



Special Issue:

Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

Protective Effect of Propolis Extract Against Nickel Chloride and/or Carbon Tetrachloride Induced Alterations in Physiological and Endocrine Functions in Adult Male Rats

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Abstract | Nickel chloride (NiCl₂) alters thyroid hormones and causes morphophysiological changes and oxidative stress. Reactive free radicals from carbon tetrachloride (CCl₄) damage the liver and kidneys. On the other hand, propolis has antioxidant, anti-inflammatory, anti-cancer, antibacterial and hepatoprotective properties. Propolis protects thyroid and gonadal function from NiCl₂ and CCl₄. In order to assess the protective impact of propolis extract against NiCl₂ and CCl₄, 80 adult male albino rats were divided into eight groups including control group, the propolis-treated group, the NiCl₂-treated group, the CCl₄-treated group, the NiCl₂ and propolis-treated group and the CCl₄ and propolis-treated group. Blood-based ELISA kits were applied to determine T₃, T₄, TSH, LH, FSH and testosterone in the rats after four weeks of medication. Results analysis indicated a significant increase ($P \leq 0.05$) in TSH and lower T₃ and T₄ levels in the NiCl₂, CCl₄ and NiCl₂+ CCl₄ groups compared to the control group. Propolis treatment in the NiCl₂ and CCl₄ groups significantly ($P < 0.05$) lowered TSH levels compared to the control group. In contrast, the CPF-treated group had significantly higher serum levels. Our investigation concludes that propolis has a significant impact in safeguarding against hypothyroidism and gonadotoxicity generated by NiCl₂ and/or CCl₄.

Keywords | Carbon tetrachloride, Endocrine functions, Nickel chloride, Propolis, Rats

Received | July 21, 2024; **Accepted** | September 27, 2024; **Published** | November 15, 2024

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Citation | Abed NM (2024). Protective effect of propolis extract against nickel chloride and/or carbon tetrachloride induced alterations in physiological and endocrine functions in adult male rats. J. Anim. Health Prod. 12(s1): 99-106.

DOI | <http://dx.doi.org/10.17582/journal.jahp/2024/12.s1.99.106>

ISSN (Online) | 2308-2801



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INTRODUCTION

Throughout their lives, both animals and humans are constantly exposed to environmental chemicals, leading to the accumulation of these chemicals in their bodies, known as personal body burdens. These burdens can cause inflammation and tissue necrosis (Farhan, 2020). Interestingly, the increase in endocrine diseases corresponds to the elevated production of manufactured chemicals (Kumar et al., 2020).

Nickel is a metallic element with a silvery-white colour, known for its hardness, malleability, and ductility (Adriano, 2001). It belongs to the iron group of elements and is naturally present in small amounts in various food items. Notably, chocolate and fats contain exceptionally high concentrations of nickel. Consumption of vegetables grown in polluted soils also increases nickel uptake in the body, as plants can accumulate nickel (Iyaka, 2011). In the Earth's crust, nickel is a heavy metal that occurs at a concentration of around 0.009%, existing as soluble and

However, human activities, such as mining and industrial operations, have raised environmental nickel concentration. These activities are associated with producing various items like clocks, mobile phones, coins, and cardiovascular stents (Schmidt and Goebeler, 2011). Nickel and its compounds are highly carcinogenic and toxic to humans and laboratory animals (Kasprzak and Salnikow, 2007). Also, nickel is a carcinogen and toxic agent affecting the liver, immune system, nerves, genes, kidneys, and lungs. It also has reproductive and nephrotoxic effects (El-Shafei, 2011). Studies have shown that acute, subchronic, and chronic doses of nickel can affect various metabolically active human and animal tissues (Gupta et al., 2008). Nickel chloride (NiCl_2), a major endocrine disruptor, can alter thyroid hormone and TSH levels, induce morphophysiological changes in the thyroid gland, and cause thyroid oxidative stress in pregnant Wistar rats (Salah et al., 2021). Carbon tetrachloride (CCl_4), commonly used in laboratories to induce experimental liver and kidney damage, produces reactive free radicals that react with membrane lipids, leading to organ damage (Goodarzi and Akbari, 2016).

Propolis, a naturally occurring substance from beehives, contains numerous ingredients. Different types of propolis have been found to include active ingredients such as phenolic acids, terpenes, vitamins, amino acids, and various vital metals and elements. The chemical composition of propolis varies depending on the bee species, plant origin, collecting region, and climate impact. Various bee species, including *Apis mellifera* and *Meliponini* stingless bees, contribute essential ingredients to propolis (Anjum et al., 2019; Zulkiflee et al., 2022). Over 500 chemicals have been identified in propolis, including flavonoids, phenolic compounds, polyphenols, terpenes, terpenoids, coumarins, steroids, amino acids, and aromatic acids. Propolis also contains essential oils, vitamins A, B complexes, C, and E, as well as significant minerals like calcium, copper, magnesium, iron, zinc, sodium, potassium, and aluminium, all of which play essential roles in biological activity (Ahangari et al., 2018; Abdullah et al., 2020). Recent research has extensively examined the beneficial effects of propolis extracts on various types of tissues, demonstrating their antioxidant, anti-inflammatory, anti-cancer, anti-bacterial, and hepatoprotective properties. Propolis is commonly used as a functional food to enhance public health and prevent chronic ailments such as atherosclerosis, type 2 diabetes mellitus, chronic kidney disease, and Alzheimer's disease (Anjum et al., 2019; Pasupuleti et al., 2017; Rivera-Yañez et al., 2018; Samadi et al., 2017; Zakerkish et al., 2019). This study aims to examine the protective effects of propolis on the disruption of thyroid and gonad functions caused by NiCl_2 and CCl_4 in adult male albino rats.

EXPERIMENTAL ANIMALS

This research utilized eighty adult male albino rats weighing approximately 250 ± 25 g. Before conducting the tests, the rats underwent a fifteen-day acclimatization in the animal housing facility. The rats were maintained under optimal conditions throughout the study, with a temperature of $25 \pm 2^\circ\text{C}$ and a 12-hour light/dark cycle. They were given standard pellets as their food source and unrestricted tap water access.

STUDY DESIGN

After the acclimatization phase, the albino rats were divided into eight groups, each comprising ten rats, with an equal distribution as follows:

- Control group: Animals in this group were administered corn oil (2ml/kg)/day for four weeks.
- Propolis-treated group: Animals in this group received oral doses of Propolis at 70 mg/kg BW daily for four weeks using a metallic stomach tube.
- NiCl_2 treated group: Animals in this group received NiCl_2 daily at a dose of 0.75 mg/kg BW for four weeks.
- CCl_4 treated group: Animals in this group received intraperitoneal injections of CCl_4 in corn oil (1:1) at a dose of 2 ml/kg BW daily for four weeks.
- NiCl_2 + CCl_4 treated group: Animals in this group were daily administered NiCl_2 and CCl_4 for four weeks.
- NiCl_2 and Propolis treated group: Animals in this group received NiCl_2 daily and then oral doses of Propolis for four weeks.
- CCl_4 and Propolis treated group: Animals in this group were daily administered CCl_4 and then oral doses of Propolis for four weeks.
- NiCl_2 + CCl_4 and Propolis treated group: Animals in this group were administered NiCl_2 + CCl_4 and then oral doses of Propolis (70mg/kg BW) daily for four weeks.

After the experimental period of one month, the rats were euthanized under light chloroform anaesthesia. A Y-shaped incision was made in the rat's abdomen, and blood was collected from the posterior vena cava, which opens into the right ventricle. The blood was then placed in plain tubes and centrifuged for 15 minutes at a speed of 3000 rpm to separate the serum, which was stored at a temperature of -4°C until further use. The concentrations of T3, T4, TSH, LH, FSH, and testosterone were measured in the serum.

HORMONAL TESTS

Estimation of T3, T4, and TSH: The concentrations of T3 and T4 were determined using ELISA kits obtained from Monobind Inc., located in Lake Forest, CA, 92630, USA.

The specific kit used had the product code L25-300. TSH was measured using an ELISA kit from Calbiotech Inc., a life science company based in the USA. The kit used for TSH measurement had the product code TS227T.

Measurement of luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentration (ng/ml): The concentration of LH in the serum was measured using an ELISA kit provided by Calbiotech Inc. in the USA. The specific kit used for LH measurement had the catalogue number LH231F. Similarly, the serum's follicle-stimulating hormone (FSH) concentration was measured using an ELISA kit supplied by Foresight, a division of ACON Laboratories, Inc., based in CA 92121, USA. This information was reported in the study by [Abed and Alkalby \(2018\)](#).

Measurement of testosterone (T) concentration (ng/ml): The total testosterone concentration in serum or plasma was quantitatively determined using a microplate enzyme immunoassay kit provided by Bioactiva Diagnostics GmbH in Germany. This information was cited from the study conducted by [Abed and Alkalby \(2018\)](#).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) to identify any significant differences, with a significance level set at $p \leq 0.05$. The statistical software utilized for this analysis was Sigma Statistical, specifically [SPSS \(2010\)](#).

RESULTS AND DISCUSSION

EFFECT OF PROPOLIS ON THYROID AND GONADAL HORMONES IN MALE ALBINO RATS TREATED WITH NiCl_2 AND/OR CCl_4

The results of thyroid function tests revealed a significant increase ($P \leq 0.05$) in TSH concentration and a significant decrease in T3 and T4 concentrations in the serum of the NiCl_2 , CCl_4 , and $\text{NiCl}_2 + \text{CCl}_4$ treated groups compared to the control group, as shown in [Table 1](#), [Figures 1 and 2](#).

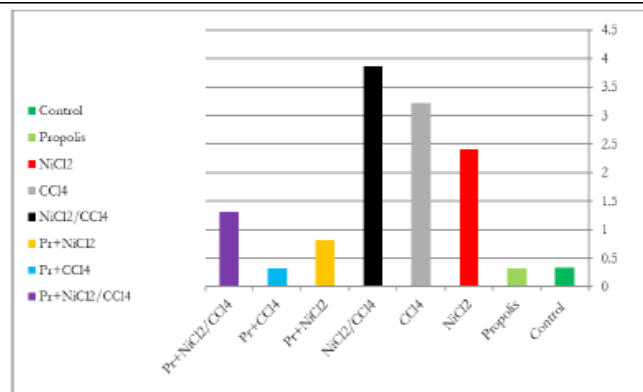


Figure 2: The effect of Propolis on T4 concentration in male rats who received NiCl_2 and/or CCl_4 .

On the other hand, the NiCl_2 and CCl_4 groups treated with propolis showed a significant decrease ($P \leq 0.05$) in TSH concentration compared to the control group, but the levels were still significantly lower ($P \leq 0.05$) than those of the NiCl_2 and CCl_4 groups. No significant differences were observed in TSH concentrations in the propolis groups treated with 70 mg/kg BW compared to the control group, as shown in [Figure 3](#).

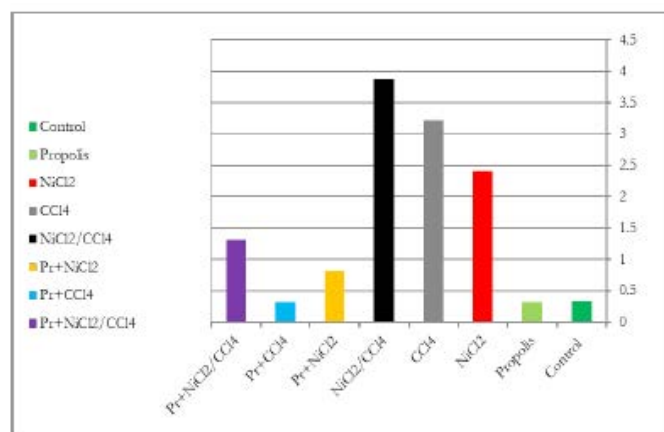


Figure 3: The effect of Propolis on TSH concentration in male rats who received NiCl_2 and/or CCl_4 .

EFFECT OF PROPOLIS ON TESTOSTERONE, ESTRADIOL, AND PROGESTERONE CONCENTRATIONS IN MALE ALBINO RATS TREATED WITH NiCl_2 AND/OR CCl_4

The data presented in [Table 2](#) showed a statistically significant increase ($P \leq 0.05$) in serum testosterone levels in the propolis-treated group compared to the NiCl_2 , CCl_4 , and $\text{NiCl}_2 + \text{CCl}_4$ treated groups and the control group. However, no significant differences in testosterone levels were observed between the propolis-treated NiCl_2 , CCl_4 , and $\text{NiCl}_2 + \text{CCl}_4$ groups compared to the control group, as shown in [Figure 4](#).

We also observed a significantly decreased ($P < 0.05$) in LH levels in the NiCl_2 , CCl_4 , and $\text{NiCl}_2 + \text{CCl}_4$ treated groups

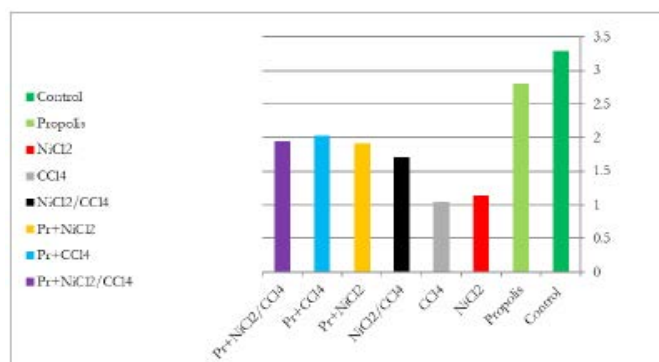


Figure 1: The effect of Propolis on T3 concentration in male rats who received NiCl_2 and/or CCl_4 .

compared to the propolis and control groups (Table 2). No significant difference in LH levels was observed between the propolis-treated NiCl₂, CCl₄, and NiCl₂+ CCl₄ groups and the control group (Figure 5).

Table 1: Effect of propolis on thyroid hormones of male rats treated with NiCl₂ and/or CCl₄.

Pa-ram-eter	Group	Mean	Std. de- viation	Std. error	95% Confi- dence Interval for Mean	
					Lower bound	Upper bound
T3	Control	3.2900	1.33621	0.42255	2.3341	4.2459
	Propolis	2.7930	0.41344	0.13074	2.4972	3.0888
	NiCl ₂	1.1300	0.35917	0.11358	0.8731	1.3869
	CCl ₄	1.0500	0.36591	0.11571	0.7882	1.3118
	NiCl ₂ /CCl ₄	1.7100	0.41218	0.13034	1.4151	2.0049
	Pr+ NiCl ₂	1.9110	0.37534	0.11869	1.6425	2.1795
	Pr+CCl ₄	2.0210	0.16703	0.05282	1.9015	2.1405
	Pr+NiCl ₂ / CCl ₄	1.9440	.36075	0.11408	1.6859	2.2021
T4	LSD	0.58				
	Control	9.4570	0.95664	0.30252	8.7727	10.1413
	Propolis	9.7000	0.61464	0.19437	9.2603	10.1397
	NiCl ₂	6.0300	1.33504	0.42218	5.0750	6.9850
	CCl ₄	5.4500	1.31000	0.41426	4.5129	6.3871
	NiCl ₂ /CCl ₄	4.0900	1.36663	0.43217	3.1124	5.0676
	Pr+ NiCl ₂	8.3800	1.18491	0.37470	7.5324	9.2276
	Pr+CCl ₄	8.5680	1.34776	0.42620	7.6039	9.5321
TSH	Pr+NiCl ₂ / CCl ₄	7.3580	1.01777	0.32185	6.6299	8.0861
	LSD	1.077				
	Control	0.3397	0.04581	0.01449	0.3069	0.3725
	Propolis	0.3270	0.05832	0.01844	0.2853	0.3687
	NiCl ₂	2.4080	0.52340	0.16551	2.0336	2.7824
	CCl ₄	3.2240	1.60180	0.50653	2.0781	4.3699
	NiCl ₂ /CCl ₄	3.8700	2.07421	0.65592	2.3862	5.3538
	Pr+ NiCl ₂	0.8120	0.12568	0.03974	0.7221	0.9019
	Pr+CCl ₄	0.3270	0.05832	0.01844	0.2853	0.3687
	Pr+NiCl ₂ / CCl ₄	1.3130	0.41406	0.13094	1.0168	1.6092
	LSD	0.986				

Note: T3= Triiodothyronine, T4= Thyroxine, TSH= Thyroid-stimulating hormone.

FSH levels showed a significant decrease ($P \leq 0.05$) in the CCl₄ and NiCl₂+ CCl₄ treated groups compared to the control group, as shown in Figure 6. However, there was no significant change in FSH concentration between the NiCl₂ and the control groups.

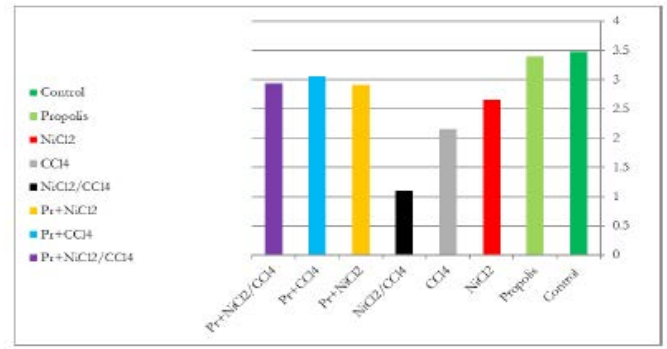


Figure 4: The effect of Propolis on Testosterone concentration in male rats who received NiCl₂ and/or CCl₄.

Table 2: The effect of propolis on T, LH and FSH concentration in male rats treated with NiCl₂ and/or CCl₄.

Pa-ram-eter	Group	Mean	Std. Devia- tion	Std. error	95% confi- dence interval for mean	
					Lower bound	Upper bound
T	Control	7.9300	0.29078	0.09195	7.7220	8.1380
	Propolis	7.8900	0.04508	0.14256	7.5675	8.2125
	NiCl ₂	4.0100	0.70781	0.22383	3.5037	4.5163
	CCl ₄	4.1800	0.47796	0.15114	3.8381	4.5219
	NiCl ₂ / CCl ₄	3.5300	0.41913	0.13254	3.2302	3.8298
	Pr+ NiCl ₂	7.0900	0.61001	0.19290	6.6536	7.5264
	Pr+ CCl ₄	6.6090	0.63555	0.20098	6.1544	7.0636
	Pr+NiCl ₂ / CCl ₄	4.3000	0.38006	0.12019	4.0281	4.5719
LH	LSD	0.48				
	Control	3.0500	0.62370	0.19723	2.6038	3.4962
	Propolis	2.9470	0.32911	0.10407	2.7116	3.1824
	NiCl ₂	0.9280	0.37169	0.11754	0.6621	1.1939
	CCl ₄	0.8120	0.12994	0.04109	0.7190	0.9050
	NiCl ₂ / CCl ₄	0.4650	0.30668	0.09698	0.2456	0.6844
	Pr+ NiCl ₂	2.8300	0.42177	0.13337	2.5283	3.1317
	Pr+ CCl ₄	1.5520	0.26233	0.08296	1.3643	1.7397
FSH	Pr+NiCl ₂ / CCl ₄	1.5560	0.12066	0.03816	0.4697	0.6423
	LSD	0.624				
	Control	3.4780	0.37410	0.11830	3.2104	3.7456
	Propolis	3.4030	0.41215	0.13033	3.1082	3.6978
	NiCl ₂	2.6520	0.21275	0.06728	2.4998	2.8042
	CCl ₄	2.1490	0.49323	0.15597	1.7962	2.5018
	NiCl ₂ / CCl ₄	1.0990	0.31061	0.09822	0.8768	1.3212
	Pr+ NiCl ₂	2.9120	0.32516	0.10282	2.6794	3.1446
	Pr+ CCl ₄	3.0560	0.41969	0.13272	2.7558	3.3562
	Pr+NiCl ₂ / CCl ₄	2.9330	0.24887	0.07870	2.7550	3.1110
	LSD	0.347				

Note: T= Testosterone, LH= Luteinizing hormone, FSH= Follicle-stimulating hormone.

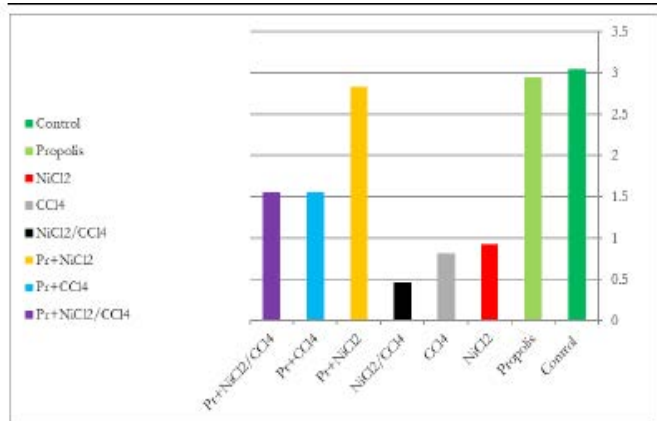


Figure 5: The effect of Propolis on LH concentration in male rats who received NiCl₂ and/or CCl₄.

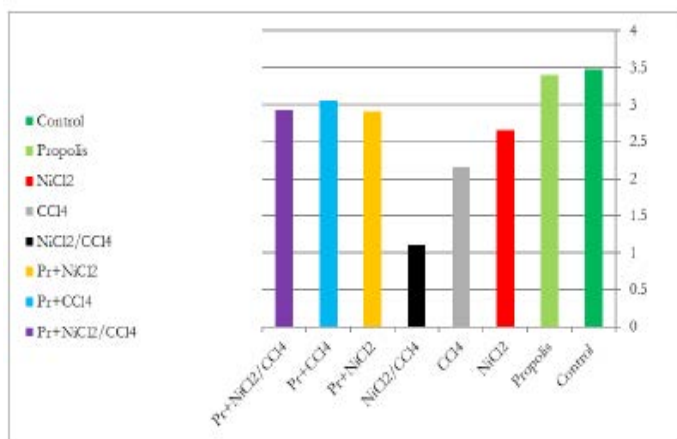


Figure 6: The effect of Propolis on FSH concentration in male rats who received NiCl₂ and/or CCl₄.

Finally, no significant changes in FSH serum levels were observed in the groups treated with propolis containing NiCl₂, CCl₄, and NiCl₂+ CCl₄ compared to the control group.

DISCUSSIONS AND RECOMMENDATIONS

The current study found that rats treated with NiCl₂ and/or CCl₄ exhibited hypothyroidism, as evidenced by a marked reduction in serum T₃ and T₄ levels. Several researchers (Badr et al., 2021; Salah et al., 2021) have documented hypothyroidism in rats treated with NiCl₂ and CCl₄. Thyroxine (T₄) and triiodothyronine (T₃) are essential for proper human growth and development as well as for the functioning of adult physiology. The identification of substances that have adverse effects by interfering with the thyroid system is difficult due to many obstacles. Endocrine disruptors such as NiCl₂ and/or CCl₄ interfere with the endocrine system, particularly the thyroid gland, by inhibiting thyroperoxidase. This interference impairs the ability of follicular cells to produce T₄ and T₃, even

when sufficient iodine is present (Chakraborty, 2021). Several chemical compounds have significant structural similarities to T₄ and T₃, which consequently interfere with the binding of thyroid hormones to their receptors or transfer proteins, leading to subclinical hypothyroidism (Farokhi and Taravati, 2014).

The administration of Propolis to animal groups treated with NiCl₂ and/or CCl₄ resulted in a notable enhancement in thyroid hormone levels. This improvement can be attributed to the presence of antioxidant and polyphenolic compounds in Propolis and its lack of significant adverse effects. These compounds effectively act as potent antioxidants by eliminating free radicals and inhibiting fat peroxidation, thereby preserving the integrity of the cell membrane (Pahlavani et al., 2020). Abu-Almaaty et al. (2019) and El-Amawy et al. (2021) have proved that Propolis possesses antioxidant properties that counteract oxidative stress and protect organs from damage caused by Aluminum Silicate and heavy metals. Furthermore, they have shown that Propolis exerts a protective effect on many organs in albino rats. The findings demonstrated a notable reduction in blood LH, FSH, and testosterone levels in the NiCl₂ and/or CCl₄ groups. These outcomes align with previous studies conducted by Adedara et al. (2019), Rizvi et al. (2020), and Keshtmand et al. (2021). The decrease in LH, FSH, and testosterone levels may be attributed to oxidative damage to the hypothalamus, which is responsible for the secretion of gonadotropin-releasing hormone (GnRH). GnRH stimulates the release of LH and FSH by the pituitary gland, which subsequently inhibits testosterone production in the testes of rats. The administration of CCl₄ decreased the levels of T hormone and gonadotropins (FSH and LH). It caused testicular shrinkage and degeneration of the germinal layer in male rats (Elsawy et al., 2019). CCl₄ modulates pituitary secretions by influencing the central nervous system, particularly the hypothalamus nuclei, resulting in decreased synthesis of sex hormones in the testes (Keshtmand et al., 2021). However, CCl₄ also harms the cells responsible for producing and storing cholesterol and fatty acids, hence decreasing the production of sex hormones. The liver has a direct role in the production and breakdown of sex hormones in both humans and animals (Kur et al., 2020).

Nickel is a poisonous substance that can harm the blood, immune system, nerves, genes, kidneys, and liver. In addition to being a carcinogenic agent, it also induces reproductive toxicity and pulmonary toxicity (Deng et al., 2016). Studies have shown that nickel can cause damage to the standard structure of the testicles, with the extent of the damage varying depending on the dose of nickel administered. This damage affects practically all measurable characteristics of the testicles. Temamogullari et al. (2021) discovered a notable reduction in the size and weight

ratios of the testes, epididymides, seminal vesicles, and prostate gland following the ingestion of nickel sulfate. Nickel influences follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels by directly affecting the anterior pituitary gland. This leads to an atypical release of hormones in the hypothalamic-pituitary-gonadal axis (HPGA). Experimental investigations present conflicting findings regarding the impact of nickel on follicle-stimulating hormone (FSH) and luteinizing hormone (LH), potentially attributable to variations in nickel dosage. Small amounts of nickel impact reproductive function, disrupting the hormone axis that regulates the hypothalamus, pituitary gland, and gonads. This disruption leads to elevated follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels. Exposure to a significant amount of nickel can cause harm to the pituitary gland and other organs, leading to a reduction in the amounts of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Yang and Ma, 2021).

Nickel might impact many organs by generating reactive oxygen species (ROS), which triggers lipid peroxidation (LPO). The resulting molecules can form adducts with DNA, thereby influencing gene expression. Peroxide breakdown of unsaturated fatty acids results in malondialdehyde (MDA) synthesis. Elevated concentrations of nickel result in a moderate level of oxidative stress in the testis, leading to apoptotic cell death and DNA damage in both the testis and epididymal sperm cells. ROS, or reactive oxygen species, can produce severe pathological damage and directly impact spermatogenesis, therefore playing a crucial part in the development of male infertility (Barati et al., 2020). Administration of propolis orally, along with NiCl_2 and/or CCl_4 , resulted in the restoration of normal levels of male sex hormones. Multiple research studies have examined the efficacy of propolis in mitigating the decline in testosterone levels caused by external toxins (Çilenk et al., 2016; Hashem, 2021). The increase in testosterone hormone and gonadotropin concentration due to propolis can be attributed to specific bioactive compounds. For example, the flavonoid chrysin has been found to enhance testosterone production in the testes (Darwish et al., 2014).

Additionally, phenolic compounds like flavonoids (such as tectochrysin, chrysin, pinocembrin, galangin, apigenin, genkwanin, and kaempferol), stilbenes, flavan-3-ols (catechins), and hydroxybenzoic acids have also been identified as contributors to this improvement (Kurek-Górecka et al., 2013; Laaroussi et al., 2021). Other significant phenolic compounds in propolis include cinnamic acid and its derivatives, such as hydroxycinnamic acids, ferulic acid, caffeic acid, isoferulic acid, and p-coumaric acid. Gallic acid has also been detected in propolis from Poland (Moskwa et al., 2020; Wezgowiec et al., 2020).

Our investigation concludes that propolis has a significant impact in safeguarding against hypothyroidism and gonadotoxicity generated by NiCl_2 and/or CCl_4 , which are endocrine disruptors. The co-administration of propolis alongside an endocrine disruptor can shield the endocrine glands from the harmful impact and oxidative stress of NiCl_2 and/or CCl_4 . Additional clinical trials are necessary to evaluate the efficacy and safety of propolis prior to its utilization in humans and subsequent approval by the Food and Drug Administration (FDA).

ACKNOWLEDGEMENTS

I am extremely pleased and proud to express my sincere appreciation and gratitude to Dr. Amer M.A for his assistance in handling the animals and to Dr. Muslim Dh.M for his valuable contributions to the statistical analysis.

NOVELTY STATEMENT

Although previous studies have examined the toxic effects of substances such as nickel chloride and carbon tetrachloride on physiological and endocrine functions, few have explored the use of propolis extract as a potential protective agent. This study aims to investigate the protective properties of propolis extract against the physiological and endocrine alterations induced by these toxins, thereby contributing to the current understanding of the potential medical and environmental applications of propolis.

AUTHORS CONTRIBUTION

NMA was solely responsible for the study design, data collection, data analysis, manuscript writing, and critical review of the research.

ETHICAL APPROVAL

All procedures adhered to the guidelines set by the Scientific Committee for Animal Care and Use at the Faculty of Veterinary Medicine, Shatrah University, with the project approval number SCACU/R013/2024.

CONFLICT OF INTEREST

The author has declared no conflict of interest.

REFERENCES

- Abdullah NA, Zulkiflee N, Zaini SN, Taha H, Hashim F, Usman A (2020). Phytochemicals, mineral contents, antioxidants, and antimicrobial activities of propolis produced by Brunei stingless bees *Geniotrigona thoracica*, *Heterotrigona itama*, and *Tetrigona binghami*. Saudi J. Biol. Sci., 27(11): 2902-

2911. <https://doi.org/10.1016/j.sjbs.2020.09.014>
- Abed NM, Alkalby JM (2018). Protective effect of pumpkin seed oil on reproductive system in chlorpyrifos treated adult male rats. Bas. J. Vet. Res., 17(2). <https://uobasrah.edu.iq/research/sci research/24717>
- Abu-Almaaty AH, Abd El-Aziz YM, Omar NA, Abdeen AM (2019). Estimation of the prophylactic effect of the Egyptian propolis extract against aluminum silicate toxicity on some organs of albino rats: Growth performance and histochemical studies. Egypt. J. Hosp. Med., 76(7), 4564-4569. <https://doi.org/10.21608/ejhm.2019.45042>
- Adedara IA, Abiola MA, Adegbosin AN, Odunewu AA, Farombi EO (2019). Impact of binary waterborne mixtures of nickel and zinc on hypothalamic-pituitary-testicular axis in rats. Chemosphere, 237: 124501. <https://doi.org/10.1016/j.chemosphere.2019.124501>
- Adriano DC (2001). Nickel trace elements in terrestrial environments: Biogeochemistry, bioavailability, and risks of metals, pp. 677-705. https://doi.org/10.1007/978-0-387-21510-5_17
- Ahangari Z, Naseri M, Vatandoost F (2018). Propolis: Chemical composition and its applications in endodontics. Iran Endod. J., 13(3): 285.
- Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, Bashir MA, Tahir M, Ansari MJ, Ghramh HA, Adgaba N, Dash CK (2019). Composition and functional properties of propolis (bee glue): A review. Saudi J. Biol. Sci., 26(7): 1695-1703. <https://doi.org/10.1016/j.sjbs.2018.08.013>
- Badr GM, Algefare AI, Alfwaiares MA (2021). Antioxidant potential of Parsley leaf (*Petroselinum crispum*) essential oil on hypothyroidism and testicular injury in mice intoxicated by carbon tetrachloride. Biomed. Res. Int., 2021: 1-11. <https://doi.org/10.1155/2021/9989174>
- Barati E, Nikzad H, Karimian M (2020). Oxidative stress and male infertility: Current knowledge of pathophysiology and role of antioxidant therapy in disease management. Cell. Mol. Life Sci., 77(1): 93-113. <https://doi.org/10.1007/s00018-019-03253-8>
- Bertini I, Sigel A (2001). Handbook on metalloproteins. CRC Press. <https://doi.org/10.1201/9781482270822>
- Chakraborty A (2021). Excess iodine exposure: An emerging area of concern for male reproductive physiology in the post-salt iodization era. Asian Pac. J. Reprod., 10(3): 102. <https://doi.org/10.4103/2305-0500.316622>
- Çilenk KT, Öztürk İ, Sönmez MF (2016). Ameliorative effect of propolis on the cadmium-induced reproductive toxicity in male albino rats. Exp. Mol. Pathol., 101(2): 207-213. <https://doi.org/10.1016/j.yexmp.2016.08.004>
- Darwish HA, Arab HH, Abdelsalam RM (2014). Chrysin alleviates testicular dysfunction in adjuvant arthritic rats via suppression of inflammation and apoptosis: Comparison with celecoxib. Toxicol. Appl. Pharmacol., 279(2): 129-140. <https://doi.org/10.1016/j.taap.2014.05.018>
- Deng J, Guo H, Cui H, Fang J, Zuo Z, Deng J, Wang X, Zhao L (2016). Oxidative stress and inflammatory responses involved in dietary nickel chloride (NiCl₂)²-induced pulmonary toxicity in broiler chickens. Toxicol. Res., 5(5): 1421-1433. <https://doi.org/10.1039/C6TX00197A>
- El-Shafei H (2011). Assessment of liver function among nickel-plating workers in Egypt. East. Mediterr. Health J., 17(6): 490-494. <https://doi.org/10.26719/2011.17.6.490>
- El-Amawy AA, Zaahkous SA, Rasheed HG, Mohammed BE (2021). The protective role of propolis against multi heavy metals-induced oxidative stress-hepato-renal damage in the male of albino rats. <https://doi.org/10.21203/rs.3.rs-422622/v1>
- Elsawy H, Badr GM, Sedky A, Abdallah BM, Alzahrani AM, Abdel-Moneim AM (2019). Rutin ameliorates carbon tetrachloride (CCl₄)-induced hepatorenal toxicity and hypogonadism in male rats. PeerJ., 7: e7011. <https://doi.org/10.7717/peerj.7011>
- Farhan AS (2020). Effects of Tribulus terrestris fruits on renal and lung tissues in female mice administered with cadmium. J. World's Poult. Res., 10(3): 414-420. <https://doi.org/10.36380/scil.2020.wvj51>
- Farokhi F, Taravati A (2014). Pesticide exposure and thyroid function in adult male sprayers. Int. J. Med. Investig., 3(4): 0-0.
- Goodarzi N, Akbari M (2016). Renal structure and its concentrating ability in the Persian squirrel (*Sciurus anomalus*). Comp. Clin. Pathol., 25: 445-448. <https://doi.org/10.1007/s00580-015-2206-9>
- Gupta A, Das S, Dhundasi S, Das K (2008). Effect of garlic (*Allium sativum*) on heavy metal (Nickel II and Chromium VI) induced alteration of serum lipid profile in male albino rats. Int. J. Environ. Res. Publ. Health, 5(3): 147-151. <https://doi.org/10.3390/ijerph5030147>
- Hashem A (2021). Defensive impact of propolis against CCl₄ actuated rats testicular damage. J. Adv. Vet. Anim. Res., 8(1): 1. <https://doi.org/10.5455/javar.2021.h487>
- Iyaka YA (2011). Nickel in soils: A review of its distribution and impacts. Sci. Res. Essays, 6(33). <https://doi.org/10.5897/SREX11.035>
- Kasprzak KS, Salnikow K (2007). Nickel toxicity and carcinogenesis. Nickel and Its Surprising Impact in Nature, pp. 619-660. <https://doi.org/10.1002/9780470028131.ch17>
- Keshtmand Z, Akbaribazm M, Bagheri Y, Oliaei R (2021). The ameliorative effects of Lactobacillus coagulans and Lactobacillus casei probiotics on CCl₄-induced testicular toxicity based on biochemical, histological and molecular analyses in rat. Andrologia, 53(1). <https://doi.org/10.1111/and.13908>
- Kumar M, Sarma DK, Shubha S, Kumawat M, Verma V, Prakash A, Tiwari R (2020). Environmental endocrine-disrupting chemical exposure: Role in non-communicable diseases. Front. Publ. Health, 8. <https://doi.org/10.3389/fpubh.2020.553850>
- Kur P, Kolasa-Wołoskiuk A, Misiakiewicz-Has K, Wiszniewska B (2020). Sex hormone-dependent physiology and diseases of liver. Int. J. Environ. Res. Publ. Health, 17(8): 2620. <https://doi.org/10.3390/ijerph17082620>
- Kurek-Górecka A, Rzepecka-Stojko A, Górecki M, Stojko J, Sosada M, Świerczek-Zięba G (2013). Structure and antioxidant activity of polyphenols derived from propolis. Molecules, 19(1): 78-101. <https://doi.org/10.3390/molecules19010078>
- Laaroussi H, Bakour M, Ousaid D, Ferreira-Santos P, Genisheva Z, El-Ghouizi A, Aboulghazi A, Teixeira JA, Lyoussi B (2021). Protective effect of honey and propolis against gentamicin-induced oxidative stress and Hepatorenal damages. Oxid. Med. Cell. Longev., 2021: 1-19. <https://doi.org/10.1155/2021/9719906>
- Moskwa J, Naliwajko SK, Markiewicz-Żukowska R, Gromkowska-Kępka KJ, Nowakowski P, Strawa JW, Borawska MH, Tomczyk M, Socha K (2020). Chemical composition of Polish propolis and its antiproliferative

- effect in combination with *Bacopa monnieri* on glioblastoma cell lines. Sci. Rep., 10(1). <https://doi.org/10.1038/s41598-020-78014-w>
- Pahlavani N, Malekahmadi M, Firouzi S, Rostami D, Sedaghat A, Moghaddam AB, Ferns GA, Navashenag JG, Reazvan R, Safarian M, Ghayour-Mobarhan M (2020). Molecular and cellular mechanisms of the effects of propolis in inflammation, oxidative stress and glycemic control in chronic diseases. Nutr. Metab., 17(1). <https://doi.org/10.1186/s12986-020-00485-5>
- Pasupuleti VR, Sammugam L, Ramesh N, Gan SH (2017). Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits. Oxid. Med. Cell. Longev., 2017: 1-21. <https://doi.org/10.1155/2017/1259510>
- Rivera-Yañez N, Rodríguez-Canales M, Nieto-Yañez O, Jiménez-Estrada M, Ibarra-Baraja M, Canales-Martínez MM, Rodríguez-Monroy MA (2018). Hypoglycaemic and antioxidant effects of propolis of Chihuahua in a model of experimental diabetes. Evid. Based Complement. Altern. Med., pp. 1-10. <https://doi.org/10.1155/2018/4360356>
- Rizvi A, Parveen S, Khan S, Naseem I (2020). Nickel toxicology with reference to male molecular reproductive physiology. Reprod. Biol., 20(1): 3-8. <https://doi.org/10.1016/j.repbio.2019.11.005>
- Salah I, Adjroud O, Elwej A (2021). Protective effects of selenium and zinc against nickel chloride-induced hormonal changes and oxidative damage in thyroid of pregnant rats. Biol. Trace Elem. Res., 200(5): 2183-2194. <https://doi.org/10.1007/s12011-021-02815-x>
- Samadi N, Mozaffari-Khosravi H, Rahmanian M, Askarishahi M (2017). Effects of bee propolis supplementation on glycemic control, lipid profile and insulin resistance indices in patients with type 2 diabetes: A randomized, double-blind clinical trial. J. Integr. Med., 15(2): 124-134. [https://doi.org/10.1016/S2095-4964\(17\)60315-7](https://doi.org/10.1016/S2095-4964(17)60315-7)
- Schmidt M, Goebels M (2011). Nickel allergies: Paying the toll for innate immunity. J. Mol. Med., 89(10): 961-970. <https://doi.org/10.1007/s00109-011-0780-0>
- Statistical Packages for the Social Sciences (SPSS). (2010). Statistical software for windows version 19.0 Microsoft. SPSS, Chicago, Illinois, USA.
- Temamogullari F, Atessahin A, Cebi Sen C, Yumusak N, Dogru M (2021). Protective role of silibinin over nickel sulfate-induced reproductive toxicity in male rats. Pol. J. Vet. Sci., pp. 29-34. <https://doi.org/10.24425/pjvs.2020.135817>
- Wezgowiec J, Wieczynska A, Wieckiewicz W, Kulbacka J, Saczko J, Pachura N, Wieckiewicz M, Gancarz R, and Wilk KA (2020). Polish propolis chemical composition and biological effects in tongue cancer cells and macrophages. Molecules, 25(10): 2426. <https://doi.org/10.3390/molecules25102426>
- Yang J, Ma Z (2021). Research progress on the effects of nickel on hormone secretion in the endocrine axis and on target organs. Ecotoxicol. Environ. Saf., 213: 112034. <https://doi.org/10.1016/j.ecoenv.2021.112034>
- Zakerkish M, Jenabi M, Zaeemzadeh N, Hemmati AA, Neisi N (2019). The effect of Iranian propolis on glucose metabolism, lipid profile, insulin resistance, renal function and inflammatory biomarkers in patients with type 2 diabetes mellitus: A randomized double-blind clinical trial. Sci. Rep., 9(1). <https://doi.org/10.1038/s41598-019-43838-8>
- Zulkiflee N, Taha H, Usman A (2022). Propolis: Its role and efficacy in human health and diseases. Molecules, 27(18): 6120. <https://doi.org/10.3390/molecules27186120>