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Assessment of Oxidative Stress Induced by Nickel Chloride and Antioxidant Effects of Basil (*Ocimum Basilicum* L.) and Thyme (*Thymus vulgaris* L.)

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ABSTRACT

Oxidative stress (OS) mechanisms are speculated to play a significant role in Nickel-induced toxic effects and their carcinogenic potency. Although Nickel-induced oxidative damage in somatic tissues is well demonstrated, evidence of the involvement of a similar mechanism(s) in Nickel-induced testicular dysfunction and associated genotoxic effects is scarce. Hence, the present study aimed to investigate the oxidative stress induced by Nickel chloride (NiCl2) and antioxidant effects of Thyme and Basil in mice testis. The effects of Thyme and Basil on the cytotoxicity and genotoxicity induced by Nickel chloride were examined with respect to the intracellular glutathione in testis, DNA fragmentation, randomly amplified polymorphic DNA (RAPD) technique and sperms abnormality. The obtained data from this study revealed that treatment of mice with NiCl2 (20 mg/kg) for two consecutive days exhibited significantly ($p \leq 0.01$) depleted intracellular glutathione (GSH) levels by 40% below that of control group and led to apoptotic changes in the testis cells as evidenced by DNA fragmentation (24.9% compared to 5.0% in control group) as measured by diphenylamine assay and gel electrophoresis. DNA fingerprint profile showed an increase in the instability of treated mice DNA. Nickel treatment resulted in a significant decrease in sperms count and motility whereas it caused a significant increase in abnormal sperms. The treatment with Nickel and Thyme or Basil oil resulted in a significant improvement in all tested parameters, moreover, Thyme was found to be more effective than Basil.

Key Words: Germ cells, antioxidant, DNA fragmentation, RAPD-PCR, heavy metal

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INTRODUCTION

The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest *(Rice-Evans et al., 1997 and Scalbert and Williamson, 2000)*. Many epidemiological and experimental studies indicate that diets rich in antioxidants may reduce the risk of cancer and mutation *(Ames, 1983, Block, 1991 and Chalchat and Ozcan, 2008)*. There is an increasing interest in herbs and spices as sources of natural antioxidants *(Baratta et al., 1998, Lis-Balchin et al., 1998 and Dorman et al., 2003)*. Especially worthy of note are spices and herbs used for many years to enhance the sensory features of food. Culinary herbs have also been grown and used to flavor foods since antiquity. In most of these herbs, the flavor is provided by the aromatic ingredients in their essential oils and oleoresins. In addition to providing additional taste and flavor to foods, a wide spectrum of beneficial actions of spices, in particular, their antioxidant function has been documented in several previous *in vivo* and *in vitro* studies *(Madsen and Bertelsen, 1995, Lampe 2003, Srinivasan 2005 and Tsai et al., 2007)*. Thus, it may be claimed that adopting a cuisine rich in spices may boost the chemoprotective capacity of fruits and vegetables in one's diet *(Tsai et al., 2007)*. Herbs and spices such as Thyme and Basil possessing high antioxidant activity, would not only be very useful to maintain food freshness, flavor, taste and color, but also to alleviate diseases by preventing oxidative deterioration *(Lee et al., 2002 and 2005)*. Antioxidants can act by:

i. Decreasing localized oxygen concentrations.
ii. Preventing chain initiation by scavenging initiating radicals.
iii. Binding catalysts, such as metal ions, to prevent initiating radical generation.

Basil (*Ocimum basilicum* L.) and Thyme (*Thymus vulgaris* L.) are aromatic herbs that are used extensively to add a distinctive aroma and flavour to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used

as aroma additives in food, pharmaceuticals and cosmetics *(Senatore 1996, Simon et al. 1999 and Javanmardi et al. 2002)*. Basils (Ocimum spp., Lamiaceae) contain a wide range of essential oils rich in phenolic compounds *(Simon et al. 1990 and Phippen and Simon 2000)*. The main phenolic compounds in Basil are rosmarinic acid, lithospermic acid, vanillic acid, coumarinic acid, hydroksibenzoacid, syringic acid, ferulic acid, protocatheuic acid and caffeic acid *(Javanmardi et al. 2002 and Jayasingne et al. 2003)* and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins *(Phippen and Simon 1998 and Chalchat and Ozcan 2008)*. Traditionally, Basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction *(Simon et al. 1999)*.

In Thyme, the main phenolic compounds are glycuronids of apigenin, luteolin, eriodyctiol, luteolin glycosides, rosmarinic acid and quercitine *(Guillen and Manzanos 1998 and Justesen 2000)*. Thyme also possesses various beneficial effects, like antiseptic, carminative, antimicrobial and antioxidative properties *(Baranauskiene et al. 2003)*. Modern industrialization has introduced harmful metals into the environment by redistributing them from immobilized ores and minerals, thereby exposing humans and animals to more metal salts. Among the myriad environmental pollutants, Nickel, a heavy metal, merits special consideration as a potential toxic element *(Das and Dasgupta 2002)*. An impaired antioxidant defense mechanism followed by oxidative stress is the major cause of Nickel-induced toxicity, which can lead to reproductive failure *(Das and Dasgupta 2000)*. Nickel has been classified as a human carcinogen based on epidemiological evidence, which shows high incidence of nasal and lung cancers in refinery workers *(Coogan et al. 1989 and Goyer 1991)* and its potency to induce tumors in a variety of mammalian species *(Sunderman 1987, 1989)*.

Although the toxicity and carcinogenicity of Nickel compounds in humans and experimental animals are well demonstrated, the underlying mechanisms of their action remain unclear *(Sunderman et al. 1985 and Stohs and Bagchi 1995)*. The most plausible mechanism that may be operative *in vivo* is the generation of reactive oxygen species (ROS), which may initiate lipid peroxidation (LPO), oxidative damage to critical macromolecules such as proteins or DNA and cell damage or death. In mammalian cells, induction of DNA single-strand breaks, DNA protein crosslinks, sister chromatid exchanges and chromosomal aberrations has been demonstrated with various Nickel salts *(Kawanishi et al. 1989, Torreilles and Gurein 1990 and Kasprzak 1991)*. Although bioaccumulation of Nickel in testis is well demonstrated, the exact mechanisms of Nickel-induced male reproductive toxic effects are not clear *(Kakela et al. 1999 and Obone et al. 1999)*. Testicular toxicity of Nickel compounds may be related to enhanced production of reactive oxygen species, probably mediated through oxidative damage to macromolecules, including damage to DNA *(Doreswamy et al. 2004)*. Accordingly, in the present study, we investigated the propensity of Nickel to induce oxidative stress response in testis and the associated genotoxic implications *in vivo* as well as the antioxidant effects of Thyme and Basil. Furthermore, Nickel-induced genotoxic effects were ascertained by examining them on caudal sperms (counts and head abnormalities).

MATERIALS AND METHODS

Chemicals:

Thyme and Basil oils were purchased from El-Captain Company (CAP PHARMA), 6th October City, Egypt. Nickel chloride was purchased from El-Gomhouria Co., Cairo, Egypt. All other chemicals were of analytical grade.

Animals:

A total of sixty Swiss albino adult male mice, 10 – 12 weeks old and weighing 25 – 30 g. were used in this study. The animals were obtained from the Department of Animal House Colony of National Research Center. Animals were randomized and housed under ambient room-temperature and relative-humidity conditions, a commercial diet and water were provided ad libitum. All mice were acclimatized for at least one week prior to dosing.

Experimental Design:

Experiments were carried out to evaluate the antioxidant effects of Thyme and Basil against genotoxicity induced by Nickel Chloride using different cytogenetic and molecular analysis. Animals were divided into six equal groups (10 mice/group) and caged separately. Group I (control) mice were maintained on standard diet, while in groups II and III male mice were orally administered Thyme or Basil oil at 0.1 ml /mouse/day for 10 days, group IV were initially administered (intraperitoneally) Nickel chloride (dissolved in distilled water) at 20 mg/kg for two consecutive days and groups V and VI in which mice were orally administered Thyme or Basil oil at 0.1 ml /mouse/day for 10 days and then administered (intraperitoneally) Nickel chloride at 20 mg/kg for two consecutive days. All groups were divided into two subgroups A and B. Animals in subgroup A (within all treatment groups) were used for the determination of glutathione in testis and molecular analysis. In this subgroup, animals were scarified after 24 h from the last

dose and different samples were collected. Whereas, animals in subgroup B were used for the determination of sperm abnormality and sacrificed after 35 days and epididymis were removed for the sperm abnormality study.

Measurement of Intracellular Level of Reduced Glutathione in Testis:

Tissue homogenate contents of reduced glutathione was measured according to the method of *Ellman (1959)*, modified by *Silber et al. (1992)*. Testis tissues (about 0.2g) was homogenized in 600 µl hypotonic lysis buffer and centrifuged for 15 min at 11,000 rpm. The assay is based on the reduction of 5,5- dithiobis-2 nitrobenzoic acid (DTNB) by SH groups of glutathione to form 2-nitro-S-mircaptobenzoic per mole of glutathione. The concentration of GSH was quantified spectrophotometrically at 412 nm using the extinction coefficient of 13.7 mM-1 cm-1.

DNA Fragmentation Assays for Apoptosis:

Apoptotic changes in testis were evaluated calorimetrically by DNA fragmentation and by agarose gel electrophoresis according to the procedure of *Perandones et al. (1993)*. Testis samples were homogenized in 700 µl hypotonic lysis buffer and centrifuged for 15 min at 11,000 rpm. The supernatants (SN) containing small DNA fragments were separated; one-half of the volume was used for gel electrophoresis and the other half, together with the pellet containing large pieces of DNA were used for quantification of fragmented DNA by the Diphenyl amine (DPA) assay. The samples were treated with equal volumes of absolute isopropyl alcohol and NaCl to precipitate DNA. Extracted DNA was electrophoresed on 1% agarose gels containing 0.71 µg/ml ethidium bromide. At the end of the runs, gels were examined using UV transillumination. The Diphenyl amine (DPA) assay reaction was modified by *Perandones et al. (1993)* from *Burton (1956)*. The colorimetric reaction was measured spectrophotometrically at 575 nm. and the percentage of DNA fragmentation was calculated.

DNA Extraction and RAPD Analysis:

The DNA was extracted separately from each of the replications by phenol–chloroform. The integrity of extracted genomic DNA was checked by electrophoresis in 0.8% agarose gels. The RAPD reaction described by *Becerril et al. (1999)* and *Ferrero et al. (1998)* was performed using 5 ng DNA template in a total volume of 25µl. Ten primers previously selected because of their good RAPD profiles and their good in vivo/in vitro correlation were used in this study. Their sequences are, A04: 5-AAT CGG GCT G-3, A05: 5-GGT CCC TGA C-3, A07: 5-GAA ACG GGT G-3, A09: 5-GGG TAA CGC C-3, A19: 5-CAA ACG TCG G-3, A20: 5-GTT GCG ATC C-3, B02: 5-TGA TCC CTGG-3, B03: 5-CAT CCC CCT G-3, B07: 5-GGT GAC GCA G-3 and B09: 5-GGT GAC GCA G-3. The primers were obtained from the Operon Technology. The amplifications were carried out in duplicate and on different days for each of the primers used. For accuracy in the comparison of the results, the controls and the exposed DNA extract were run in the same gel. A negative control reaction without template DNA and a molecular size marker (X174-Hae III EUROBIO, France) were also included in the same gel.

Gel Electrophoresis:

Each sample of RAPD products (10 µl) was mixed with gel loading buffer and loaded onto an agarose gel (1.8%, w/v) for electrophoresis. Amplification products separated by gels were visualized and documented using the Gel Documentation system, Gel-Pro Analyzer (Media Cybernetics).

Epididymis Sperm Abnormality:

The technique of *Wyrobek et al. (1984)* was adopted for sperm abnormality study with minor modifications. Epididymis (free of fats, vas deferens and other tissues) from each side of testis of either control or treated mice was dissected out into 10 ml of 0.87% normal wormed saline separately. The content was thoroughly shaken, filtered through a silken cloth and dropped on grease-free clean slides to determine the movement and swimming ability of sperm (motility) using microscope. Spermatozoa were counted using heamocytometer and a drop of a homogenate smeared on a cleaned slide, allowed to air dry and stained by Eosin Yellow to determine the head and tail abnormalities of sperms.

Statistical Analysis:

All data were statistically analyzed using analysis of variance (ANOVA). The significance of the differences among treatment groups was determined by Waller–Duncan k-ratio *(Waller and Duncan, 1969)*. All statements of significance were based on probability of $P \leq 0.05$.

RESULTS

Effect of Nickel, Thyme and Basil on Testis Glutathione (GSH) Content:

Figure 1, shows the protective effect of both Thyme and Basil against GSH depletion induced by Nickel chloride in mice's testis. Nickel chloride treatment revealed a significant GSH-depletion by 40 % as compared to that of the control group. However, when each of Thyme and Basil were pretreatment at dose of 0.1 ml/mouse for each; significantly improved the depleted testis GSH content by 2.0 and 1.7 folds increase, respectively, as compared to that of Nickel chloride treated group and these effects were reached to the same manner of the control values. Both Thyme and Basil

treated mice showed significant increased in GSH content in testis (11.5± 0.6 and 11.3± 0.4) respectively when compared with the control group (8.3± 0.5).

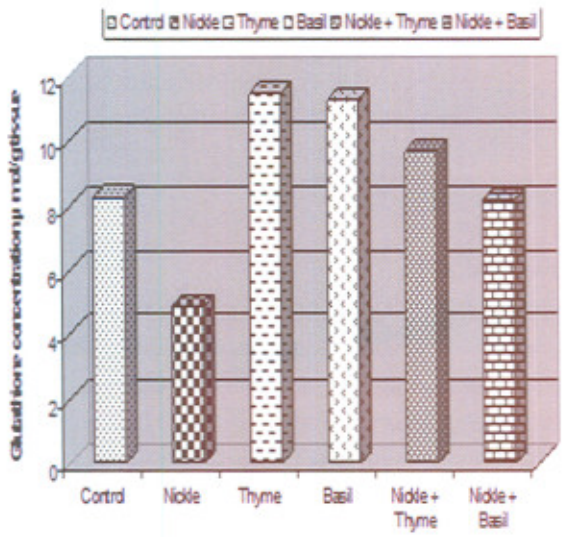

Figure 1: Effect of Nickle, Thyme and Basil on glutamine concentration in testis of mice.

Effect of Nickel, Thyme and Basil on DNA fragmentation: Nickel chloride-induced DNA damage was evaluated by measuring the level of fragmented DNA colorimetrically using Diphenylamine (DPA) and by comparing DNA profiles on agarose gel electrophoresis. The results in (Table 1) showed that NiCl2 caused marked DNA fragmentation in testis (24.9 ± 0.4) compared to control untreated mice (5.0 ±0.3) as indicated by DPA assay. Pretreatment with Thyme and Basil oils significantly decreased NiCl2-induced DNA fragmentation to 12.3% and 14.6 % in testes, respectively.

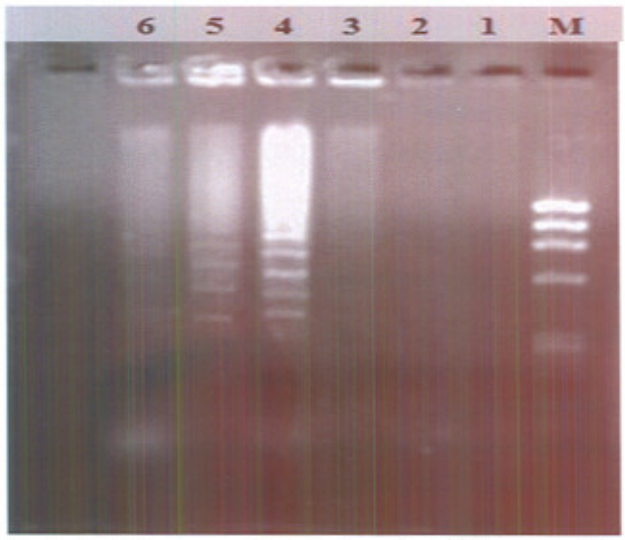

Figure 2: 1.2% agarose gel electrophoresis of DNA extracted from mice testis treated with, Nickel chloride, Thyme and Basil. Lane M: DNA molecular weight marker. Lane 1: control mice. Lane 2: Thyme-treated mice. Lane3: Basil-treated mice. Lane 4: Nickel chloride-treated mice. Lane 5: Nickel chloride with Basil. Lane 6: Nickel chloride with Thyme.

DNA fragmentation in response to NiCl2 treatment was also detected by gel electrophoresis as DNA ladder representing a series of fragments that is multiples of 180–200 bp. (Figure 2). Treatment with Thyme and Basil led to significant protection against NiCl2-induced DNA fragmentation reached to 63 % and 52 % respectively in testis. The results indicated that, Thyme and Basil oil could counteract NiCl2-induced apoptosis in mice.

RAPD Profiling:

Ten random 10-mer primers were used to analyze instability in the genome of mice testis using RAPD-PCR fingerprinting and the presence of changes in the RAPD profiles obtained from the exposed population depending on the primer used. Of the 10 primers, six produced reproducible and scorable amplification fingerprints. One of the six primers (OP A20) produced similar RAPD fingerprints for controls and treatment groups rendering it uninformative in revealing alterations in testis DNA. The remaining five primers detected changes in the RAPD profiles of the testicular DNA compared with untreated controls. A total of 37 loci of different bands were amplified by the six primers, with an average about six loci per primer of the 37 loci that were amplified, 17 bands were polymorphic giving (45.9%) polymorphism, the sizes of bands ranged from 881593-bp. DNA profiles presented in (Figure 3) were generated using three primers and a mixture of four individuals from each replicate. Profiles generated by these primers revealed differences between control and exposed individuals, with visible changes in the number and size of amplified DNA fragments. The most obvious result was the appearance of new bands in DNA samples of the group treated with Nickel chloride alone. The number of new bands was 9 bands (B02-280, B02188-, B09113-, B09166-, B09421-, B09504-, B07-565, B07682- and B07752-) which resulted from the genetic alteration in DNA in Nickel chloride-treated group. Whereas none of these new bands were found in the genomic DNA of the samples collected from the animals of the control group or the animals that received Nickel chloride in combination with either Thyme or Basil.

Incidence of Sperm Abnormalities:

The results of the sperm abnormalities (Tables 2 and 3) revealed that the motility of sperms of the group treated with Nickel chloride showed a significant decrease (P>0.001) compared to those of the control group. The combined treatment with Thyme or Basil resulted in a significant improvement in the sperm motility although the values were still lower than the control group. The sperm count in Nickel-treated group showed a significant decline compared to those of the control group. Whereas, in animals treated with Nickel plus Thyme or Basil showed a complete recovery in

the sperm count and were comparable to the control groups (Table 2). The current results clearly indicated that treatment with either Thyme or Basil alone had no influence to induced sperm abnormality. Animals treated with Nickel chloride showed a significantly increase (23.2 %) in the frequency of abnormal sperm compared to the control group (Figure 4). Treatment with Thyme or Basil in combination with Nickel considerably reduced sperm abnormality frequencies (13.5 and 14.1 %, respectively). In spite of this reduction, the percentage of sperm abnormality was still significantly higher than the control group.

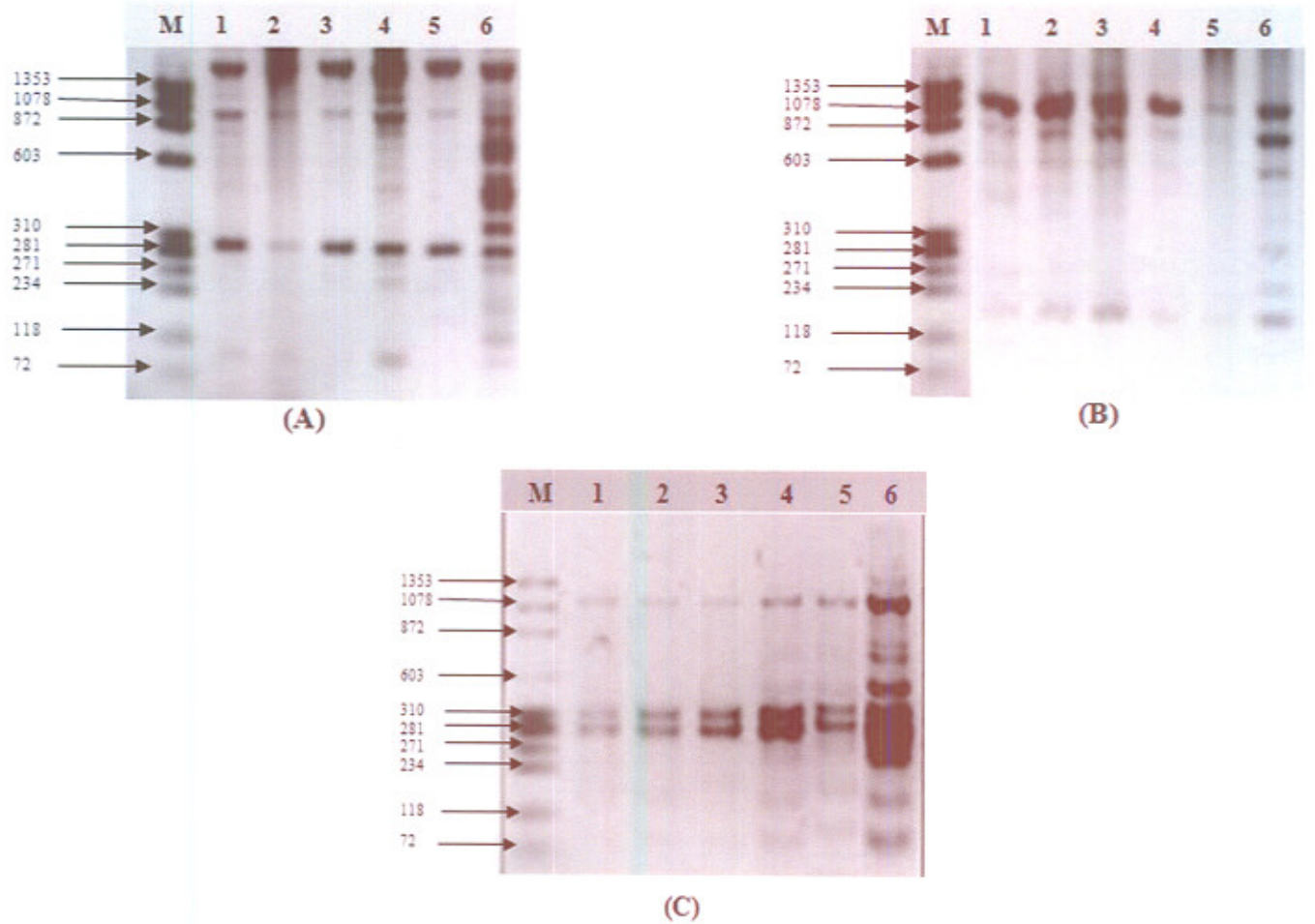

Figure 3: Comparison of RAPD fingerprinting of different testis genomic DNA: (A) represent PCR product with primer B09; (B) represent PCR product with primer B02 and (C) represent PCR product with primer B07. The DNA marker is in lane M, lane 1 represents untreated mice (control), lane 2 represents mice treated with Thyme, lane 3 represents mice treated with Basil, lane 4 represents mice treated with Thyme and Nickel, lane 5 represents mice treated with Basil and Nickel and lane 6 represents mice treated with Nickel chloride.

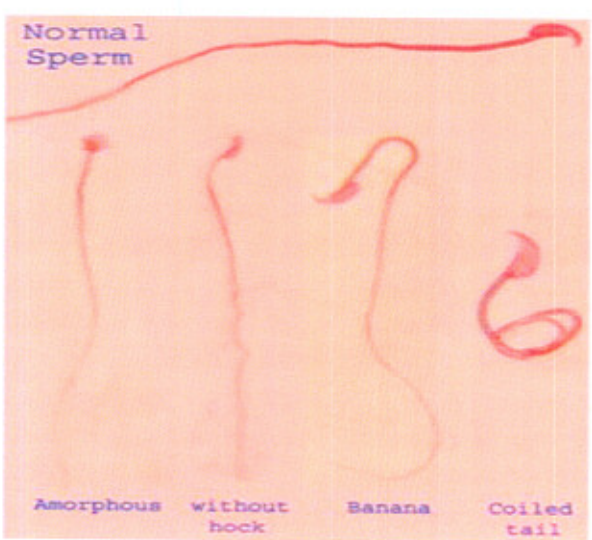

Figure 4: Showing different sperm abnormalities.

Table 1: Effect of Nickel, Thyme and Basil on DNA fragmentation of mice testis.

Treatment	DNA fragmentation %		The change
Control	5.0 ± 0.3	d	0
Nickel Chloride	24.9 ± 0.4	a	+ 19.9
Thyme	4.3 ± 0.5	d	- 0.7
Basil	5.2 ± 0.2	d	+ 0.2
Thyme + Nickel	12.3 ± 0.8	c	+ 7.3
Basil + Nickel	14.6 ± 0.3	b	+ 9.6

Means with different superscripts (a, b, c, d) between groups in the same column are significantly different at P<0.05.

Table 2: Cauda epididymal sperm motility, count and total abnormality percentage.

Groups	Sperm motility %	sperm count (x 10^6)	Total abnormalities (%)
Control	86.3 ± 1.2 a	53.3 ± 36.7 bc	0.6
Nickel	41.3 ± 4.3 d	35.3 ± 34.2 d	23.2
Thyme	84.8 ± 1.2 b	57.3 ± 26.7 ab	0.3
Basil	91.3 ± 1.2 a	63.8 ± 30.2 a	0.5
Nickle + Thyme	78.0 ± 4.1 bc	b57.8 ± 36.8 a	13.5
Nickle + Basil	74.5 ± 3.3 c	46.3 ± 12.7 c	14.1

Means with different superscripts (a, b, c, d) between groups in the same column are significantly different at P<0.05.

Table 3: Effect of different treatments on types of sperm abnormality in mice.

GROUPS	TAIL ABNORMALITIES	HEAD ABNORMALITIES			TOTAL ABNORMALITIES
		Without hock	Banana	Amorphous	
Control	3.6 ± 0.8 c	2.4 ± 0.8 d	1.2 ± 0.5 d	4.8 ± 0.5 d	12.0 ± 1.3 c
Nickel	39.0 ± 3.2 a	62.2 ± 3.5 a	51. 4 ± 2.9 a	72.2 ± 2.8 a	231.8 ± 11.2 a
Thyme	0.8 ± 0.5 c	0.8 ± 0.5 d	1.6 ± 0.8 d	2.0 ± 0.9 d	5.2 ± 1.4 c
Basil	1.6 ± 0.4 c	2.0 ± 0.6 d	2.8 ± 0.5 d	2.8 ± 0.8 d	9.2 ± 1.0 c
Nickle + Thyme	20.8 ± 2.9 b	38.0 ± 3.5 b	28.4 ± 2.2 c	48.0 ± 2.4 c	135.2 ± 7.3 b
Nickle + Basil	25.4 ± 2.2 b	21.8 ± 1.6 c	37.0 ± 3.3 b	56.6 ± 4.2 b	140.8 ± 8.7 b

Means with different superscripts (a, b, c, d) between groups in the same column are significantly different at P<0.05.

DISCUSSION

The present investigation was carried out to explore the possible antioxidant and ameliorative role of Thyme and Basil herbs on Nickel chloride-induced genotoxic effects of testicular DNA (DNA fragmentation and RAPD-profiling), sperm abnormality as well as glutathione content in testis of mice. Epidemiological findings have emphasized a potential relationship between oxidative damage in testis and sperms and testicular dysfunction *(Dawson et al., 1992 and Fraga et al., 1996)*. Currently, it is well appreciated that damage to testicular male germ cells induced by various xenobiotics, products of abnormal metabolism, or ROS can result in testicular dysfunction, leading to infertility *(Aitken, 1994 and 1995, Stohs and Bagchi, 1995, Sikka, 2001 and Doreswamy et al., 2004)*. Although much evidence indicates the involvement of oxidative stress (OS) mechanisms in Nickel-mediated toxic effects in somatic organs *(Sunderman et al., 1985, Athar et al., 1987, Sunderman, 1989, Misra et al., 1990, Stinson et al., 1992 and Chen et al., 1998)*, some studies on the propensity of Nickel to induce OS in testis are scarce *(Das and Dasgupta, 2000 and Doreswamy et al., 2004)*. More importantly, data on the concequences of DNA alteration in testis and their possible genotoxic implications are non-existent.

The present study showed that Nickel chloride induced considerable decrease of testicular glutathione, which protected cells against oxidative stress, maintaining a reducing environment in the cells and inactivating xenobiotic *(Kondo and Iada, 1997)*. Previous studies have also reported that glutathione plays a central role in a wide range of cellular functions, including protection, detoxication, transport and metabolism *(Mizutani and Yoshida, 1994 and Hassan et al., 1999)*. In this regards, *Doreswamy et al. (2004)* observed moderate elevations in the activities of few antioxidant enzymes (glutathione peroxidase, glutathione S-transferase and catalase) in testis, suggesting the induction of OS following administration of sub-lethal multiple doses of Nickel chloride. Glutathione depletion might be due to free radicals and reactive oxygen species generation produced from the interaction of Nickel with DNA *(Athar et al. 1987*

and Doreswamy et al., 2001). Furthermore, Nickel chloride caused marked DNA fragmentation in testis reaching to 25 % which was assessed biochemically (in terms of DNA laddering).

This result was in agreement with *Doreswamy et al. (2004)* who suggested that Nickel induced a significant degree of apoptosis in testis. Excessive ROS increases germ cells apoptosis and inhibits the activity of spermatozoa *(Fujii et al., 2003)*. Sheweita et al. *(2007)* reported that almost 40% of infertile males show abnormally increased ROS levels. Our data are consistent with earlier reports on Nickel chloride–induced DNA strand breaks in rat liver *(Stinson et al., 1992)* and *in vitro* cell models *(Patierno and Costa, 1985 and Kawanishi et al., 1989)*. Although the mechanism of Nickel-induced DNA damage is not clear, it may be caused by the induction of Fenton-generated hydroxyl radicals as suggested earlier in somatic cells *(Misra et al., 1990 and Stinson et al., 1992)*. *Athar et al. (1987)* have hypothesized that Nickel-induced accumulation of iron in hepatic tissue may be directly responsible for the oxidative damage to macromolecules like protein and DNA. Although speculative, similar mechanisms may also be operating in germ cells, since *Doreswamy et al., (2001)* also noted significant increases in iron in the rat testis following Nickel intoxication. A rationale for a closer look at possible generation of oxidative DNA damage by *Nickel* in the testis comes from the strong oxidation-mediating properties of the Nickel complex with protamine P2, a DNA-binding protein abundantly present in the testis and sperm heads *(Liang et al., 1999 and Bal et al., 1997)*. Pro-oxidant and antioxidant balance is vital for normal biological functioning of the cells and tissues *(Fujii et al., 2003)*.

The antioxidant system comprises enzymatic antioxidants such as SOD, GSH-Px and GSH are major enzymes that scavenge harmful ROS in male reproductive organs *(Fujii et al., 2003)*. GSH repairs oxidized and damaged molecules and play a role in regulating a variety of cellular functions. GSH levels were significantly increased in mice pretreated with Thyme or Basil. Both herbs protected testis against GSH depletion induced by Nickel chloride. The ability of both herbs (Thyme and Basil) to maintain testicular GSH homeostasis might be due to activation of the glutathione reductase enzyme as well as their anti-oxidant and free radical scavenging effects *(Lee et al., 2005, Javanmardi et al., 2003, Madsen et al., 1996 and Madsen and Bertelsen, 1995)*. The antioxidant activities of Basil and Thyme have been investigated using various model systems and assays *(Lozien et al., 2007 and Lee et al., 2005)*.

In the present study it was found that Thyme and Basil treatments significantly protected the testicular DNA and sperm quantity and quality. This protective effect of these herbs could be the result of direct free radical scavenger properties *(Lozien et al., 2007, Lee et al., 2005 and Javanmardi et al., 2003)*. Phenolic compound, which are a powerful antioxidants, found in both herbs could also react with membrane phospholipid bilayers to break the chain reaction initiated by ROS *(Lee and Shibamoto, 2001)*. In particular, eugenol, thymol, carvacrol and 4-allylphenol, found in Basil and Thyme, exhibited potent antioxidant activity, comparable to the known antioxidants, BHT and a-tocopherol *(Lee and Shibamoto, 2002)*. Considering the abundance of these aroma chemicals in natural plants, the total activity may be comparable, to those of known antioxidants. Furthermore, ingestion of these aroma compounds may help to prevent in vivo oxidative damage, such as lipid peroxidation, which is associated with cancer, premature aging, atherosclerosis and diabetes *(Lee et al., 2005 and Verma and Nair, 2001)*. Further more *Stajkovi et al. (2007)* clearly demonstrate anti-mutagenic potential of Basil derivatives *in vitro* while *Dasgupta et al. (2004)* investigation has demonstrated clearly that Basil leaf can be used as a potential cancer chemo-preventive agent by virtue of its efficacy in inducing drug detoxification enzymes such as GST and DTD, as well as in blocking carcinogen-activating phase I enzymes *(Henderson et al., 2000)*. In the present study Thyme pretreatment was shown more effective than Basil. Thyme was shown to have the highest amount of phenolics whereas Basil had three times lower concentration of phenolics compounds *(Kruma et al., 2008)*.

CONCLUSION

The current results revealed that Nickel chloride induced a stressful effects on the testis functions including, depletion in glutathione content, increased in DNA fragmentation and instability, decrease in sperm motility and counts and increase sperm abnormality. Both Thyme and Basil oil proved to have protective effects. Moreover, Thyme pretreatment was more effective than Basil as Thyme have more content of polyphenole compound than Basil. Generally the protective role of both herbs can be attributed to the antioxidant effect of herbs by acting as free radical scavenger. The improvement of the activities of antioxidant systems might be one of the results of the free radical scavenging effect of antioxidant herbs. The detailed mechanisms are worthy of further investigation.

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