Volume 67, Issue 2, 2023
Journal of Scientific Research
of
The Banaras Hindu University

Alteration in THRβ-1 expression on Thyrocytes of Hypothyroidism Mice: Antioxidant Activity of Ashwagandha and Quercetin

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Abstract:**The present study is designed to investigate the protective efficacy of ashwagandha and quercetin against altered THRβ-1 expression, structural damages, and induced oxidative stress in the thyroid gland of hypothyroidism-modeled mice. Mice were orally exposed to cypermethrin for 28 days. The body weight of mice was recorded during the experiment at seven days intervals and the thyroid gland weight was recorded at the end of the experiment after sacrifices. The homogenate of thyroid tissue was prepared for biochemical examinations such as LPO, H_2O_2, NO, SOD, CAT, GSH, GST, GR, and tissues were fixed in Bouin's fluid for histopathological evaluations by double staining (hematoxylin and eosin) and immunohistochemistry (THRβ-1) methods. Cypermethrin-induced hypothyroidism showed increased body weight and decreased thyroid gland weight. The MDA concentration and activity of H_2O_2 and NO increased and the activity of CAT, SOD, GSH, GST, and GR decreased in hypothyroidism. The histopathological examinations showed decreased numbers and size of thyroid follicles in hypothyroidism mice. The immunohistochemistry showed decreased THRβ-1 expression in thyrocytes of cypermethrin-induced hypothyroidism mice. The co-administration of ashwagandha and quercetin showed amelioration efficacy in biochemical changes and histopathological deformities in hypothyroidism mice. The present study concluded that hypothyroidism caused oxidative stress and histopathological changes in the thyroid gland and the use of ashwagandha and quercetin as an antioxidant showed prevention against the damage caused by hypothyroidism.**

Index Terms:**Ashwagandha,Cypermethrin, Hypothyroidism, Oxidative stress, Quercetin, Thyroid gland**

I. Introduction

3-phenoxy benzoic acid is a metabolic byproduct of type II pyrethroid insecticides including cypermethrin. 3-PBA reaches into the blood and exerts toxicological effects governing oxidative stress, immunomodulation, endocrine disruption, and neurodegeneration (Chen et al., 2011). Cypermethrin is a type II synthetic pyrethroid insecticide and anendocrine-disrupting agent. Deltamethrin, fenvalerate, and cyhalothrin are also pyrethroids and cause endocrinedysfunction (Mnif et al., 2011). The LD50 value of cypermethrin is about 250 mg/kg body weight in rodents (Raj et al., 2013). Hyperthyroidism is characterized by higher serum thyroid hormone and lower TSH levels, whereas declined thyroid hormones and increased TSH is known as hypothyroidism,both hypo and hyperthyroidism are pathological conditions. The thyroid hormone plays an important role in the regulation of basal metabolism of the body and helps in growth and development (Hanley et al., 2016). 3-PBA caused decreased binding of TSH to its receptors resulting in increased TSH, causing decreased secretion of T3 and T4 *via* a negative feedback mechanism(Requena et al., 2019).

Oxidative stress is the state of failure of the total antioxidant capacity of the body against the generated free radicals. Induced hypothyroidism altered oxygen consumption and metabolic modifications causing the generation of superoxide, hydroxy, and hydrogen peroxide radicals subsequent toincreased lipid peroxidation and decreased glutathione resulting in oxidative damage and pathological condition. Lipid peroxidation, hydrogen peroxide, and nitric oxides are the oxidative stress markers, and glutathione, superoxide dismutase,

and catalase activity are the antioxidant capacity, which plays a crucial role in radical scavenging (Erdamar et al., 2008).

Ashwagandha (*Withania somnifera*) is an herbal plant cultivated majorly in the tropical and subtropical regions of India, Africa, and Southeast Asia. Ashwagandha plant is clearly distinguished into root, stem, leaves, flowers, and fruit. Each part of ashwagandha is rich in medicinal properties. Ashwagandha is a medicinal herb used in the ayurvedic medicine system of India since ancient times. withanolides and Withaferin A are the two active constituents of ashwagandha which governs the major medicinal properties such as antioxidant, anti-inflammatory, hepatoprotective, anti-stress, and endocrine protective (Verma and Kumar, 2011). The overdose of antioxidants could lead to toxicity and the LD50 value for ashwagandha is about 1260 mg/kg body weight (Sharada et al., 1993). Quercetin is a bioactive compound of the flavonoid group. Quercetin is found in a variety of food and food products. It is majorly found in fruits including grapes, berries, custard apples, and vegetables like onion, cauliflower, etc. Tea, red wine, and some beverages are also rich in quercetin. Quercetin is the ingredient of some medicines due to its antioxidant, anti-inflammatory, anti-stress and cardioprotective properties (David et al., 2016). A dose over 3807 mg/kg body weight could lead to toxicity and the dose is LD50 for quercetin (Dibal et al., 2020).

The aim of this study is to investigate the antioxidant effect of ashwagandha and quercetin against oxidative damage, histological alterations, and altered THRβ-1 expression in thyrocytes of cypermethrin-induced hypothyroidism mice.

II. MATERIALS AND METHODS

A. Chemicals

The chemicals and the reagents used were of analytical grade. Cypermethrin was purchased from DhanukaAgritech Pvt. Ltd. Under brand name Superkiller 25% EC. The desired concentration as per the experiment was obtained after diluting with water. The crude methanolic extract of the whole plant of ashwagandha was prepared. Quercetin, NBT, NADPH, methionine, and reduced glutathione were purchased from Himedia, India and thiobarbituric acid, hematoxylin, eosin, and the rest of the chemicals used were purchased from Central Drug House (P) Ltd. New Delhi, India

B. Animals

The adult male *Swiss albino* mice were purchased from Veterinary College Mhow, Indore, and housed in the animal caring room at the Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya Sagar, M.P. with 12-12 hours dark light cycle at 25 ± 2°C temperature. Mice were fed with standard laboratory food and water *ad libitum*. This work was approved by the animal ethical committee with approval number- 379/GO/ReBi/S/01/CPCSEA.

C. Experimental Design

The animals are randomly divided into six groups after acclimatization for 7 days. All the doses are given according to per kg body weight. The proposed experimental design used in the experiment is shown in the table below (**Table. 1**).

S. No.	Group	Name	Dose
1.	Group I	C	Food and water *ad libitum*
2.	Group II	Cy	Cypermethrin, 15 mg/kg (Jin et al., 2011)
3.	Group III	CyA	Cypermethrin 15 mg/kg and Ashwagandha 200 mg/kg (Amaravathi and Srilatha, 2010)
4.	Group IV	CyQ	Cypermethrin 15 mg and Quercetin 150 mg/kg (Wong et al., 2020)
5.	Group V	AS	Ashwagandha 200 mg/kgbody weight
6.	Group VI	QU	Quercetin 150 mg/kg body weight

Table 1: Experimental design.

D. Body and Thyroid gland Weight

The body weight of the animals was recorded at the interval of seven days (0, 7, 14, 21, and 28 days) from the beginning with the help of weighing balance (Sartorius, BP210 S), and the thyroid gland weight was taken when mice were sacrificed, and individual values were noted down.

E. Sample preparation

The blood sample was taken through direct cardiac puncture after anesthesia and collected in the clot-activating tubes and centrifuged at 2000rpm for 15 min to separate serum. The collected serum was used to estimate serum thyroid hormone levels using ELISA.The thyroid gland tissue samples were collected for biochemical estimations and histopathological examinations after sacrifices. For the biochemical analysis of the thyroid gland, tissues were homogenized in 0.02M tris HCl pH 7.4. The thyroid tissues were homogenated in 10% (w/v) and then centrifuged at 12,500 rpm for 30 minutes at 4°C. The collected supernatant was stored at -20°C for further estimation. The thyroid sample for histopathological investigation was collected and stored in Bouin's fluid and then followed the procedure for paraffin block preparation and then double staining and immunohistochemistry evaluation.

F. Biochemical Estimations

The biochemical examinations were performed to determine the induced oxidative stress and suppressed activity of the endogenous antioxidant system under the hypothyroidism condition. MDA assay was determined by thiobarbituric acid assay and using the method of placer (Placer *et al.,* 1966). The obtained optical density from the spectrophotometer at 548 nm, was recorded and the results were expressed as nmol MDA/mg Protein.

The activity of Hydrogen peroxide was estimated by following the method given by Sinha (Sinha, 1972) and measured absorbance at 570 nm. H_2O_2 concentration was expressed in μM/mg protein.

The activity of Nitric oxide was estimated by following the method of Griess (Sun et al., 2003), and the absorbance was taken at 570 nm. The concentration of nitric oxide was calculated against a standard curve plot of sodium nitrate and the values were expressed as µM/mg protein. The activity of catalase was determined as per the method of Bergmeyer (Bergmeyer, 1983). The rate of H_2O_2 decomposition for 180 sec at 240 nm was recorded and expressed as µmol/min/mg/protein.

The activity of SOD measured the photochemical inhibition of nitro blue tetrazolium (Beauchamp *et al.,* 1971). Take absorbance was taken at 560nm and expressed results in unit/mg protein. One unit of the enzyme is 50% enzyme inhibiting reaction rate. The levels of glutathione (GSH) in the tissue homogenates were determined spectrophotometrically by using the method of Sedlak and Lindsay (Sedlak *et al.,*1968). The absorbance was read against the blank at 412 nm and the results were expressed as µmol/mg protein.

The specific activity of Glutathione-S-Transferase (GST) was evaluated spectrophotometrically by following the method of (Seyyedi *et al.,* 2005). The absorbance was measured at 340 nm for 5 min and the results were expressed as µmol/min/mg protein.

The activity of Glutathione reductase was measured by (Carlberg and Mannervic 1975). The reaction was started by the addition of tissue supernatant and NADPH was recorded as a decrease in absorbance at 340 nm for 10 min. Nonspecific oxidation of NADPH was calibrated by the absence of GSSG. The unit of the enzyme activity was defined as µmole NADPH/min and the activity of the enzyme was expressed as units /mg protein. The protein concentration in the supernatants was determined by following the method described by Lowry (Lowry *et al.,* 1951).

G. Histological Examinations

The histological examination of the thyroid gland included a dehydration process in ascending alcohol series from 30% to absolute followed by fixation in Bouin's fluid for 48 hours. The dehydrated tissues were embedded in wax following xylene, xylene: wax (1:1 and 1:3), and pure was at 62°C in the oven and then blocks were prepared in wax. The prepared blocks were cut into thin sections of 5µm and fixed over albumin-coated glass slides. The prepared slides were used for the double staining procedure followed by hematoxylin and eosin staining. The prepared slides were observed under 100X and 400X magnification and histological changes were recorded (Carleton et al., 1980).

The thin sections were incubated with primary polyclonal antibody THRβ-1, secondary goat anti-rabbit antibody and, 3,3′-Diaminobenzidine (DAB) then mounted with DPX after counterstaining with hematoxylin (Srivastava and Chaturvedi, 2010).

H. Data analysis

All statistical data analyses were performed using one-way ANOVA (Analysis of variance), and the data were expressed as mean ± SE (Standard Error). The student t-test was applied for comparison between the control and exposed groups. The level of significance at *p<0.05 was considered significant.

III. RESULTS

A. Body and Thyroid gland Weight

The body weight increased significantly (**p<0.01) in the cypermethrin-induced hypothyroid group as compared to the control group and decreased significantly (*p<0.05) in the ashwagandha and quercetin co-administrative group. The thyroid gland weight decreased significantly (***p<0.001) in the cypermethrin-induced hypothyroid group as compared to the control group and increased significantly (**p<0.01) in the ashwagandha and quercetin co-administrative groups. Only ashwagandha and quercetin administration showed non-significant changes in gain in body weight and thyroid gland weight (**Table. 2**).

Group	Body Weight	Thyroid Weight
C	0.7±0.06	0.08±0.001
Cy	1.15±0.05**	0.054±0.002***
CyA	0.95±0.05*	0.065±0.001**
CyQ	1.00±0.09*	0.063±0.001**
AS	0.65±0.05[a]	0.080±0.001[a]
QU	0.85±0.05[a]	0.081±0.001[a]

Table 2:Ashwagandha and quercetin co-administrative groups (CyA and CyQ) showed a preventive role in body weight and the thyroid gland weight against the increased body weight in hypothyroidism. The values represent Mean±SE (n=6). **p<0.01 and ***p<0.001 (significance difference between control and hypothyroid group for body weight and thyroid weight respectively) *p<0.05 and **p<0.01 (significance difference between CyA and CyQ versus hypothyroid group for body weight and thyroid weight respectively). [a] represents non-significant changes.

B. Hormonal Estimation

The level of thyroid hormones (T3 and T4) decreased significantly (**p<0.01)) in the cypermethrin-exposed group as compared to the control and administrative groups (AS and QU) and increased significantly (*p<0.05 and **p<0.01 respectively) in the ashwagandha and quercetin co-administrative groupsas compared to the cypermethrin-exposed group. The serum level of TSH increased significantly (**p<0.01) in the cypermethrin-exposed group as compared to the control and administrative groups (AS and QU) and decreased significantly (*p<0.05 and **p<0.01 respectively) in the ashwagandha and quercetin co-administrative groupsas compared to the cypermethrin-exposed

group. The levels of free thyroid hormones (fT3 and fT4) decreased significantly in the cypermethrin-exposed group as compared to the control and administrative groups (AS and QU) and increased significantly in the ashwagandha and quercetin co-administrative groups as compared to the cypermethrin-exposed group (**Table. 3**).

	T3 (ng/dl)	T4 (µg/dl)	TSH (µUI/ml)	fT3 (pg/dl)	fT4 (ng/dl)
C	85±3.65	4.62±0.23	2.59±0.21	2.22±0.06	1.46±0.05
Cy	41.5±3.34**	2.95±0.20**	4.36±0.23**	1.40±0.04***	0.93±0.03**
CyA	59.7±3.75*	4.09±0.24*	3.57±0.14*	1.79±0.07*	1.18±0.03*
CyQ	48.5±3.54**	2.91±0.13**	4.03±0.09**	1.88±0.04*	1.16±0.04*
AS	73.2±2.32*	4.0±0.14*	3.25±0.15*	2.47±0.03*	1.54±0.06[a]
QU	51.5±2.42**	3.8±0.14*	3.54±0.13*	2.58±0.05*	1.57±0.05[a]

Table 3: Co-administration of ashwagandha and quercetin (CyA and CyQ) showed a preventive role in thyroid hormone dysfunctions. The values represent Mean±SE (n=6). ***p<0.001 and **p<0.01 (Significant difference between cypermethrin-exposed and control group for fT3 and T3, T4, TSH, and fT4 respectively) **p<0.01 and *p<0.05 (significant difference between CyA and CyQ versus cypermethrin-exposed group).

C. Biochemical Examinations

1) Lipid Peroxidation

The MDA concentration increased significantly (***p<0.001) in the thyroid gland of the cypermethrin-induced hypothyroid group as compared to the control and decreased significantly (*p<0.05 and **p<0.01) in the ashwagandha and quercetin co-administrative groups as compared to the cypermethrin-induced hypothyroidism group.

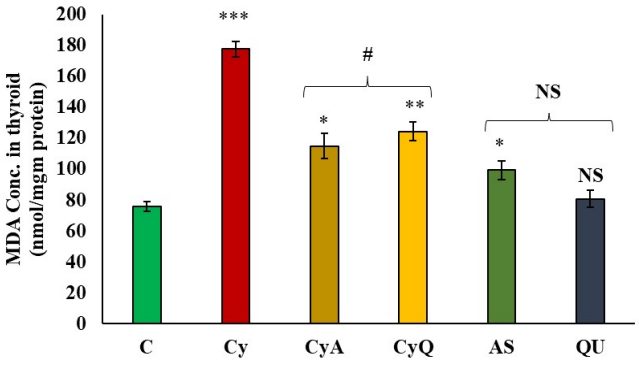


Figure1: Co-administration of ashwagandha and quercetin (CyA and CyQ) on the MDA concentration in the thyroid gland against cypermethrin-induced hypothyroidism mice. The values represent Mean±SE (n=6). ***p<0.001 (significance difference between control and hypothyroidism group) *p<0.05 and **p<0.01 (significance difference between CyA and CyQ versus hypothyroidism group respectively) #p<0.05 (significance difference between CyA versus CyQ).

2) H_2O_2 Activity

The H_2O_2 activity significantly increased (**p<0.01) in the thyroid gland of the cypermethrin-induced hypothyroidism group as compared to the control group and decreased significantly (*p<0.05) in the ashwagandha and quercetin co-administrative groups as compared to the cypermethrin-induced hypothyroidism group.

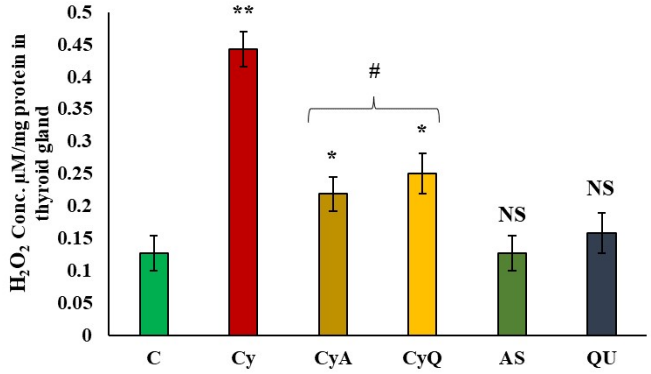


Figure 2: Co-administration of ashwagandha and quercetin (CyA and CyQ) on the H_2O_2 concentration in the thyroid gland against cypermethrin-induced hypothyroidism mice. The values represent Mean±SE (n=6). **p<0.01 (significance difference between control and hypothyroidism group) *p<0.05 (significance difference between CyA and CyQ versus hypothyroidism group respectively) #p<0.05 (significance difference between CyA versus CyQ).

3) Nitric Oxide (NO) Activity

The activity of nitric oxide increased significantly (**p<0.01) in the thyroid gland of the cypermethrin-induced hypothyroidism group as compared to the control group and decreased significantly (*p<0.05) in the ashwagandha and quercetin co-administered groups as compared to the cypermethrin-induced hypothyroidism group.

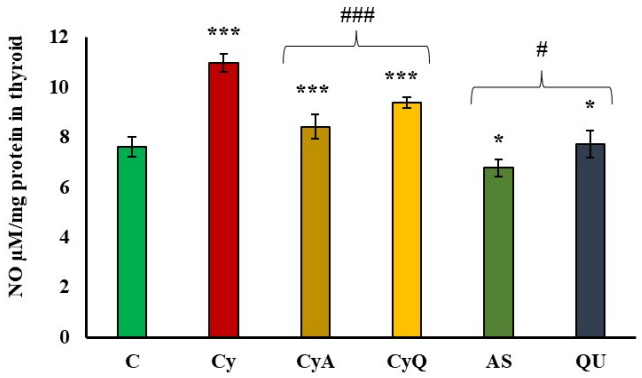


Figure 3: Co-administration of ashwagandha and quercetin (CyA and CyQ) on the nitric oxide activity in the thyroid gland against cypermethrin-induced hypothyroidism mice. The values represent Mean±SE (n=6). **p<0.01 (significance difference between control and hypothyroidism group) *p<0.05 (significance difference between CyA and CyQ versus hypothyroidism group respectively) #p<0.05 (significance difference between CyA versus CyQ).

4) Superoxide Dismutase (SOD) Activity

The activity of superoxide dismutase decreased significantly (***p<0.001) in the thyroid gland of the cypermethrin-induced hypothyroidism group as compared to the control group and increased significantly (**p<0.01) in the ashwagandha and quercetin co-administration groups as compared to the cypermethrin-induced hypothyroidism group.

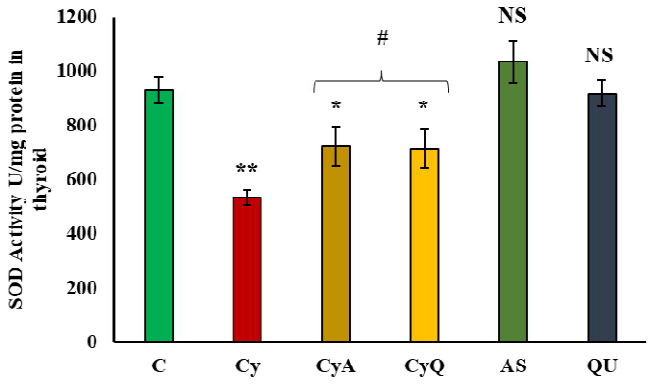


Figure 4: Co-administration of ashwagandha and quercetin (CyA and CyQ) on the SOD activity in the thyroid gland against cypermethrin-induced hypothyroidism mice. The values represent Mean±SE (n=6). ***p<0.001 (significance difference between control and hypothyroidism group) **p<0.01 (significance difference between CyA and CyQ versus hypothyroidism group respectively) ##p<0.01 (significance difference between CyA versus CyQ).

5) Catalase Activity

The catalase activity significantly decreased (**p<0.01) in the thyroid gland of the cypermethrin-induced hypothyroidism group as compared to the control group and increased significantly (*p<0.05) in the ashwagandha and quercetin co-treated groups as compared to the cypermethrin-induced hypothyroidism group.

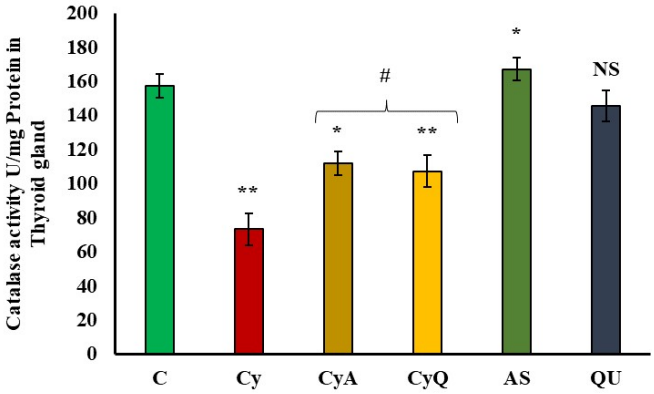


Figure 5: Co-administration of ashwagandha and quercetin (CyA and CyQ) on the catalase activity in the thyroid gland against cypermethrin-induced hypothyroidism mice. The values represent Mean±SE (n=6). **p<0.01 (significance difference between control and hypothyroidism group) *p<0.05 (significance difference between CyA and CyQ versus hypothyroidism group respectively) #p<0.05 (significance difference between CyA versus CyQ).

6) GSH Activity

The activity of glutathione significantlydecreased (**p<0.01) in the thyroid gland of the cypermethrin-induced hypothyroidism group as compared to the control group and increased significantly (*p<0.05) in the ashwagandha and quercetin co-administration groups as compared to the cypermethrin-induced hypothyroidism group.

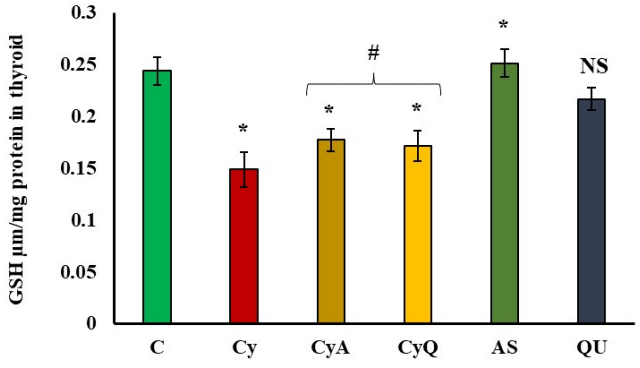


Figure 6: Co-administration of ashwagandha and quercetin (CyA and CyQ) on the GSH activity in the thyroid gland against cypermethrin-induced hypothyroidism. The values represent Mean±SE (n=6). **p<0.01 (significance difference between control and hypothyroidism group) *p<0.05 (significance difference between CyA and CyQ versus hypothyroidism group respectively) #p<0.05 (significance difference between CyA versus CyQ).

7) Glutathione-S-transferase (GST) Activity

The activity of glutathione-S-transferase (GST) significantlydecreased (**p<0.01) in the thyroid gland of the cypermethrin-induced hypothyroidism group as compared to the control group and increased significantly (*p<0.05) in the ashwagandha and quercetin co-administrative groups as compared to the cypermethrin-induced hypothyroidism group.

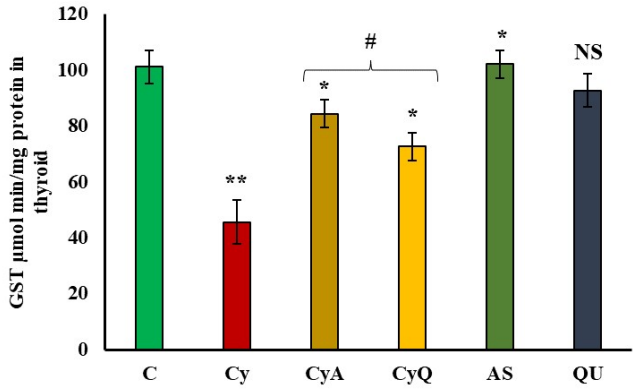


Figure 7: Co-administration of ashwagandha and quercetin (CyA and CyQ) on the activity of GST in the thyroid gland against cypermethrin-induced hypothyroidism. The values represent Mean±SE (n=6). **p<0.01 (significance difference between control and hypothyroidism group) *p<0.05 (significance difference between CyA and CyQ versus hypothyroidism group respectively) #p<0.05 (significance difference between CyA versus CyQ).

8) Glutathione Reduce (GR) Activity

The GR activity significantly decreased (***p<0.001) in the thyroid gland of the cypermethrin-induced hypothyroidism group as compared to the control group and increased significantly (*p<0.01) in the ashwagandha and quercetin co-administrative groups as compared to the cypermethrin-induced hypothyroidism group.

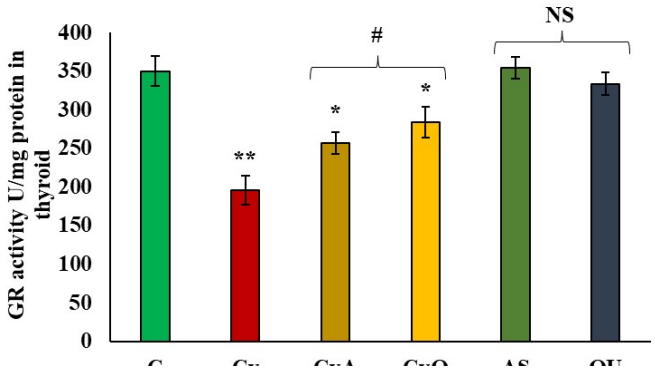
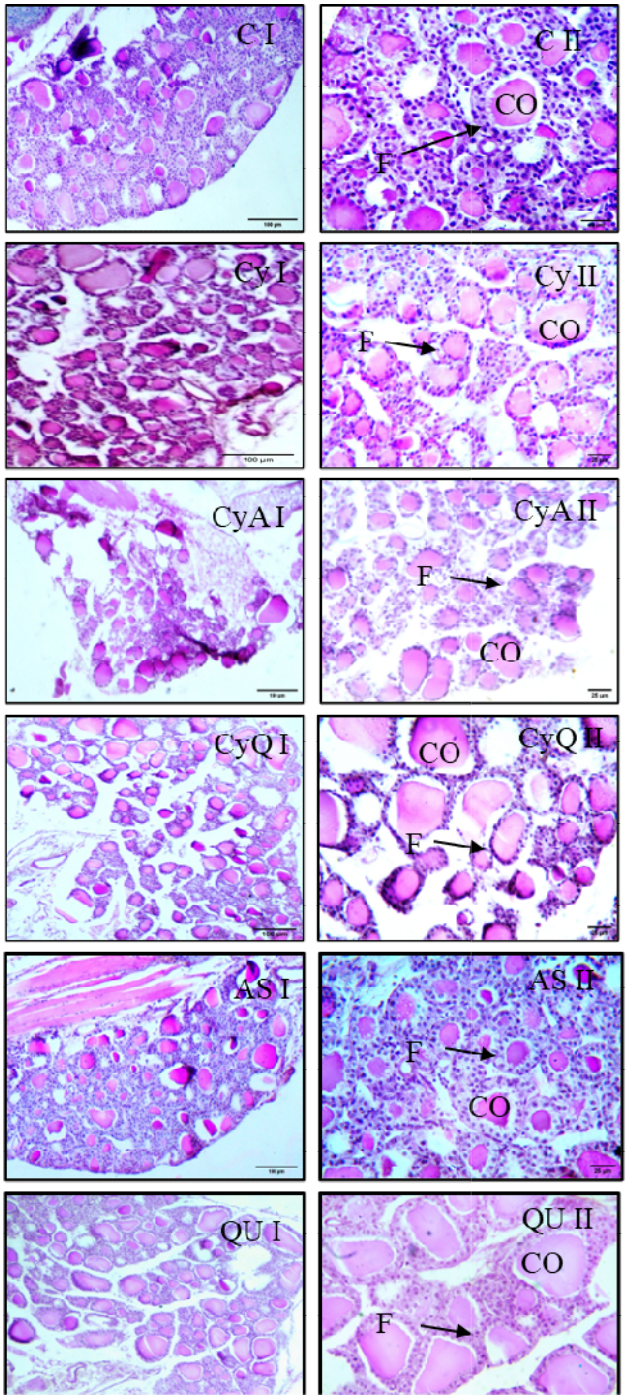


Figure 8: Co-administration of ashwagandha and quercetin (CyA and CyQ) on GR activity in the thyroid gland against cypermethrin-induced hypothyroidism. The values represent Mean±SE (n=6). ***p<0.001 (significance difference between control and hypothyroidism group) **p<0.01 (significance difference between CyA and CyQ versus hypothyroidism group respectively) ##p<0.01 (significance difference between CyA versus CyQ).

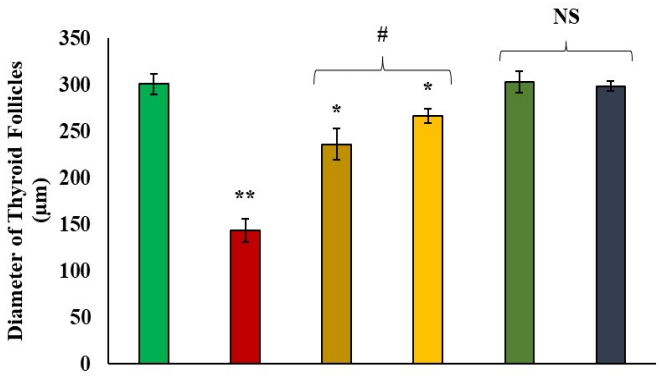
D. Histological and Immunohistochemical Examination

1) Histology of Thyroid Gland

The hematoxylin and eosin staining of the thyroid gland showed a follicular diameter.Due to cypermethrin-induced hypothyroidism,the structureof the thyroid gland is damaged and thyroid follicles appeared to have variable shape. Some follicles seemed shrunkenand decreased concentration of colloid in thyroid follicular cells as compared to the control thyroid structure. Co-administration of ashwagandha and quercetin repaired the thyroid follicles and increased the concentration of colloid**(Fig. 9A)**. The follicular diameter significantly decreased (**p<0.01) in the cypermethrin-induced hypothyroidism group as compared to the control and ashwagandha and quercetin co-administration showed significantly preventive (*p<0.05) follicular diameter as compared to the cypermethrin-induced hypothyroidism group**(Fig. 9B)**.



(A)

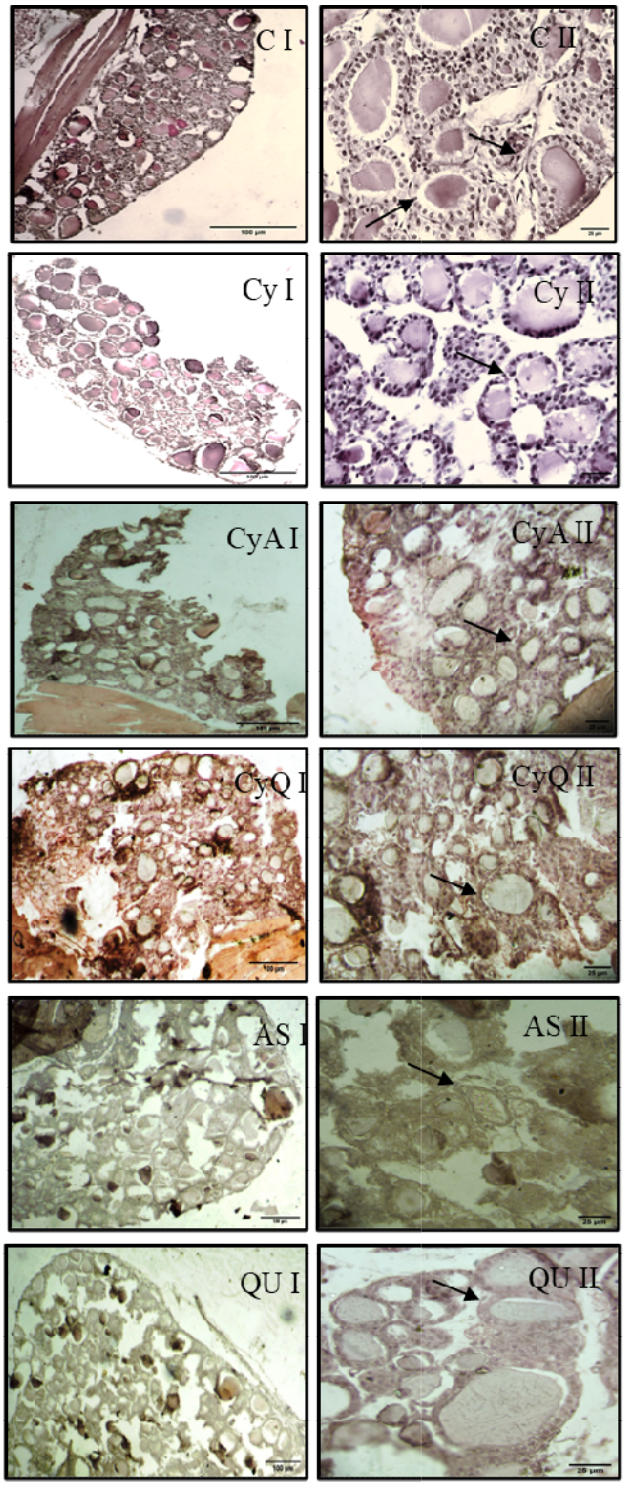


(B)

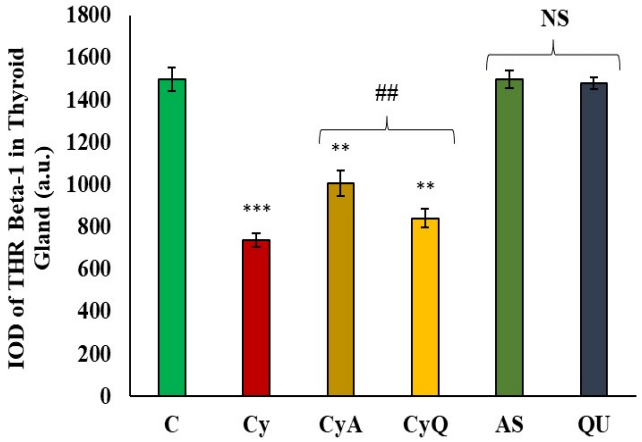
Figure 9 (A & B): The lower and higher magnification (I and II respectively) of double staining showed the damage in thyroid follicles in the cypermethrin-induced hypothyroid group (B)as compared to control (A) and ashwagandha and quercetin co-administration groups (C and D) showed a preventive role in follicular damage**(9A)**. Co-administration of ashwagandha and quercetin (CyA and CyQ) showed the decreased diameter of thyroid follicles in the cypermethrin-induced hypothyroidism mice. The values represent Mean±SE (n=6). **p<0.01 (significance difference between control and hypothyroidism group) *p<0.05 (significance difference between CyA and CyQ versus hypothyroidism group respectively) #p<0.05 (significance difference between CyA versus CyQ)**(9B)**.

2) Immunohistochemistry of Thyroid Gland

The immunohistochemistry of the thyroid gland showed the expression of THRβ-1 in the thyroid gland. The expression ofTHRβ-1 significantly decreased (***p<0.001) in the cypermethrin-induced hypothyroidism group as compared to the control group and significantly increased (**p<0.01) in ashwagandha and quercetin co-administrative groups as compared to the cypermethrin-induced hypothyroidism group**(Fig. 10 A)**.THRβ-1 showed lower integrated optical density (IOD)(***p<0.001) in follicular cells of the cypermethrin-induced hypothyroidism group and higher IOD showed in the ashwagandha (**p<0.01) and quercetin (**p<0.01) co-administrative groups as compared to cypermethrin-induced hypothyroidism group**(Fig. 10 B)**.



(A)



(B)

Figure 10A & B:immuno-histochemical localization of THRβ-1 in the thyroid gland of mice in lower and higher magnification (I and II respectively). **(10A)** cypermethrin-induced hypothyroidism groupshoweddecreased expression of THRβ-1as compared to control (A) and ashwagandha and quercetin co-administration groups (C and D) showed increased expression as compared to the cypermethrin-induced hypothyroidism group. Co-administration of ashwagandha and quercetin (CyA and CyQ) showed the decreased expression of THRβ-1in the cypermethrin-induced hypothyroidism group. The values represent Mean±SE (n=6). ***p<0.001 (significance difference between control and hypothyroidism group) **p<0.01 (significance difference between CyA and CyQ versus hypothyroidism group respectively) ##p<0.01 (significance difference between CyA versus CyQ)**(10B)**.

IV. DISCUSSION

Natural dietary products are rich in antioxidants and possess free radical scavenging activity. Various phytoconstituentshelp in the prevention of damage caused by oxidative stress generated due to exposure to pollutants and carcinogens in daily life. Ashwagandha and quercetin are the antioxidants used for their therapeutic/preventive properties. Cypermethrin is an environmental toxicant contaminating the air, water, and soil.Humans and others are exposed to cypermethrin *via* different routes of administration with a variety of products used containing the same.The endocrine system is responsible for the chemical control and coordination of the body with different hormones. alterations in the endocrine system resulting in altered growth and development. The endocrine system is sensitive to chemical toxicants and pollutants.

The results of the present study are according to previous research works when a survey was conducted on 330 patients with primary hypothyroidism. Researchers found that the decreased thyroid hormones caused the accumulation of fat, salt, and water which increased the body weight. In most cases, hypothyroidism results in obesity (Ríos-Prego et al., 2019). Similarly, when 0.025% methimazole was administered to rats for 22 successive days induced hypothyroidism, caused a gain in body weight under hypothyroidism, and decreased the thyroid gland weight (Herwig et al., 2014).

In the present study, it is found that the 15 mg/kg body weight oral exposure of cypermethrin to mice causes decreased synthesis and secretion of thyroid hormones resulting in hypothyroidism. The induced hypothyroidism causes alterations in the oxidative stress markers and enzymatic and non-enzymatic antioxidant defense resulting in oxidative stress. In the present study it is found that the decreased thyroid hormones, increased thyroid stimulating hormone and decreased free thyroid hormones after exposure to cypermethrin. The results of the present study are according to the results found when rats were administered to 20 mg of cypermethrin for 14 days and 20 mg of lambda-cyhalothrin for 21 days to mice (Yousef et al., 2019; Al-Amoudi, 2018)

The results of the present study showed increased MDA concentration (***$p<0.001$), H_2O_2 activity (**$p<0.01$), and NO activity (**$p<0.01$) which are the markers of induced oxidative stress, and decreased GSH (**$p<0.01$), GST (**$p<0.01$), and GR (***$p<0.001$) activity showed decreased activity of the antioxidant system of the body under hypothyroidism. The results of the present study are according to the results of the previously available research works. It has been previously found that alterations in thyroid hormones or hypothyroidism have been associated with oxidative stress (Resch et al., 2002). The increased MDA concentration in hypothyroidism may be due to altered lipid metabolism in thyroid cells. The increased MDA along with the increased activity of H_2O_2 and NO suggested that in the hypothyroid condition the free radical generation accelerates and caused damage (Torun et al., 2009). MDA plays a reciprocal role against the activity of SOD and catalase, which helps in reducing the levels of generated free radicals. SOD caused suppression of superoxide anions and catalase caused catalytic degradation of harmful hydrogen peroxide into water and both prevent the cellular damage against the induced oxidative stress under hypothyroidism (Baskol et al., 2007). The increased free radical generation caused the decreased activity of the endogenous antioxidant system. GSH, GST, and GR constitute the antioxidant system that prevents oxidative damage. In the present study, the induced hypothyroidism caused decreased activity which results in the accumulation of free radicals in thyroid cells (Mancini et al., 2016). The 0.05% propylthiouracil-induced (PTU) hypothyroidism in adult rats caused significant changes in the oxidative stress markers and declined antioxidant enzymatic activity in testes. The finding supports to the present results in mice thyroid glands under cypermethrin-induced hypothyroidism. In rat testes, MDA concentration and H_2O_2 activity increased signifies oxidative stress, and declined activity of SOD, catalase, and GSH signifies decreased antioxidant enzyme activity. The findings support the present results in the thyroid gland's increased oxidative stress markers and decreased antioxidant enzyme activity found (Choudhury et al., 2003).

In the present study, it is found that cypermethrin-induced hypothyroidism caused changes in the histological structures of the thyroid gland. Under hypothyroidism, the follicular diameter is reduced and the gland becomes underactive. The reduced follicular size caused decreased thyroid hormone synthesis. The results of the present study are supported by the results found when rats were exposed to synthetic pyrethroid similar to cypermethrin, lambda-cyhalothrin caused structural damage to thyroid follicles and caused the reduced size of the thyroid follicles. The reduced size of thyroid follicles may be due to oxidative stress in the thyroid gland (Al-Amoudi, 2018).

The co-administration of ashwagandha and quercetin against hypothyroidism caused oxidative damage and showed significant results and therapeutic potential. The results of the present study showed ashwagandha showed significant therapeutic potential against the damage caused by hypothyroidism in the thyroid gland. From the results of the present study and previous research work, it is found that the methanolic extract of ashwagandha regulated thyroid functions and prevents damage by hypothyroidism in rats. Ashwagandha co-administration prevents structural damage to thyroid follicles under hypothyroidism and restores the follicular diameter (Abdel-Wahhab et al., 2019; Alam et al., 2012). In the present study, results revealed that quercetin co-administration in hypothyroidism prevents oxidative damage caused by hypothyroidism. The results of the present study are similar to the results obtained by other researchers when albino rats were co-administered with a 50mg/kg body weight oral dose of quercetin for 4 weeks in cadmium-induced hypothyroidism showed prevention from hypothyroidism (Badr et al., 2019). Similarly, it is found that quercetin supplementation prevents oxidative damage in rat testes and germ cells in mice. Quercetin supplementation in hypothyroid mice restores the histological integrity of the thyroid gland structure. (Altintas et al., 2015; Bu et al., 2011).

The thyroid hormone receptor β-1 belongs to the nuclear receptor family (Mangelsdorf et al., 1995). They modulate transcription factors by binding to the thyroid hormone receptor element of the target gene (Munoz and Bernal, 1997). The

present immunohistochemistry results showed decreased expression of THRβ-1 in the cypermethrin-induced hypothyroidism group as compared to the control group. The co-administration of ashwagandha and quercetin in hypothyroidism cause increased expression toward normal. The decreased expression in hypothyroidism is possibly due to the decreased affinity of the follicular cells for thyroid hormones and generated oxidative stress and, apoptosis. The present findings are supported by the previous findings that decreased TR isoforms expression in hypothyroidism is responsible for hypofunction of the thyroid gland (De Paul et al., 2008). The previous findings revealed that thyroid hormone receptors have significant functioning in the growth, metabolism, antioxidant capacity, and reproduction of mice (Forrest andVennström, 2000).

CONCLUSION

The results of the present study and the previously available research work on different model with different antioxidants may conclude that exposure to cypermethrin cause alterations in thyroid hormones resulting in hypothyroidism causing the generation of free radicals, oxidative stress, and structural damage in the thyroid gland. The immunohistochemistry results showed that expression of THRβ-1 decreased in thyrocytes of hypothyroid mice. The co-treatment with ashwagandha and quercetin helped in the amelioration of oxidative stress resulting in restoring the structural damage caused under hypothyroidism. Ashwagandha and quercetin also play an important role in amelioration against immuno expression of THRβ-1.

ACKNOWLEDGMENT

We the thankful to the Department of Zoology, School of Biological Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar M.P. for providing the facility during the research works. The author also wants to thank the UGC Non-NET Fellowship for financial support.

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