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Protective efficacy of Spinacia Oleracea extract against sodium arsenite induced oxidative stress and imbalance in the level of thyroid hormones of Albino rats

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ABSTRACT- Arsenic metal is omnipresent in the environment in both organic and inorganic forms. It is a potent endocrine disruptor because the oxidative stress caused by it creates alternations in the level of hormones. The aim of present study was to investigate protective role of spinacia oleracea (spinach) extract to against oxidative stress induced by sodium arsenite ($AsNaO_2$) in the thyroid of albino rats. In this study twenty albino rats were randomly divided into four groups. Group I kept was kept as control. Group II rats were administered a single oral dose of 10 mg/kg body weight of sodium arsenite on day 1 and left for 30 days. Group III rats were given an oral dose of 50 mg/kg body weight of spinach extract and kept as positive control group. Group IV was administered a single oral dose of 10 mg/kg body weight of sodium arsenite on day 1 and 50 mg/kg body weight of spinach extract for next 30 days. Sodium arsenite treatment generated oxidative stress and declined the level of natural antioxidants i.e. CAT, SOD, GPx and altered the concentration of the thyroid hormones (T3 & T4). But after spinach extract supplementation the level of arsenic induced lipid peroxidation decreased significantly and level of natural antioxidants CAT, SOD, GPx was also restored. This shows that spinach extract acts as a powerful natural antioxidant and aids in recovery of arsenic induced thyrotoxicity in albino rats.

Keywords - Sodium arsenite, spinach, thyroid hormones, oxidative stress, antioxidants

I. INTRODUCTION:

Arsenic has been classified as a class I human carcinogen by International Agency for Research on Cancer (IARC). It is a nonessential trace element and a silent toxicant (Zhou Q 2018). Drinking water contaminated with arsenic possess serious global holocaust. Major routes of exposure to arsenic in living organisms are an oral inhalation and through dermal contact (Kulshrestha et al. 2014).

Among heavy metals arsenic is the 33rd element and in the periodic table it is categorized in Group 15. Arsenic is commonly referred to as 'King of poisons' and 'poison of kings' (Hughes et al. 2011). Its compounds lacks smell, taste, colour and odour. The presence of arsenic in food, water and air cannot be immediately predicted. Thus, it creates serious health hazards to living organisms (Mandal and Suzuki, 2002).

National Research Counsil (2001) declared that drinking water with arsenic contamination is more toxic than the dietary sources of arsenic. Because its bioavalibility from water is greater than the vegetables or grains. Anthropogenic activities such as manufacture of glass, wood preservatives, pesticides, semiconductors, herbicides are major exposure sources of arsenic to living organisms (Akter et al. 2005).

The toxicity of arsenic depends upon its oxidation state because inorganic arsenic in its trivalent state is more toxic than the pentavalent state (P.B. Tchounwou et al. 2003). The amount of dose, duration and route of exposure are some important parameters which predicted the extent of its toxicity in the living system (Abernathy et al. 1999). Arsenic (As) even can replace 'phosphate group' from its original position and can alter pathway of many biochemical processes (M.F. Hughes 2002) It is also reported that due to arsenic induced cellular toxicity over 200 enzymes gets inactivated (R.A. Goyer 1996).

Among endocrine glands, thyroid is one of the most important gland which synthesizes `two important metabolic hormones i.e. tri-iodothyronine (T3) and thyroxine (T4). These hormones affect the functioning of every cell in the body by controlling the metabolic rate (Canaris GJ 2000). Arsenic (As) acts as an endocrine disruptor because it disrupts retinoic acid receptor and

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thyroid hormone receptor-mediated gene regulation. Hence it directly inhibits the synthesis of thyroid hormones (Jennifer C. Davey et al. 2008).

Recently medical professionals and scientists have shown keen interest in phytochemical protective agents as these plants based remedies are loaded with tremendous health benefits. These products/extracts are cheaper and safe without any side effects as compared to the synthetic drugs. Ayurveda recommended 'Spinach' as an excellent nutraceutical because it contains all essesstial natural antioxidants (Sah et al. 2017).

Spinach (Spinacia Oleracea) belongs to family "Amaranthaceae" which includes perennial herbs that's why spinach is available throughout the year (Nikam et al. 2016). Among the list of world's healthiest vegetables, spinach ranked at the top because it not only contains vitamins and minerals but is also a rich source of health promoting phytonutrients such as carotenoids (beta-carotene, lutein, zeaxanthin) and flavonoid and hence spinach is reffered as 'Power food' (Segheloo AE et al. 2014 and Tehseem et al. 2014).

Spinach is truly among the one of the natures most perfect foods. Besides its nutritional value, it has also been credited with many biological activities like it acts as anthelmentic, virus inhibitor, hepatoprotective, antioxidant and reducing risk of multiple cancers (Adam G et al. 2008). Spinach has been given the designation of 'Poor man's vegetable' because it is cost effective. It has been shown to possess a broad spectrum of biological and pharmacological properties. It shows anti-inflammatory, anti-mutagenic, chemo-protective as well as anti-neoplastic effects (Yadav et al. 2013).

II. MATERIALS AND METHODS:

Precurement of animals: Albino rats weighing 100-150 grams were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. They were kept and acclimatized to the laboratory conditions for 15 days under optimal condition of light and temperature. They had ad libitium acess to tap water and the standard rat feed in form of pellets purchased from M/S Aashirwad industries, Ltd., Chandigarh. The animals were handled with humane care in accordance with the guidelines of the Institutional Animal Ethical Committee.

Source of chemicals: Sodium arsenite ($NaAsO_2$) was bought from Himedia, Mumbai. It was dissolved in double glass distilled water and administered orally.

Preparation of Spinach extract (SE): Spinach extract (SE) was made by the method of Islam et al. (2009).

Experimental Design: Twenty albino rats were randomly divided into four groups (n=5).

Group I (Control): Rats were kept as control.

Group II (As): Rats were administered a single oral dose of 10 mg/kg bw of sodium arsenite (AsO_2) and left for 30 days.

Group III (SE): Rats were given an oral dose of 50 mg/kg body weight of spinach extract (SE) for 30 days.

Group IV (As+SE): Rats were given an oral dose of sodium arsenite (10 mg/kg bw of sodium arsenite) on day 1 and then spinach extract (50 mg/kg bw of spinach) was administered till 30 days.

Autopsies were done after 30 days of treatment.

Blood sample collection: On the day of autopsy blood was collected from rats by cardiac puncture under anesthesia. The blood samples were collected in vacutainer blood collection tubes and centrifuged at 3000 rpm for 10 mins to obtain the serum.

Hormonal analysis: The serum level of thyroxin (T4), triiodothyronine (T3) and thyroid- stimulating hormones (TSH) were measured with commercially available radio-immunoassay kit (RIA) according to the manufactures instructions.

Biochemical studies: Thyroid gland homogenates were prepared with the help of tissue homogenizer in 3 ml of phosphate buffer and the lipid peroxidation (MDA), catalase (CAT), superoxide dismutase (SOD) and gluthathione peroxidase (GP_X) were estimated by the methods of Wilbur et al. (1949), Aebi (1984), Das et al. (2000) and Rotruck et al. (1949) respectively.

Statistical analysis:

The data was analysed by using student's t –test using graphpad software and verified by one way ANOVA test. The data was stastistically analyzed and presented in the table as mean ± SD.

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III. RESULTS AND DISCUSSIONS:

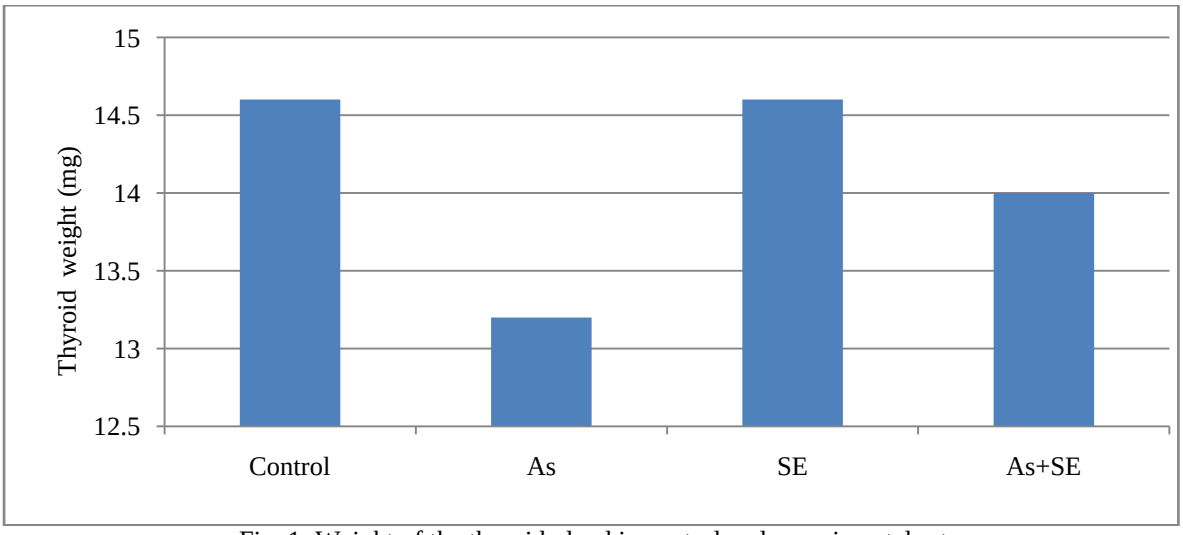

Fig. 1: Weight of the thyroid gland in control and experimental rats.

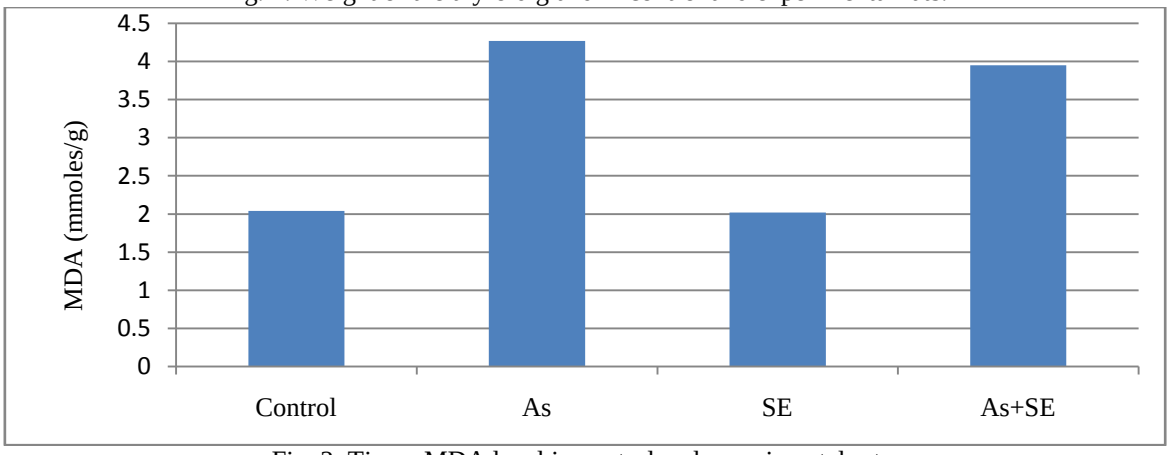

Fig. 2: Tissue MDA level in control and experimental rats.

Fig. 3: Tissue CAT concentration in control and experimental rats.

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Fig. 4: Tissue superoxide dismutase (SOD) concentration in experimental and control rats.

Fig. 5: Tissue glutathione peroxidase (GPx) concentration in control and experimental rats.

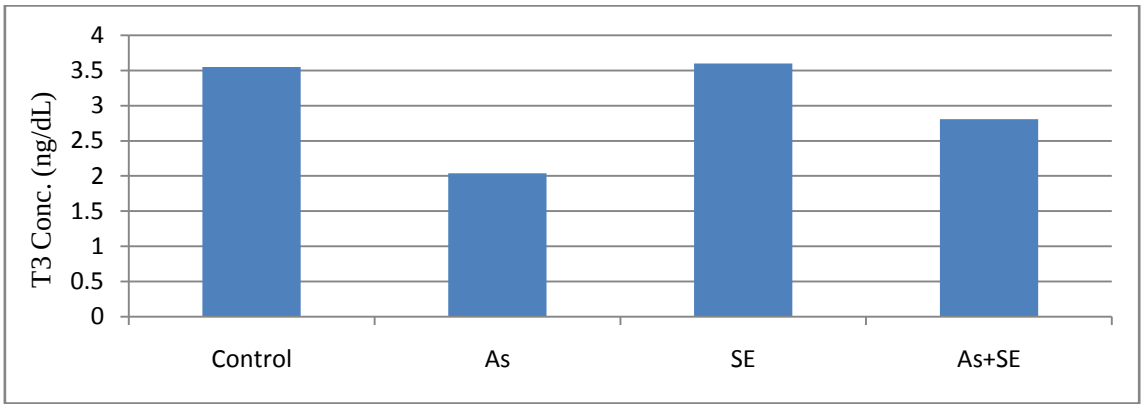

Fig.3: T3 concentration in control and experimental rats.

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Fig. 4: T4 concentration in control and experimental rats

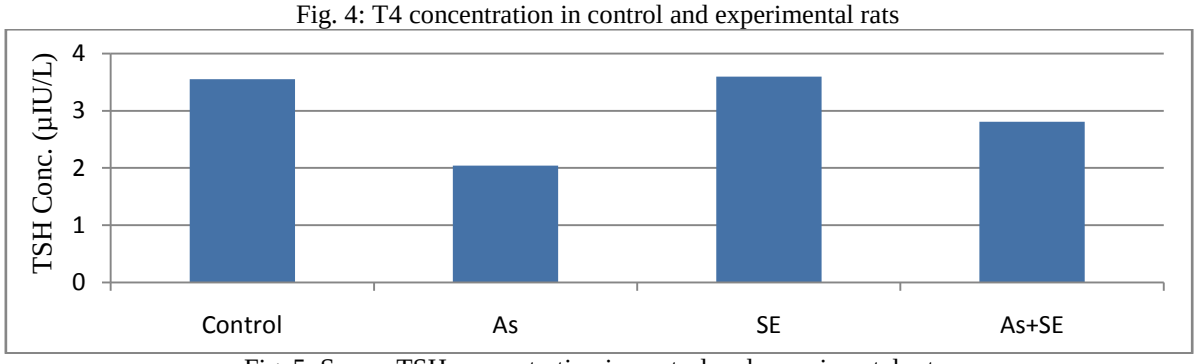

Fig. 5: Serum TSH concentration in control and experimental rats.

IV. DISCUSSION

Drinking water is the major source of arsenic exposure to the living organisms. Some other sources of arsenic exposure are man-made activities i.e. non ferrous rock mining, smelting, coal combustion, pesticide application, wood combustion. All these anthropogenic activities release arsenic into land or soil in the form of solid wastes (Roy et al. 2014). Acute or chronic exposure of As can lead to oxidative stress in the various organs. 'Oxidative stress' is basically the disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defence mechanism (Vivek Kumar, 2006).

Plants-derived antioxidants are a large group of natural products with reducing or radical scavenging capacity. They clean up the free radicals within the cells and prevents the oxidation of cellular content. Lomnitski et al. (2003) reported that spinach is rich source of natural antioxidant which have numerous health benefits. These plant based antioxidants acts as chemo-protective, anti-cancerous, anti-oxidative and anti-aging. The purpose of the present study was to find out the protective efficacy of spinach extract (SE) against sodium arsenite induced oxidative stress in thyroid gland of albino rats.

Thyroid is a butterfly shaped gland located at the base of the neck in front of the trachea. It secretes tri-iodothyronine (T3) and thyronine (T4) which travel through the blood stream and controls metabolic rate, development and growth. Unnikrishnan et al. (2011) reported that thyroid disorders is one the most common health problem globally. The functioning of thyroid gland may be modulated by natural (present in water soil) or synthetic compounds (Sabra & Di Cristofano, 2019). Davey J.C (2007) reported that arsenic is first metal which is classified as "Endocrine disruptor" because it has tendency to alter the enzyme regulation.

In the present study arsenic (As) exposed rats experienced 'Hypothyroidism' which was evidenced biochemically by significant decrease in serum T3 and T4 level and significant rise in TSH level as compared to control group. Thyroid gland itself is an organ of 'oxidative nature' because huge amount of reactive oxygen species, especially hydrogen peroxide (H_2O_2) are produced in the thyroid under physiological conditions (Peepre et al. 2014). Many studies have shown that after arsenic exposure, the production of reactive oxygen species became much higher than its normal limit, which may be the principal cause of the low level of T3 and T4 hormones in hypothyroidic rats. Ahangarpour et al. 2017 observed increase in level of TSH, leptin, ROS, MDA which clearly shows

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that oxidative stress inhibits the synthesis and release of thyroid hormones.

Arsenic directly interferes in production and functioning of thyroid hormones because after arsenic exposure the blood and serum level of selenium (Se) decreases. The conversion of thyroxine (T4) into tri-iodothyronine (T3) depends upon the selenium containing enzyme 'deiodinase'. Thus arsenic intoxificated thyroid hormones remain in their inactivated form showing that arsenic induced toxicity interferes in the functioning of thyroid hormones (Gailer J 2009, George CM et al. 2013, Meltzer et al. 2002).

Thyroid peroxidase (TPO) or iodide peroxidase is an enzyme normally found in follicular cells of thyroid gland it controls two major processes (i) organification of iodine and (ii) it also participates in coupling of two iodothyrosyl residues in thyroglobulin for formation of thyroid hormones (Jagminder et al. 2016).

The results of present study are in accordance to the study of Dominic et al. (2015) which reported that arsenic exposure directly inhibits the activity of thyroid peroxidase (TPO) enzyme because it binds to the sulfhydryl group of TPO and stops formation of thyroid hormones being the major reason behind arsenic induce 'Hypothyriodism'.

Hultbery et al. (2001) also reported that arsenic creates oxidative stress as it binds with sulfhydryl group of various proteins and enzymes. It also causes hindrance in metabolism of glutathione (GSH) which is a super-antioxidant present in the living beings. In the present study, a significant elevation in MDA concentration and significant reduction in the activities of CAT, SOD and GPx were observed in arsenic treated rats as in compare to control rats.

Oxidative stress is basically a disturbance in the balance between the production of reactive oxygen species (free radicals) and anti-oxidant defence mechanisms (antioxidants enzymes). The cells under the oxidative stress unable to cope with existing reactive oxygen species (ROS). The power of the cellular antioxidants i.e. catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) decreased to fight with these free radicals (Ali et al. 2017). In present study the level of all above mentioned antioxidants decreased which may be one of the possible reasons behind arsenic caused cellular damage.

The phytochemicals present in green leafy vegetables are the rich source of antioxidants because they reduce the risk of chronic disorders by protecting against free radical damage and detoxify the cells (Patra et al. 2001, Santra et al. 2000). Among green leafy vegetables, Spinacia Oleracea (Spinach) is the most beneficial vegetable because it possesses many health benefits. It controls normal blood pressure, improves bone health, maintains good digestive system, lowers the risk of asthma and acts as excellent antioxidant (Yadav et al. 2013).

Thyroid hormone replacement therapy or iodine supplementation are the most common way to treat the thyroid problems but the ideal herbal therapy is safe way to cure thyroid diseases. In our experiment after, spinach extract (SE) supplementation a significant improvement in level of T3 and T4 hormones was observed whereas the level of thyroid stimulating hormone (TSH), malondialdehyde (MDA) declined which shows that spinach extract has ability to reverse the toxic effects of arsenic to some extent. The reduction in malondialdehyde (MDA) level shows that after treatment with natural antioxidants present in spinach the process of lipid peroxidation slowed down which significantly protected the thyroid gland from oxidative damage and maintained the normal formation and secretion of thyroid hormones. The results of the present study are in agreement with the results of previous study which reported that micronutrients present in spinach act as free radicals scavengers and reduce the arsenic induced oxidative stress in lungs, liver and kidneys of rats (Islam et al. 2009).

The results of present study are also supported by Umar et al. 2007 who reported that hexane extract of spinach acts as potent antioxidant and reduces the arsenic induced lipid peroxidation in various organs of rat. It also significantly decreased the accumulation of arsenic in spleen, liver, kidney, lungs, intestine and skin of albino rat.

Similarly, the results of present study are in agreement with previous studies, which reported that spinach leaves act as super-antioxidants and have ability to remove the reactive oxygen species (ROS) generated due to toxicity caused by methotrexate in liver of albino rats. It also improves the level of master antioxidant 'glutathione' by balancing the level of oxidants/antioxidants (Farah et al. 2012). The work of Janella et al. (2019) also supports the results of the present study which reported that the flavonoids and carotenoids present in spinach improves liver health and shows hepatoprotective role against acetamenophen induced liver toxicity.

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V. CONCLUSION

The present study was undertaken to evaluate the efficacy of spinach extract against sodium arsenite induced oxidative stress in thyroid gland. Our results show that arsenic administration generates oxidative stress in thyroid gland which was clearly assessed by the high level of malondialdehyde (MDA). Thyroid antioxidant defence system fails to combat this oxidative stress and the level of T3 and T4 are significantly reduced. But spinach extract supplementation improves the synthesis and secretion of thyroid hormone. It also boosts up the level of antioxidants: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

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