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Deterioration of reproductive performance and seminal plasma biochemistry in male rats due to aluminum exposure

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Abstract
Aluminum (Al) comes in a variety of forms and is a known environmental xenobiotic that causes cytotoxicity and reproductive toxicity via the release of free radicals. Antioxidant properties of propolis have been widely recognized. Therefore, the purpose of this research was to determine whether or not propolis may mitigate the harmful effects of aluminum chloride ($AICI_{3)}$ on male rat reproductive function. The first group was used as a comparison. The second group was given 34 mg iara-3 per kg of body weight (1/25 LD_{50}). The third group received 50 milligrams of propolis per kilogram of body weight every day. The fourth group received both $AICI_3$ and propolis.
Seventy days of treatment were completed. The effects of $AICI_3$ on male reproductive function were reflected in a diminution in testicular, seminal vesicle, and epididymal weights as well as sperm concentration, motility, testosterone, and 17-ketosteroid reductase, cytochrome P450 (CAT), and glutathione (GSH) activities. In contrast, TBARS levels were found to be elevated in sperm and testes that had died or were aberrant. Histopathological analyses indicated obvious changes in the testes of the $AICI_3$ treatment group, where significant lesions were produced in the seminiferous tubules. Testosterone, glutathione, 17-ketosteroid reductase, catalase, and glutathione were all enhanced, while the number of dead and defective sperm was lowered. The results demonstrated that propolis counteracted the negative effects of $AICI_3$.

Keywords: Free Radicals, Compensation, Testosterone, Male Rats, Propolis, Aluminium chloride, Reproductive Toxicity

1. Introduction
Uncertainty vaccinations, buffered aspirins, phosphate binders, antiperspirants, and allergen injection are just a few of the numerous medicinal applications for aluminum compounds. Aluminum can enter the human body through a number of different routes, including the food we eat and the water we drink [1, 2]. When taken with fruit juices or citric acid, however, aluminium absorption in the intestines and excretion in the urine are dramatically increased, even in otherwise healthy people. It has been shown that parenteral administration of aluminum chloride (AICI3) to animals causes fetal harm. After 16 weeks of treatment with 34 mg/kg BW of AICI3 orally, rabbits showed a decrease in ejaculate volume, sperm concentration, total sperm output, sperm motility, packed sperm volume, total functional sperm fraction, normal and live sperm, and an increase in dead and abnormal sperm, according to the results [3, 4].
Excessive aluminium intake is linked to organ accumulation and, in humans and animals alike, harm to testicular tissues. It has been shown that elevated aluminum levels in seminal plasma and human spermatozoa reduce sperm motility and viability. Male mice and rats exposed to aluminum developed testicular aluminum accumulation, necrosis of spermatocytes/spermatids, and a significant decrease in fertility. Long-term exposure to $AlCl_3$ in drinking water had a negative impact on male rats' reproductive health, aggression, and sexual behavior. Aluminum toxicity has been shown to affect male reproduction through mechanisms involving androgenic hormones. Discovered that testosterone and adenosine 30, 50 -cyclic monophosphate (cAMP) levels in the testicles were reduced after aluminum administration, while nitric oxide (NO) production was significantly increased [5, 6]. They showed that high levels of NO stimulated inducible nitric oxide synthase (NOS), suggesting that NOS plays a role in aluminium's teratogenicity. The enzyme NOS converts the amino acid L-arginine into nitric oxide, a gas that plays a role in a wide range of physiological and pathological processes in living organisms. Overproduction of NOS in response to various stresses can lead to the release of large quantities of NO metabolites, which have been shown to impair sperm cell speed and motility and increase morphological abnormalities. A NO-related substance also inhibited testosterone production in male rats. Our research shows that aluminum increases lipid peroxidation in rabbit plasma, testes, brain, kidney,

lung, and liver, and in cultured rabbit sperm.

Mammalian tissues contain several enzymes scavenging reactive oxygen species (ROS), including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and glutathione S-transferase (GST), as well as reduced glutathione, to regulate the protect cells under stress conditions and the level of ROS. Compounds like propolis also help in the removal of harmful reactive oxygen species from the body [7, 8].

Honeybees create propolis, a resinous sticky material used as a sealant and sterilant in beehive nests and a folk remedy since ancient times. In recent times, its many biological properties including its antimicrobial, anti-inflammatory, anticarcinogenic, antioxidative, hepatoprotective, and immunomodulatory effects have been uncovered. It has been shown that propolis has around 300 different substances, including phenolic aldehydes, polyphenols, sequiterpene quinines, coumarins, steroids, amino acids, and inorganic compounds. The biological action of propolis is mostly due to phenolic components like flavonoids. Since flavonoids may scavenge free radicals, they are also responsible for antioxidant action. There is evidence that propolis may prevent the production of superoxide anion. Propolis is also known to inhibit the oxidation of glutathione, an antioxidant produced in the liver [9].

Aluminum toxicity has been studied intensively in recent years, but there is still a lack of data on its effects on reproduction and sperm quality. Furthermore, the protective effects of propolis against the reproductive performance declines in aluminium-treated rats have not been investigated. Therefore, the purpose of this research was to establish whether or not aluminium chloride is hazardous to the reproductive system of adult male rats [10].

2. Substances and Techniques

Chemicals

The Aldrich chemical Company in Milwaukee, Wisconsin, USA, supplied the aluminum chloride ($AlCl_3$), while the Jarrow Formulas in Los Angeles, California, USA, supplied the propolis. As far as I can tell, the experiment only employed analytical-grade chemicals. $AlCl_3$ was administered at a dosage of 34 mg/kg body weight (1/25 LD_{50}). When given orally to rats, the LD_{50} for Al was determined to be between 380 and 400 mg/kg body weight. The standard dosage of propolis is 50 milligrams per kilogram of body weight. In accordance with the results of the prior trials, this dosage was used.

Structure of an Experiment

For this study, we used the services of 40 male Wistar Albino rats (weighing between 180 and 200 g on average). The Faculty of Medicine at Alexandria University in Egypt provided the animals used in this study. All experimental procedures have been authorized by the institutional review board, and the methodology follows NIH standards (NIH). Groups of five animals were housed in a cage with free access to food and water.

The animals were acclimated for two weeks before being split into four groups. As a reference point, we utilized the first group. In contrast, group 2 received oral administration of aluminum chloride (34 mg/kg bw, 1/25 LD_{50}), group 3 received propolis (50 mg/kg BW), and group 4 received both aluminum chloride and propolis (34 mg/kg bw, 50 mg/kg BW). Each rat received its daily dosage orally for a total of 70 days. The length of the tests is 70 days to allow for the epididymal maturation of sperm and the conclusion of the spermatogenic cycle.

At the conclusion of the treatment period, all of the rats in each group were put to sleep. The animals were slaughtered, and blood was taken from their trunks and frozen as soon as possible. Plasma samples were collected by centrifugation at 860g for 20 minutes and kept at -60 C° until measurements. Heparin was employed as an anticoagulant. The testes and other genitourinary organs were removed surgically, cleaned of any excess tissue, and weighed. Relative organ weights were determined. Body weight was divided by 100 to get the percentage of organ weight. Testicular samples were promptly preserved in 10% buffered formalin for further histological analysis.

Male attributes

Employee After removing the left epididymis, 0.2 ml of calcium and magnesium-free Hank's solution was put in a petri dish and heated to 37 degrees Celsius. For 15 minutes at 37 degrees Celsius, the tissue was chopped using scalpels before its motility was evaluated. One drop of the suspension was put on a preheated microscope slide, and a 22 ×22 mm coverslip was placed on top. A phase-contrast microscope was used to examine at least 10 microscopic areas at 400 × magnification to determine the proportion of motile sperm. After taking off the lid, the spermatozoa solution was left to air-dry. Staining with 1% eosin Y and 5% nigrosin, followed by examination at 400, revealed no morphological irregularities in the sample. Similar to what was said before, three hundred spermatozoa were analyzed from various locations for each sample.

After a careful weigh-in, the right epididymis and testicular tissues were placed into the freezer for further analysis. The complete epididymis and testis samples were thawed at room temperature, and then homogenized in 0.49 ml of a 0.9% NaCl solution containing 2% Triton X-100. By utilizing an eosin-nigrosine blue staining combination, the percentages of viable, nonviable, and aberrant spermatozoa may be determined, as explained.

Evaluation of 17-ketosteroid reductase, an enzyme involved in steroidogenesis

After being homogenized in a 10-volume solution of 0.25 M-sucrose containing 0.05 mM-EDTA and 5 mM mercaptoethanol, buffered with 0.05 M potassium phosphate (pH 7.4) using a Tekmar model TR-10, West Germany homogenizer, the testicular tissues were centrifuged at 399 degrees Celsius in a cooling centrifuge. The 17-ketosteroid reductase enzyme was then extracted and crudely prepared from aliquots of the supernatant in accordance with the protocol.

Estimating Testosterone Levels in the Blood

After collecting plasma, testosterone levels were measured using a kit from Roche Diagnostics GmbH. D-68298 Canada called an electrochemiluminescence immunoassay (ECLIA). The lowest measured testosterone level was 0.02 ng/ml.

Analysis of reduced glutathione, thiobarbituric acid reactive compounds, and antioxidant enzymes in the testicles

The first step in the formation of mercapturic acid is a

conjugation process with glutathione, which is catalyzed by glutathione S-transferase (GST; EC 2.5.1.18). GST activity was evaluated using these standards. The substrate utilized was p-nitrobenzyl chloride. Using a UV-Double Beam spectrophotometer, the absorbance was determined to be 310 nm. H2O2 is neutralized by the enzyme catalase. breakdown of H_2O_2, the substrate of the enzyme, was used to determine the CAT activity, which was measured spectrophotometrically at 240 nm. Lipid peroxidation was evaluated by measuring the production of thiobarbituric acid reactive compounds (TBARS). For 1 hour at 100 degrees Celsius, tissue supernatant and plasma were combined ml TBA (0.70%) and ml TCA (30%). Centrifugation was used to separate the precipitate after cooling. At 535 nm, makes up 99 percent of TBARS, hence the extinction co-efficient of MDA (1.56×10^{-5} M^{-1} cm^{-1}) was used to determine the TBARS concentrations in the samples. Commercial glutathione reduction kits were used to measure glutathione (GSH) levels.

Estimation of Protein Content

The method described here was used to determine the protein content of testicular tissues using bovine serum albumin as a standard.

Histopathology sectioning and prepping

The tissues from the rat testicles were removed and promptly preserved in 10% formaldehyde saline. Paraffin blocks were made from tissue sections that had been treated (paraffin method). Rotatory microtome sections were placed on glass slides for examination. Hematoxylin and eosin (H&E) stain was used to examine the tissue slices. Light microscopy was used to analyze the tissue sections.

Number crunching

The data are presented as the mean standard deviation of ten independent measurements. One-way analysis of variance (ANOVA) was used for statistical analysis to see whether there were statistically significant differences between the various treatment groups. Post hoc Tukey's test comparisons were performed for each treatment effect that was statistically significant. P values of 0.05 or 0.01 were chosen as the threshold for statistical significance. The SPSS 8 statistical software program was used for all analyses.

Discussing the findings

The relative weights of the testes, seminal vesicle, and epididymis were significantly ($P \leq 0.009$) reduced in mice treated with $AlCl_3$ compared to controls and animals treated with propolis alone (Table 1). However, when propolis was included with $AlCl_3$, the toxic effects were reduced, and the tissue weights returned to the levels seen under control conditions. The weight of the sampled tissues was not significantly altered by treatment with propolis alone. Furthermore, there was no difference in prostate weight across treatments (Table 1). Mice treated with $AlCl_3$ showed a decrease in testicular, seminal vesicle, and epididymal weights similar to those seen in rabbits. Aluminum chloride consumption was also associated with reduced relative testes and seminal vesicle weights and absolute.

Male rats given $AlCl_3$ had considerably ($P < 0.009$) lower sperm concentration and motility (%) and higher rates of dead and aberrant sperm compared to the control and propolis groups (Table 2). Propolis treatment alone had no detectable impact on sperm concentration or motility but significantly reduced the number of aberrant and dead sperm compared to the control group ($P < 0.009$). However, compared to the AlCl3 group, the percentage of dead and aberrant sperm was much lower in the propolis treatment group, and the decline in sperm concentration and motility was greatly attenuated when propolis was used in conjunction with $AlCl_3$.

The consumption of aluminum chloride has been shown to reduce sexual activity in male rats. In the testes of aluminium nitrate-treated mice, spermatocytes and spermatids necrosed. Reductions in testicular and epididymal weights, as well as testicular and spermatid counts, and epididymal sperm counts, were also seen. We have previously shown that $AlCl_3$ reduces sperm quality *in vivo*, and we have also shown *in vitro* that aluminum inhibits the activity of the enzyme aconitase, which catalyzes and binds citrate through the interm ate cis-aconitate in the Krebs cycle. Sperm motility and viability may so reflect alterations in mitochondrial activities.

In this research, we see how aluminum chloride affects sperm movement (Table 2). results showed that elevated levels of aluminum in human spermatozoa and seminal plasma were associated with lower sperm motility and viability. Sperm motility is essential for conception because it allows the sperm to go up the female reproductive canal to the egg. As a result, the drop in testosterone synthesis after aluminum treatment may have contributed to the observed decline in sperm motility. $AlCl_3$ treatment also significantly boosted the production of thiobarbituric acid-reactive compounds in the testes (Table 4). Motility, midpiece abnormalities, and sperm-oocyte fusion may all be negatively affected by elevated TBARS levels.

Based on the data in Table 3, testicular protein and plasma testosterone levels were both significantly lower in AlCl3-treated rats compared to controls ($P \leq 0.05$ and $P \leq 0.01$, respectively). Propolis, on the other hand, significantly raised testosterone and protein levels while counteracting the debilitating effects of $AlCl_3$ in group 4.

Rat and mouse studies have shown that exposure to aluminum increases the prevalence of fetal malformations. The research conducted showed that testosterone levels in mice were reduced both in the blood and in the testicles after being exposed to aluminum. The scientists hypothesized that low testosterone concentrations and high aluminum buildup in the testes would account for the dramatic decline in male libido that followed aluminium delivery. It was found, however, that significant amounts of aluminum in aluminium-treated mice were visible by week 3, much before the consequences on male libido and fertility were widespread. This disparity was rationalized on the grounds that elevated aluminum levels do not directly impact the enzymes responsible for androgen production or cause any noticeable disruption to the hypothalamic-pituitary-gonadal axis. After 70 days of treatment with $AlCl_3$, however, a substantial decrease in 17-ketosteroid reductase activity was observed in the current investigation (Table 3).

Our findings corroborate a decrease in testosterone as a contributing factor to the deterioration of sperm quality in AlCl3-treated rats (Table 2). (Table 3). alluded to the possibility that Al-induced NO acts as a testosterone suppressant. Additionally, data showing that NO inhibits LH-stimulated steroidogenesis in Leydig cells was presented. Steroidogenic enzyme activities were reduced due to the increase in testicular NO that was triggered by

stress. The findings of suggested that the primary second messenger cAMP, which mediates the effects of Al, might be directly inhibited by exposure to excessive NO compounds generated by Al.

Table 1: Sex organ weights in male rats treated with $AlCl_3$, propolis, and $AlCl_3$ + propolis, expressed as a percentage of total body weight (g/100 g)

Parameter	Topical divisions for experimental purposes			
	Con.	$AlCl_3$	Prop.	Prop+$AlCl_3$
Testes	0.72±0.092	0.58±0.059**	0.78±0.0738***	0.66±0.089
Vesicle in the Seminal Fluid	0.35±0.059	0.20±0.079**	0.39±0.104***	0.31±0.117
Epididymis	0.27±0.034	0.19±0.035**	0.30±0.034***	0.24±0.039
Prostate	0.16±0.022	0.14±0.20	0.16±0.016	0.15±0.01

Sex organ weights in male rats treated with AlCl3, propolis, and AlCl3 + propolis, expressed as a percentage of total body weight (g/100 g).

Table 2: Variations in AlCl3, propolis, and AlCl3 + propolis-treated male rat sperm concentration (Sp. Conc., ×106 /ml), motility (%), and dead (%) and aberrant (%) sperm

Parameter	Topical divisions for experimental purposes			
	Con.	$AlCl_3$	Prop	Prop+$AlCl_3$
Sp. Conc.	211± 14.4	138±7.00**	223.± 11.0##	200 ± 8.4##
Motility	77.4± 199	56.9±1.63**	80.6± 1.63##	70.5 ± 1.42##
Dead	24.4± 2.94	46.1±1.19**	19.2± 1.77##	28.8± 2.00##
Abnormal	15.6± 21.95	22.3±2.311**	12.6±1.26##	15.7±1.236##

Mean ± standard deviation (n=10 per treatment group) values are provided.
Differential between the experimental and control groups at *P < 0.05 and **P < 0.01 level of significance, respectively.

Table 3: AlCl3, propolis, and AlCl3 + propolis increased the plasma testosterone concentration (ng/ml), the activity of the testicular 17- ketosteroid reductase enzyme (U/min/mg protein), and the testes protein content (mg/g tissue) in male rats

Parameter	Topical divisions for experimental purposes			
	Con.	$AlCl_3$	Prop	Prop+$AlCl_3$
Testosterone	1.31 ±0.243	1.00±0.114*	1.51±0.112*#	1.15±0.063#
The 17-Ketosteroid Reductase enzymatic process	14.6 ±2.01	10.2±1.12**	19.1±1.148**##	12.8±2.02*#
Quantity of Protein	76. ±4.3	60 ± 4.6**	105±5.2**##	75 ± 5.1##

Mean ± standard deviation (n=10 per treatment group) values are provided.
Mean ± standard deviation (n=10 per treatment group) values are provided.
Differential between the experimental and control groups at *P < 0.05 and **P < 0.01 level of significance, respectively.
Less testosterone was produced due to a lack of gonadotropin-stimulated cholesterol-to-pregenolone steroidogenesis in Leydig cells. In addition, our prior research established that aluminium chloride may produce ROS in rabbit testes, and the current research confirmed that TBARS can be created in rat testes (Table 4). Male infertility has been linked to an increase in reactive oxygen species (ROS), which may be harmful to sperm. Therefore, it is possible that generated free radicals are responsible for $AlCl_3$'s spermatoxic impact.
Male reproductive systems may be negatively affected by aluminum, although the underlying processes are unknown. However, $AlCl_3$ injection decreased testosterone levels suggesting that aluminum-induced NO may be a testosterone suppressor. One discovery showing that NO inhibits LH-stimulated steroidogenesis in Leydig cells provided evidence for NO important function in regulating androgen production. Steroidogenic enzyme activities were reduced due to the increase in testicular NO that was triggered by stress.
$AlCl_3$ treatment substantially ($P \leq 0.009$) reduced the activity of 16.99-ketosteroid reductase, the enzyme responsible for the conversion of androstenedione to testosterone, in rats compared to the control group (Table 3). On the other hand, compared to the control group, 17-ketosteroid reductase activity was considerably ($P \leq 0.01$) higher in the treatment group that received propolis. That is because Table 3 shows that propolis improved sperm proliferation by increasing the process of steroidogenesis and, by extension, testosterone synthesis (Table 4 and Fig. 3). Compared to the $AlCl_3$ group, 17-ketosteroid reductase activity was significantly ($P \leq 0.01$) increased when propolis was present. The decrease in 17-ketosteroid reductase activity in $AlCl_3$-treated animals suggests that the compound has an unfavorable effect on testosterone hormone production, which in turn has an adverse effect on fertility (Table 3).
As a sign of tissue injury, damage, and rewound healing, the protein level of testicular tissue is closely monitored by medical professionals during the course of therapy.
The levels of thiobarbituric acid-reactive compounds (TBARS) were raised in AlCl3-treated animals compared to controls, and the activities of catalase (CAT) and glutathione S-transferase (GST) were decreased in their testicles. While CAT and GST were significantly increased in propolis-treated animals ($P \leq 0.01$), glutathione (GSH) was significantly increased ($P \leq 0.05$), and tyrosine aminotransferase (TBARS) was significantly decreased ($P \leq 0.049$).In the combination group, propolis was able to increase GSH levels, CAT and GST activities, and overall antioxidant capacity

Table 4: Changes in thiobarbituric acid-reactive substances (TBARS; nmol/g tissue), reduced glutathione (GSH; mM/g tissue), and catalase (CAT; mol/h/g tissue) and glutathione S-transferase (GST; lmol/min/g tissue) activities were observed in the testes of rats treated with AlCl3, propolis, and AlCl3 + propolis.

Parameter	Topical divisions for experimental purposes			
	Con.	$AlCl_3$	Prop	Prop +$AlCl_3$
CAT	6.96±1.139	30.1±0.577**	9.95±1/049**##	5.89±0.792##
GST	1.08±0.101	0.59±0.153**	1.43±0.298**##	0.96±0.124##
TBARS	71.8±5.58	195.9±10.61**	60.4±6.17*##	97.4±9.57*##
GSA	6.02±0.694	4.15±0.691*	8.25±0.901*##	5.74±1.862#

Mean ± standard deviation (n=10 per treatment group) values are provided.
Differential between the experimental and control groups at *P < 0.05 and **P < 0.01 level of significance, respectively.
#P < 0.05 and ##P < 0.01 indicate a statistically significant contrast to the $AlCl_3$-intoxicated control group.
as compared to the norm or the control group. In comparison

to the AlCl3 group, propolis was able to considerably ($P \leq 0.01$) lower TBARS levels; nevertheless, it was unable to get them down to control values (Table 4). When oxygen radical generation exceeds the antioxidant capability of the stressed tissue, oxidative stress ensues. Inducers of oxidative stress include several circumstances or events linked to male infertility. Testicular oxidative stress is increased by X-irradiation, for example, or by exposure to environmental toxicants, and by the physical circumstances of varicocele and cryptorchidism, leading to an increase in germ cell death and consequent hypospermatogenesis. Substantial alterations in the dynamics of testicular microvascular blood flow, endocrine signaling, and germ cell death have been linked to chronic stress. Developing more effective antioxidant therapy for instances of hypospermatogenesis may be helpful since oxidative stress in the testes seems to be a common trait in many of the factors behind male infertility.

Testicular glutathione (GSH), glutathione S-transferase (GST), and catalase levels were all shown to be lower in AlCl3-toxicated rats, among other indications of oxidative stress (Table 4). The current study's findings are consistent with those of prior research on AlCl3-induced oxidative stress, which pointed to free radical generation as the mechanism through which aluminum produced oxidative stress. Despite this, Al is a pro-oxidant both *in vitro* and *in vivo*, which is why it is classified as a non-redox active metal. Previous research has linked aluminium exposure to increased reactive oxygen species (ROS) through mechanisms including electron leakage and heightened electron chain activity. Lipid peroxidation is a byproduct of ROS's assault on membrane lipids and almost all other cellular components. Therefore, it is plausible to postulate that oxidative stress is involved in the aluminium-induced dysfunction of the testicles. Lipid peroxidation (LPO) has been identified as a major form of oxidative damage and has been linked to the toxicity of several environmental contaminants and drugs of abuse. An increase in TBARS levels was seen in the testes, and the authors believe this may be due to oxidative cell damage caused by free radicals, which aluminum's toxicity relies on to be effected (Yousef, 2004).

Spermatozoa are vulnerable to oxidative damage as a result of spermatozoa's high susceptibility to producing reactive oxygen species, their low antioxidant capacity.

well as lipid peroxidation, which is linked to an increase in cell enzyme, inactivation, the formation of spermicidal chemicals and permeability.

Aluminum has been proven to affect free radical-related enzyme activity, according to previous research. testicles, liver, kidneys, and brains of rabbits all showed elevated lipid peroxidation and decreased activity of antioxidant enzymes after injection of aluminum. *In vitro* cultivation of rabbit sperm has produced similar outcomes. Increased lipid peroxidation confirms the biological impact that aluminium has on the body, suggesting that it enhances oxidative stress by reducing the activities of free radical scavenging enzymes like catalase and GST.

The most prevalent reason of infertility is poor sperm function, which is difficult to diagnose and treat due to its complex interplay with a wide range of environmental, physiological, and genetic variables. The fact that we still do not know enough about what causes normal and aberrant sperm function contributed to this problem. Poor sperm function and infertility have received a lot of attention as of late, and one possible cause is oxidative damage caused by free radicals in spermatozoa. This highlights the critical role of factors that might provide spermatozoa with protection. Evidence was revealed in the current research that propolis might shield sperm, testes, and enzymes against the harmful effects of AlCl3. Propolis was tested *in vitro* to see whether it had any impact on human spermatozoa that had been exposed to benzopyrene and exogenous reactive oxygen species (hydrogen peroxide; H_2O_2, adenosine 50 - diphosphate; ADP and ferrous sulfate; $FeSO_4$). Researchers showed that propolis may significantly lower intracellular oxidants, protecting genomic DNA from damage caused by benzopyrene, H_2O_2, and H_2O_2 in combination with ADP and $FeSO_4$. Treatment of sperm with H_2O_2, ADP, and $FeSO_4$ was the sole factor that led to an increase in membrane damage, as determined by measuring the synthesis of (LDH) and (TBARS). They found that propolis protected sperm membranes against oxidative damage, as measured by a decrease in TBARS production and a rise in LDH release. Additionally, they found that propolis's antioxidant capacity of its active components is connected, at least in part, to the protective impact it demonstrated in human spermatozoa, indicating that propolis may have a role in protecting against male infertility. Propolis contains a number of phenolic chemicals, including flavonol galangin, hydroxycinnamic acids, caffeic acid, p-cumaric acid, ferulic acid, and caffeic acid phenethyl ester, all of which may contribute to the observed biological effects of propolis. It is believed that the flavonol galangin has antioxidant properties similar to those of the hydroxycinnamic acids (caffeic acid, pcumaric acid, and ferulic acid).

We also discovered that when propolis was given to animals with propetamphos, the amounts of malondialdehyde (MDA) in the plasma and tissues (liver, kidney, and brain) decreased, and the levels of antioxidant enzyme parameters (SOD, CAT, and GSH-Px) increased or decreased, respectively. Propolis may be able to prevent lipid peroxidation by mopping up the free radicals that cause it. Propolis may also work by preventing the production of free radicals by inhibiting the enzyme xanthine oxidase. Research indicates that propolis may inhibit xanthine oxidase.

We also found that the levels of reduced glutathione (GSH) in rats treated with AlCl3 and propolis were maintained at normal levels (Table 4). Overall, our results suggest that propolis' antioxidative capabilities can protect rats from AlCl3-induced harm.

This study further shown that elevated GST and CAT activities and elevated GSH levels in the testes were the result of propolis therapy alone, which reduced TBARS levels. These findings corroborate those of previous studies showing that propolis lowers malondialdehyde (MDA) levels and increases antioxidant enzyme activity (SOD, GSH-Px, and CAT). Moreover, Sobocanec found that propolis boosted CAT activity.

According to the results of the histological analysis, the testes of healthy individuals are enclosed in a capsule made of thick fibrous tissue called the tunica albuginea. The testis is segmented into lobules by thin fibrous septa, and inside each lobule are a number that are bordered by interstitial

tissue. In the middle of each tubule are spermatozoa, which have developed from. The Sertoli cells are located on the basal lamina between the spermatogonia. Leydig cells are the primary; cell type in the interstitial supporting tissue between the seminiferous tubules. They may be found individually or in groups, and they are scattered throughout the dense network of blood and lymph vessels (Fig. 1). As AlCl3-treated testes were seen, a number of changes were apparent. Some seminiferous tubules have a jumbled design overall. Exfoliated germ cells accumulated in the damaged tubules as a result of these abnormalities. We found that some tubules had stopped developing. In addition, the nuclei of several germ cells were very tiny and poorly pigmented. Significant vascular dilatation and congestion were seen in the interstitium. Interstitial tissue showed Leydig hyperplasia, with Leydig cells clustering together to form thick clumps that surround the majority of the seminiferous tubules (Fig. 2). Testicular sections from animals given propolis alone were not significantly different from control sections (Fig. 3). In rats treated with aluminum and propolis, the testes remarkably restored their original structure, and the usual image of seminiferous tubules was recovered. Most of the cytoplasmic vacuolation was no longer present, and the germ cells looked to be of uniform shape. The majority of nuclear envelopes changed to vesicles (Fig. 4).

Aluminium levels rise steadily in rat testes as the animals mature. Light microscopy of silver-stained paraffin slices of testes indicated abundant intracytoplasmic black-stained tiny granular inclusions in Leydig cells. Those who have researched the impact of intraperitoneal injection of AlCl3 on the testes of rats have found that the histological alterations seen in the testes of treated rats (Fig. 2) are consistent with their findings. Testes revealed histological disturbance involving extensive destruction inside the seminiferous tubules and vascular degeneration on the spermatogenic and sertoli cells cytoplasm. There were fewer spermatids and no sperm in the lumen of the seminiferous tubules, and the germinal epithelium was thinner there. Further, electron microscopic analyses revealed the presence of irregular structures in the aluminium-treated group.

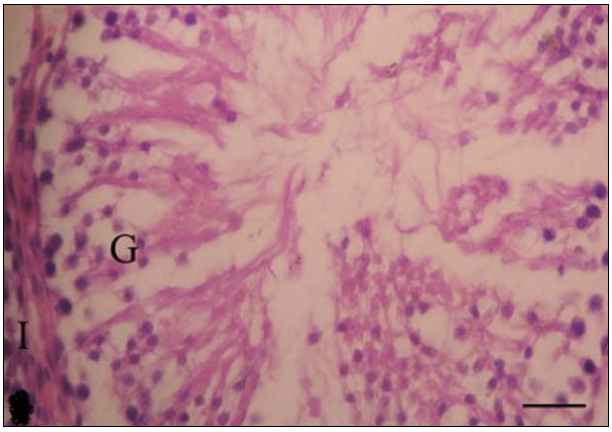

Fig 1: Control testis slice exhibiting cells in a photomicrograph (G). IHC and H&E (400 ×)

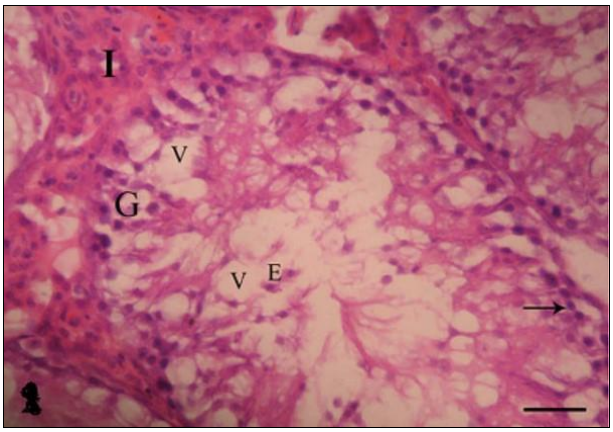

Fig 2: Testis slice treated with aluminum oxide reveals germ cells (G), exfoliated germ cells (E), hyperplasia of Leydig cells (I), and vacuolation in a photomicrograph (V). H&E stain (400 ×).

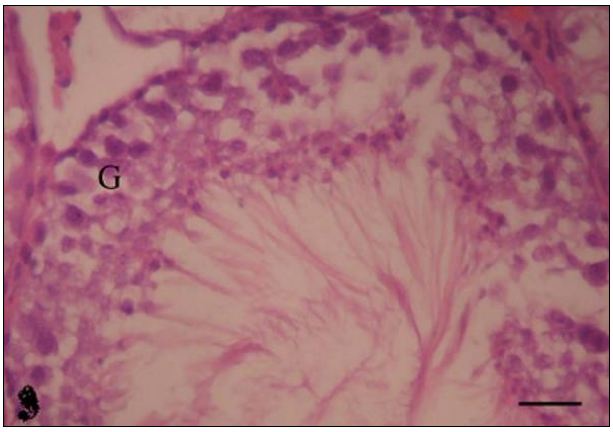

Fig 3: Germ cells, as shown in a photomicrograph of a testis that has been treated with propolis (G). Micrograph of an H&E stain (400 ×)

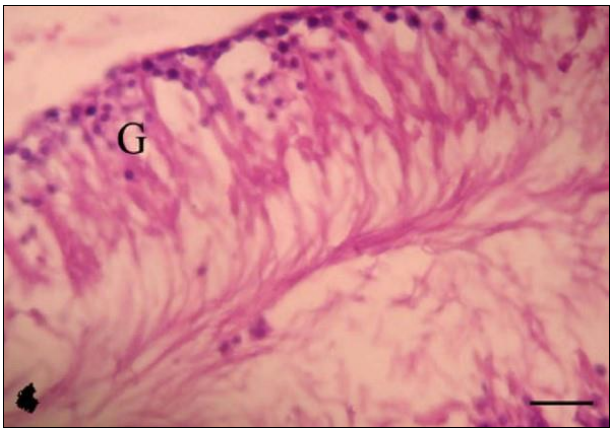

Fig 4: A photomicrograph of germ cells in a portion of test is that has been treated with aluminum and propolis (G). IHC and H&E (400 ×)

Changes in the cytoplasm of sertoli cells include nuclear membrane abnormalities, mitochondrial damage, a drop in ribosome numbers, and an uptick in lysosome abundance.

Negative effects and histological abnormalities of testicular tissues were seen after 2 weeks of aluminum therapy, including an increase in the rough endoplasmic reticulum in primary spermatocyte cytoplasm. At week 5, the spermatogenetic loss in the aluminum-treated group was seen as necroses in the spermatids and spermatozoa. Prolonged buildup of aluminum in the testes of the mice was principally responsible for the damage produced by aluminum. The scientists hypothesized that these alterations might have been a contributing factor in the pregnant rat population decline.

4. Conclusion

There are a wide variety of clinical, pathophysiological, and neurobehavioral effects that may result from aluminum exposure. Additionally, various clinical diseases contribute to the accumulation of aluminum in certain target organs, such as the testes. Despite all of the studies on aluminum's toxicity, we still don't have nearly enough information on how to avoid exposure during pregnancy. In this study, we found that aluminum chloride exposure led to dysfunctional testes, decreased sperm quality, and lower testosterone levels. Also, it led to a decline in glutathione and antioxidant enzymes in testes and an increase in lipid peroxidation. It's possible that the antioxidant activity of propolis mitigated the reproductive toxicity of aluminum chloride. The major component in reducing aluminum-induced toxicity is awareness of aluminum. In addition, eating a diet high in propolis may help counteract the negative effects of aluminum on reproduction.

5. References

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