It was purchased from the local market of agricultural supplies and used in experiments with an oral LD_{50} of 217 mg/kg for male rats (EPA 2002).

To preserve it until use, *Buchanania lanzan* oil (BLO) was purchased at a nearby medical plant store in Botany Garden, Sagar. The oil was a "rough" shop product; neither was it standardized nor was it made in a lab.

Animals and Experimental Design: At the Pasteur Institute in Kouba, Algeria, 32 male Wistar rats between the ages of 8 and 12 weeks were obtained. Two weeks were spent acclimating the animals before the experiment began. Individually housed in plastic cages with sawdust bedding, the animals were kept in an air-conditioned animal house with a controlled photoperiod of 12 hours of light and 12 hours of darkness, a controlled temperature of 22 2 C, and free access to pellet food and cleaning, running water.

The animals were then divided into four groups, each with eight rats, and given daily doses of paracetamol and *Buchanania lanzan* oil (5/7 weekly) for 45 days straight as follows:

Group I: (C), which served as control, was administered orally with 0.5ml/kg/day of distilled water.

Group II: (OP,), animals were given a dose of Organophosphate at the level of 27 mg/kg/day (DL50/8), this dose was selected by referring to the studies of Mondal *et al.* (2014) and Chakroun et al. (2016).

Group III: (BLO), rats received 0.5 ml/kg/day of *Buchanania lanzan* oil. The dose was chosen based on the works of Mansour *et al.* (2013) and Mosbah *et al.* (2014).

Group IV: (BLO+OP,), animals were given 0.5 ml/kg/day of *Buchanania lanzan* oil, and then after 30 minutes, received 27 mg/kg/ day of Organophosphate.

All rats were handled in accordance with the standard guide for the care and use of laboratory animals. The study was approved by the Ethics Committee of the Faculty of Sciences, University of Harisingh Gour Sagar (Registration number: DB07/2021)

Body and Organ Weights: For the course of the study, body weight was noted every day. Animals were sedated, and sacrificed at the conclusion of the treatment period, and the reproductive organs (testicles, epididymis, and seminal vesicles) were swiftly removed, the connective tissue was removed, and the organs were weighed.

Semen Characteristics: Each rat's left testis and epididymis were removed in order to count and count the spermatids. The testis was cut into small pieces with scissors after the tunica albuginea was removed, and the homogenate was combined with a vortex mixer after being dissolved in 10 ml of 0.9% NaCl and 0.5% Triton X-100. After performing a second dilution (1:9 v/v), one drop of the suspension was added to a hemocytometer (Malassez) chamber, and the number of spermatids that were homogenization-resistant was counted under a microscope at a magnification of 40. (Robb *et al.* 1978, Blazak *et al.* 1993). Similar to how the right caudal epididymis was homogenized and sperm counted before, the left caudal epididymis was chopped into minute pieces and placed in 10 ml of 0.9% NaCl with 0.5% Triton X-100 (Amman *et al.* 1976). The number of sperm count per gram of organ was used to express the results as a mean SD.

The analyses of sperm motility, viability, and morphology were carried out utilizing Linder et al. (1995) and Liobet et al (1995). Briefly, a rat was slaughtered, and sperm were collected as soon as feasible. Each animal's right caudal epididymis was removed and put in a warmed petri dish with 2 ccs of Hank's solution at 37 C. The tissue was cut with a scalpel blade to release the sperm, and after 15 minutes in an incubator at 37 C, the sperm motility was assessed. After stirring the suspension, 20 µL were deposited between a coverslip and a heated microscope slide. Motile and non-motile sperms were manually counted in at least 10 different, randomly chosen fields while being observed under a microscope at a magnification of 40. After removing the coverslip and allowing the spermatozoa suspension to dry in the air, the sample was stained with 1% eosin Y/5% nigrosin and checked for viability and morphological abnormalities under a 40x magnification. For each sample, 300 sperm tozoa from various fields were analyzed as previously mentioned.

Testosterone and TBARS Measurements: The blood samples were taken in EDTA tubes at the time of sacrifice, centrifuged for 15 minutes at 3000 rpm, and the plasma was then kept at 20 C for further examination. The enzyme-linked immunosorbent assay (ELISA) kits, which were acquired from DRG Diagnostics, GmbH in Germany, were used to determine the plasma testosterone content. Thiobarbituric acid reactive substances (TBARS) were measured using Tappel and Zalkin's technique (1959).

Statistical Analysis: Data are analyzed using SPSS (version 14.0, Chicago, IL) for Windows and reported as mean values SD. Using a one-way analysis of variance, comparisons between animal species were made (ANOVA). At p, differences were deemed significant.

RESULTS: In this study, OP caused a significant decrease ($p< 0.001$) or less in body weight gains **Table 1**, absolute reproductive organ weights (testis, epididymis, and seminal vesicles) **Table 2**, spermatid number and sperm count **Table 3**, motility and viability at $p< 0.01$ **Table 4**, as well as in testosterone level **Table 5**, while abnormal sperm **Table 4** and TBARS level (Table Treatment with BLO alone led to a significantly higher body weight gain ($p< 0.01$), testis, epididymis, and sperm count (at least $p< 0.001$) when compared to the OP, -treated group. Also, it has noted a considerable increase in the weight of seminal vesicles and the number of spermatids **Tables 2** and **3**.

As a comparison to the PCM group, the addition of BLO to the OP, diet improved the weights of the reproductive organs (testis, epididymis, and

seminal vesicles) **Tables 1** and **2**. The negative effects of OP, on testosterone level **Table 5** and semen parameters (spermatids number, sperm count, motility, viability, sperm morphology) **Table 3** and **4** were also significantly reversed.

TABLE 1: EFFECT OF ORGANOPHOSPHATE (OP,), *BUCHANANIA LANZAN* OIL (BLO), AND THEIR COMBINATION ON BODY WEIGHT DURING 45 CONSECUTIVE DAYS OF TREATMENT

Group	3rd day	21st day	45th day	Bodyweight gain %
Control	236.7±21.14	267.4 ± 23.24	294.4 ± 25.26	29.23 ± 9.71
OP	289.3± 27.37	293.3 ± 20.71	310.3± 36.55	9.81± 5.78
BLO	251.3±18.41	291.7 ± 21.95	313.1 ± 33.31	24.23± 9.17
OP+BLO	273.1 ± 27.04	289.6± 22.18	311.7± 29.34	13.55± 6.71

TABLE 2: EFFECT OF ORGANOPHOSPHATE (OP), *BUCHANANIA LANZAN* OIL (BLO), AND THEIR COMBINATION ON REPRODUCTIVE ORGAN WEIGHTS AFTER 45 CONSECUTIVE DAYS OF TREATMENT

Group	Testis	Epididymis	Seminal vesicle
Control	1.67±0.042	0.493 ± 0.019	0.929± 0.020
OP	1.01 ± 0.021	0.207± 0.021	0.493± 0.019
BLO	1.63 ± 0.048	0.389 ± 0.016	0.991± 0.017
OP+BLO	1.51± 0.046	0.419± 0.021	0.729± 0.026

TABLE 3: EFFECT OF ORGANOPHOSPHATE (OP), *BUCHANANIA LANZAN* OIL (BLO), AND THEIR COMBINATION ON SPERMATID NUMBER AND SPERM COUNT AFTER 45 CONSECUTIVE DAYS OF TREATMENT

Group	Sperm count (.106//g epididymis	Spermatids number (.106//g testis)
Control	316.52± 9.54	193.25 ±5.79
OP	193.6 ± 5.81	140.4 ± 4.34
BLO	321.5 ± 6.66	221.49 ± 6.72
OP+BLO	276.2 ± 6.31	178.08 ± 5.32

TABLE 4: EFFECT OF ORGANOPHOSPHATE (OP), *BUCHANANIA LANZAN* OIL (BLO), AND THEIR COMBINATION ON SPERM MOTILITY, VIABILITY, AND ABNORMALITIES AFTER 45 CONSECUTIVE DAYS OF TREATMENT

Group	Motility %	Viability %	Abnormal %
Control	73.81±7.94	81.37 ± 8.88	16.00± 2.82
OP	64.19 ± 5.93	68.00 ± 9.01	26.01± 3.54
BLO	69.19 ± 11.31	79.6± 6.64	13.73 ± 2.64
OP+BLO	78.04 ± 7.89	79.81 ± 6.39	19.81 ± 4.21

TABLE 5: EFFECT OF ORGANOPHOSPHATE (OP), *BUCHANANIA LANZAN* OIL (BLO), AND THEIR COMBINATION ON PLASMA TESTOSTERONE AND TBARS LEVELS AFTER 45 CONSECUTIVE DAYS OF TREATMENT

Group	Testosterone ng/ml	TBARS nmol/ml
Control	5.27 ± 0.32	0.47 ± 0.09
OP,	1.41± 0.57	1.17± 0.07
BLO	5.19 ± 0.29	0.54 ± 0.04
OP+BLO	4.43 ± 0.31	0.59± 0.02

Group BLO+OP (at least $p < 0.05$). Also, the key oxidative stress indicator, TBARS levels **Table 5** were markedly decreased ($p < 0.001$) following BLO administration. These results suggest that co-administration of BLO with OP can mitigate and/or control all of the harmful reprotoxic effects brought on by OP; this protective action may

DISCUSSION: Due to the rapid population expansion, insecticides are being used more frequently worldwide to manage agricultural pests and meet food needs, but often their negative consequences outweigh their beneficial ones. Because exposure to these chemicals in the environment and at work can have major health effects like endocrine disruption and fertility issues [30].

Our research supports earlier findings made on laboratory animals in which two neonicotinoid insecticides were tested, imidacloprid in rats [31] and OP in mice. The earlier findings were the decrease

in body weight gain and the absolute reproductive organ weights (testes, epididymis, and seminal vesicles) observed in OP-treated rats [4].

Weight reductions in the testis, epididymis, seminal vesicles, prostate, and vas deferens were observed in rats given nicotine (0.3 and 0.4 mg/100 g/b.w.) either orally or intraperitoneally for 30 days, according to Londonkar *et al.* (1998, 2000). These alterations could be the result of decreased pituitary FSH and LH production and the absence of androgens.

Also, in the current study, compared to the CRL group, OP significantly alters sperm characteristics by lowering testicular spermatid counts, increasing abnormal sperm morphology, and decreasing epididymal sperm counts, motility, and viability. There is a correlation between the negative effects of OP on sperm quality and a drop in serum testosterone levels. Zhang *et al.* reported similar results.

In mice treated with OP and by Bal *et al.* (2012) in developing male rats treated with imidacloprid, the authors found a direct correlation between the dose of imidacloprid and the level of sperm degradation. In light of the current study, our earlier research on nicotine which is chemically similar to neonicotinoids like acetamiprid at a level of 1 mg/kg for two months, found similar effects on reproductive function [7]. Also, it was demonstrated by Perry *et al.* (2007) and Singh *et al.* (2016) that exposure to organophosphate pesticides was linked to lower sperm concentration, motility, and testosterone levels as well as greater levels of luteinizing hormone and sex chromosome aneuploidy in semen.

The decrease in the expression of the testicular steroidogenic acute regulatory (TSAR) protein, which aids in the transfer of cholesterol in mitochondria and testosterone production, may have contributed to the drop in plasma testosterone levels in this study (Manna *et al.* 2001). Additionally, the reduction in testosterone levels under OP exposure, which is involved in the regulation of genital tract dimensions, structure, and function (steroidogenesis, spermatogenesis, and spermiation), may be the cause of the decrease in reproductive organ weights, testicular spermatid number, and epididymal sperm count. It is well known that oxidative stress plays a major role in several diseases. Current research suggests that numerous pesticides may cause hazardous symptoms, the reduction in plasma testosterone levels observed in this study may be attributable to the inhibition of the steroidogenic (3b-HSD and 17b-HSD) enzymes' activities or/and a reduction in the expression of the testicular steroidogenic acute regulatory (TSAR) protein, which aids in the transfer of cholesterol in mitochondria and is accompanied by an increase in reactive oxygen species (ROS) that damage various membrane components of cells, particularly an accumulation of lipid.

Additionally, the current findings revealed a significant rise in thiobarbituric acid-reactive substances (TBARS) in the plasma of OP-treated rats **Table 5**. This increase in TBARS could be partially attributed to a decline in the activities of antioxidant enzymes like SOD, CAT, and GSH-PX as well as an excessive production of free radicals/ROS, which suggests that free radicals-induced oxidative cell injury may be a mediator of the reproductive toxicity of therefore, a disruption in the functional integrity of membrane structures and fluidity of the mitochondria and other cytoplasmic organelles through peroxidation of phospholipids, proteins, and nucleotides in the testis may result in a decrease in sperm motility and viability and an increase in sperm abnormalities **Table 4**. (Nagda and Bhatt 2011).

According to our findings, earlier research (Zhang *et al.* 2011, Bal *et al.* 2012, Mosbah *et al.* 2015) demonstrated that neonicotinoid insecticides (acetamiprid, imidacloprid) and nicotine cause an imbalance in oxidative/anti-oxidative status by depleting antioxidant defense systems and elevating TBARS levels in the testis.

Recent studies have shown how amazingly helpful *N. sativa* is in treating a variety of illnesses and promoting the body's natural healing process (Hussain and Hussain 2016). Amin et Sahak (2016), Sahak *et al.*

The co-administration of BLO concurrently with OP in the current study significantly reduced the unfavorable effect of OP to within control levels,

which most likely stems from the antioxidant activities of its bioactive components. Using *Buchanania lanzan* oil and ascorbic acid separately can prevent oxytetracycline-induced hepato-renal damage in rabbits, according to a study by Abdel-Daim and Ghazy (2015). Moreover, they both demonstrated complementary antioxidant and hepatoprotective effects. The antioxidant activity of essential oils from *Buchanania lanzan* and its impact on the state of antioxidant enzymes have been the subject of more recent investigations (Ammari *et al.* 2012, Alenzi *et al.* 2013, Haron *et al.* 2014, Ahmad and Beg 2016). Thymoquinone (TQ), carvacrol, 4-terpineol, anethole, thymoquinone, and thymol are discovered to have appropriate radical scavenging properties. scavenging characteristics. Also, it appears that thymoquinone (the main component of seed oil extract) has a protective effect against various xenobiotics through its antioxidant action and capacity to increase the activities of antioxidant enzymes in animals (Mohamadin *et al.* 2010).

The findings of this study clearly show that BLO treatment brought all changed semen parameters in OP-treated rats close to the levels of normal status. This considerable protective impact of BLO may be attributed to its ability to lower TBARS levels **Table 5.** Moreover, BLO alone enhanced some semen properties **Table 3** and **4.**

According to these findings, *Buchanania lanzan* is recognized for its ability to improve the fertility of male rats during reproduction (Al-Sa'aidi *et al.* 2009). Furthermore, it was discovered that both the crude fixed oil of *Buchanania lanzan* and thymoquinone (TQ) boosted antioxidant defense systems and inhibited membrane lipid peroxidation (Kanter 2011). Therefore, it was demonstrated by Parandin *et al.* (2012) that an alcoholic extract of N. Higher doses of *N. sativa* seed, in particular, could boost male rats' testosterone, LH, and reproductive potential. In a different study, El-Tohamy *et al.* (2010) tested the positive benefits of *Buchanania lanzan*, *Raphanus sativus*, and *Eruca sativa* on rabbit immunity, reproductive performance, and antioxidant status. They concluded that *Buchanania lanzan*, more so than *Raphanus sativus* and *Eruca sativa*, offers the most potential for enhancing immunity, boosting semen parameters, and lowering the production of free radicals. Higher doses of *Buchanania lanzan* seed, in particular, may boost testosterone, LH, and reproductive potential.

The effectiveness of *Buchanania lanzan's* protective role against reproductively induced toxicity and oxidative stress caused by some pesticides, including chlorpyrifos, nicotine, and mancozeb, has recently been demonstrated, supporting the current work [7], Lina *et al.* 2014, Ping *et al.* 2014, Barakat and El-Masry 2016).

In conclusion, it appears that OP may affect reproductive function by causing oxidative stress in the testes, and that combining BLO with OP may be able to partially or totally undo these effects.

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