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To Study the Neuroprotective Effect of Thyroxine (T_4) in Alcohol Induced Peripheral Neuropathy (PN) of an Experimental Animal

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Abstract

Introduction: Peripheral neuropathy (PN) is defined as derangement in structure and function of peripheral motor, sensory and autonomic neurons. Thyroid hormone or hormone derivatives have experimental promise for neuroprotective and neuroregeneration effects. *Methods:* In the current experimental study the animals were divided into different groups and peripheral neuropathy (PN) was induced in rats by administration of ethanol 10 g/kg twice a day p.o, for 10 weeks. Induction of PN was assessed by measuring thermal & cold nociception, motor co-ordination, locomotor activity, mechanical nociceptive threshold, sciatic nerve conduction velocity, anti-oxidant activity, biochemical and liver function test. *Results:* Alcohol induced PN rats showed impaired thermal, cold hyperalgesia, motor co-ordination, loco-motor activity, mechanical nociception threshold, decreased nerve conduction velocity, anti-oxidant and Nitric oxide (NO) level. Administration of ethanol to normal rats brought about significant changes in histology of sciatic nerve. It characterized by demyelination and degeneration of nerve fiber density. Alcoholic treatment also causes significant changes in normal liver function. It brought about increase in SGOT & SGPT level. Treatment group rats were administered with two different doses of Thyroxine (T_4) 0.5 & 1.0 mg/kg s.c thrice weekly for 10 weeks. Treatment with T_4 shows significant improved in motor co-ordination, locomotor activity, mechanical, hot and cold nociception threshold, nerve conduction velocity, anti-oxidant and NO level. T_4 has also showed remyelination, regeneration of nerve fiber density and significant decrease in SGOT &SGPT level. *Conclusion:* The Thyroxine (T_4) effectively ameliorated many of the behavioral, biochemical, electrophysiological and histological manifestations of alcohol induced PN by its neuroprotectve effect.

Key words: Peripheral neuropathy; Alcohol; Thyroxine; Behavioral study; Electrophysiology; Histopathology.

1. INTRODUCTION

Neuropathic pain is a chronic pain condition accompanied by significant pathological changes in the nervous system. It is defined as "pain initiated or caused by a primary lesion or dysfunction in the nervous system, either central nervous system or peripheral nervous system". The two main symptoms of neuropathic pain are hyperalgesia, an increased response to normally painful stimuli and allodynia, a painful response to a usually non-noxious stimulus. Recent studies indicate that 2-3% of the population in the world suffer from neuropathic pain. As the onset of neuropathic pain may be delayed after nerve injury, pain may still be present after healing is complete. This makes proper diagnosis and early treatment difficult [1].

Peripheral neuropathy (PN) is defined as derangement in structure and function of peripheral motor, sensory and autonomic neurons. Chronic alcoholism and diabetes mellitus are the most common etiologies of PN but the primary worldwide cause of PN is leprosy. Other common causes of PN include genetic origin, metabolic disorders, infection and traumatic, inflammatory, ischemic, toxic or drug induced (Iatrogenic) insults. Due to the diverse origin of the pathology, PN exhibit different clinical forms: acute, chronic, demyelinating or axonal and *symmetrical* (metabolic-diabetes, pellagra; toxic-ethanol, Isoniazid; immune mediated) or *asymmetrical* (nerve compression, neuroma, plexus neuropathies, etc.).

Alcohol is the third most globally accepted addictive substance after nicotine and caffeine. Alcohol abuse has many long-term effects which can result in early death and increases incidences for serious illness. Chronic alcohol consumption produces painful PN for which there is no reliable successful therapy, mainly due to lack of understanding of its pathobiology. Long term excessive drinking of alcohol causes nerve damage and is characterized by spontaneous burning pain, hyperalgesia and allodynia. The mechanism behind alcoholic neuropathy is not well understood, but several explanations have been proposed, these include activation of spinal cord microglia after chronic alcohol consumption, oxidative stress leading to free radical damage to nerves, activation of -mGlu5 receptors in the spinal cord and activation of the sympathoadrenal and hypothalamo-pituitary-adrenal (HPA) axis [2].

Current treatment options for PN include antidepressants, anticonvulsants, tramadol, and capsaicin. These agents are modestly effective for symptomatic relief, but they do not affect the underlying pathology nor do they slow progression of the disease. Currently, no effective treatment is available to prevent or treat. Henceforth, alternative treatments are being researched upon.

The principal thyroid hormones, triiodothyronine (T_3) and T_4 have non-genomic and genomic actions that are relevant to repair of certain features of the pathophysiology of brain damage. Thyroid hormone coincidentally improved nerve conduction, impulse transmission and transduction of sound signals from the cochlea to the brainstem in the auditory system. Thus, Thyroid hormone or hormone derivatives have

experimental promise as neuroprotective and neuroregeneration effects [3]. Hence the present study was under taken to study the neuroprotective effect of T_4 as an alternative to prevent and maintenance of peripheral neuropathic pain.

2. MATERIAL AND METHODS

2.1. Experimental animals

Healthy Wistar rats, weighing about 160-200g at the start of the experiment were used as experimental animals in the present study. The selected animals were divided to four groups: group I: normal control, group II: positive control received only ethanol, group III: positive control + T_4 (0.5 mg/kg s.c thrice weekly) and group IV: positive control + T_4 (1mg/kg s.c thrice weekly) for 10 weeks. The use of animals in these experiments was authorized by IAEC (Institutional Animal Ethical Committee). Throughout the experiment rats were conducted in accordance with the CPCSEA guidelines.

2.2. Study design

The absolute alcohol of 100% was diluted to 35% using distilled water. The diluted 35% of alcohol was given (10g/kg, p.o. twice a day for 10 weeks) to the selected rats. The T_4 of 0.5mg/kg and 1.0mg/kg was given thrice a week s.c to the rats for 10 weeks. At the end of the study body weight, non-invasive nerve conduction velocity [4] and behavioural studies [5,6,7,8] were measured and blood was withdrawn from retro orbital sinus under light anaesthesia for the measurement of liver function tests. Rats were sacrificed and sciatic nerve and liver was isolated for the estimation of antioxidant properties and histopathology [9-13].

2.3. Statistical analysis

All data were expressed as mean ± SEM and analysed with one way analysis of variance between the groups and followed by Dunnett's Multiple Comparison Test were used to assess differences between the groups. Probability values ***$p<0.001$ were considered significant.

3. RESULTS

3.1. *Body weight (%)*

The % change of body weight of normal rats was found to be 19.322 ± 1.057 %. Body weight of ethanol treated rats was found to be -7.672 ± 1.949 % which was found to be significantly lower than that of normal rats. Body weight of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 10.427 ± 1.821 & 7.404 ± 0.803 % respectively which was higher compared that of ethanol treated rats (Figure 1).

3.2. Behavioural studies

3.2.1. *Motor co-ordination(Rota rod)*

Alcohol induced peripheral neuropathy impaired motor co-ordination as evaluated by the walking time on a rotating rod and the number of falls. Fall of time of normal rats was found to be 4.863 ± 0.018 min. fall of time of ethanol treated rats was found to be 1.120 ± 0.333 min which was compared lower than normal rats. Fall of time of rats treated with T_4 0.5mg/kg and 1.0 mg/kg was found to be 2.823 ± 0.237 and 3.985 ± 0.151 min which was higher compared that of ethanol treated rats (Figure 2).

3.2.2. *Hot Hyperalgesia*

Tail flick latency in hot water was found to be 18.000 ± 0.621 sec. A significant decrease in latency in hot water was produced in ethanol treated rats 8.778 ± 0.676 sec. There is significant increase latency in hot water in T_4 0.5mg/kg and 1.0mg/kg treated rats was found to be 11.333 ± 0.558 & 14.389 ± 0.599 sec. respectively compared with ethanol treated rats (Figure 3).

3.2.3. *Cold Hyperalgesia*

Tail flick latency in cold water was found to be 18.944 ± 0.434 sec. A significant decrease in latency in cold water was produced in ethanol treated rats 11.333 ± 0.344 sec. There is significant increase latency in cold water in T_4 0.5mg/kg and 1.0mg/kg treated rats was found to be 13.778 ± 0.562 and 17.889 ± 0.467 sec respectively compared with ethanol treated rats (Figure 4).

3.2.4. *Locomotion activity (Actophotometer)*

Locomotor activity of normal rats was found to be 259.133 ± 7.113 counts in 10 min. A significant decrease in locomotor activity was produced in ethanol treated rats 210.7 ± 6.9614counts in 10 min. There is significant increase locomotor activity in T_4 0.5mg/kg and 1.0mg/kg treated rats was found to be 236.467 ± 8.569 and 259 ± 5.592counts in 10 minrespectively compared with ethanol treated rats (Figure 5).

3.2.5. *Randollsellito*

The mean baseline paw-withdrawal threshold of normal rats was found to be 194.611 ± 3.673 g. A significant decrease in mechanical nociceptive threshold was produced in ethanol treated rats 107.667 ± 2.616 g. There is significant increase of paw-withdrawal threshold in T_4 0.5mg/kg and 1.0mg/kg treated rats was found to be 167.333 ± 2.327and 182.167 ± 3.994g respectively compared with ethanol treated rats (Figure 6).

3.2.6. *Von Frey*

The mean baseline paw-withdrawal threshold of normal rats was found to be 8.527 ± 0.149 g. A significant decrease in mechanical nociceptive threshold (i.e. hyperalgesia) was produced in ethanol treated rats 5.602 ± 0.204 g. There is significant increase of paw-withdrawal threshold in T_4 0.5mg/kg and 1.0mg/kg treated rats was found to be 6.796 ± 0.204 and 7.317 ± 0.238 g respectively compared with ethanol treated rats (Figure 7).

3.3. Electrophysiological study

3.3.1. *Sciatic nerve conduction velocity*

Nerve conduction velocity of normal rats was found to be 44.000 ± 1.826 m/s. Nerve conduction of ethanol treated rats was found to be 22.500 ± 0.992 m/s which was found to be significantly lower than that of normal rats. Nerve conduction velocity of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 29.500 ± 1.945 & 32.333 ± 2.486 m/s respectively which was higher compared that of ethanol treated rats (Figure 8).

3.4. ***Biochemical parameters***

3.4.1. *Triglycerides*(TG):

TG level of normal rat was found to 1.283 ± 0.032mmol / L. TG level of ethanol treated rats was found to be1.820 ±

0.055mmol / L which was significantly higher than that of normal rats. TG level of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 1.399 ± 0.043 & 1.269 ± 0.053 mmol / L respectively which was lower compared that of ethanol treated rats (Figure 9).

3.4.2. *Reduced glutathione in liver*

GSH level in liver of normal rats was found to be 335.301 ± 11.625µg /mg of protein. GSH level of ethanol treated rats was found to be 171.034 ± 26.444µg /mg of proteinwhich was significantly lower than that of normal rats. GSH level of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 268.404 ± 14.923 &316.950 ± 15.071µg /mg of protein of proteinrespectively which was significantly higher compared that of ethanol treated rats (Figure 10).

3.4.3. *Reduced glutathione in sciatic nerve*

GSH level in sciatic nerve of normal rats was found to be 70.851 ± 2.216µg /mg of protein. GSH level of ethanol treated rats was found to be 25.564 ± 1.109µg /mg of protein which was significantly lower than that of normal rats. GSH level of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 31.787 ± 1.154 & 35.619 ± 1.650µg /mg of protein of protein respectively which was significantly higher compared that of ethanol treated rats (Figure 11).

3.4.4. *Catalase activity in liver*

Catalase activity in liver of normal rats was found to be 78.719 ± 4.632 µ mol/min/mg of protein. Catalase activity of ethanol treated rats was found to be 38.0.61 ± 2.359 µ mol/min/mg of protein which was significantly lower than that of normal rats. Catalase activity of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 54.574 ± 0.476 & 65.741 ±0.992 µ mol/min/mg of proteinrespectively which was significantly higher compared that of ethanol treated rats (Figure 12).

3.4.5. *Catalase activity in sciatic nerve*

Catalase activity in sciatic nerve of normal rats was found to be 65.385 ± 1.266 µ mol/min/mg of protein. Catalase activity of ethanol treated rats was found to be 24.895 ± 2.442 µ mol/min/mg of protein which was significantly lower than that of normal rats. catalase activity of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 35.741 ± 0.792&45.741 ± 0.992 µ mol/min/mg of protein respectively which was significantly higher compared that of ethanol treated rats (Figure 13).

3.4.6. Nitric oxide (NO) level in sciatic nerve

Nitric oxide level of normal rat was found to 25.847 ± 1.341µg/ml. NO level of ethanol treated rats was found to be 66.893 ± 2.870µg/ml which was significantly higher than that of normal rats. NO level of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 53.291 ± 1.270 & 42.415 ± 2.762 µg/ml respectively which was lower compared that of ethanol treated rats (Figure 14).

3.5. Liver function test

3.5.1. *SGOT*

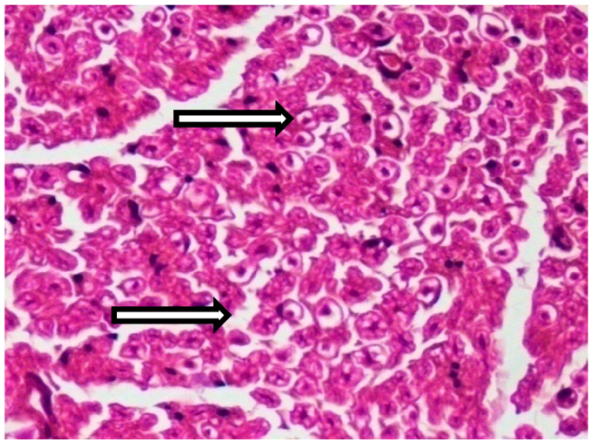
SGOT level of normal rat was found to be 2233.197 ± 103.368nKat/L. SGOT level of ethanol treated rats was found to be 3847 ± 345.470nKat/L which was significantly higher than that of normal rats. SGOT level of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 2944.755 ± 216.493 & 2511 ± 115.673 nKat/L respectively which was lower compared that of ethanol treated rats (Table 1).

3.5.2. *SGPT*

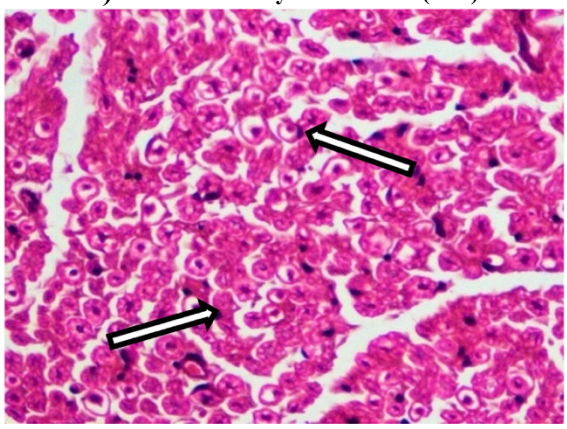
SGPT level of normal rat was found to be 956.525 ± 33.143nKat/L. SGPT level of ethanol treated rats was found to be 1526.277 ± 133.681nKat/L which was significantly higher than that of normal rats. SGPT level of rats treated with T_4 0.5mg/kg and T_4 1.0 mg /kg was found to be 1039.069 ± 77.084 & 1111.889 ± 31.855nKat/L respectively which was lower compared that of ethanol treated rats (Table 2).

3.6. Histopathology of sciatic nerve

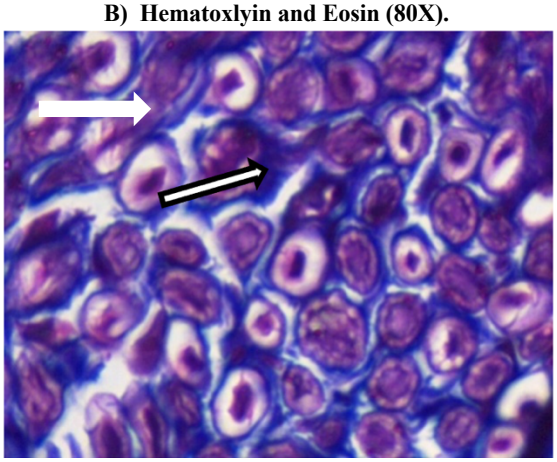
3.6.1. Group: 01 (Normal control)



A) Hematoxlyin and Eosin (80X).



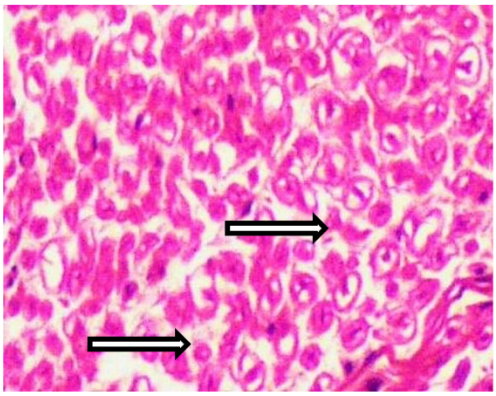
B) Hematoxlyin and Eosin (80X).



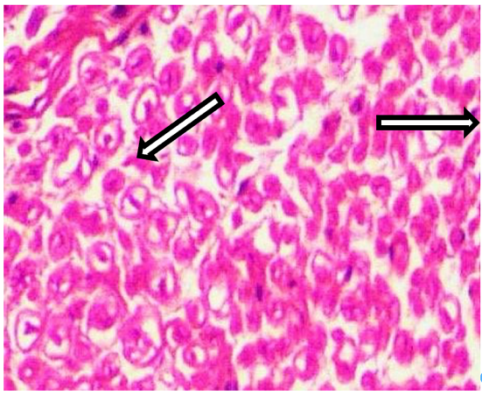
C) Masson'strichrome (160X).

Figure 15. Histology of rat sciatic nerve of normal control group: (A) light microscopytransverse section showing closely packed nerve fibers and an occasional endoneurial blood vessel. (B)light microscopytransverse section showing individual nerve fibers and a central axon surrounded by a sheath of myelin.(C) transverse section of special stain for collagen highlights the endoneurial matrix separating the nerve fibers and collagenous component is stained blue.

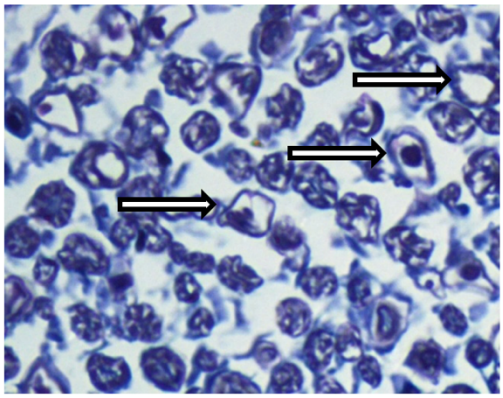
3.6.2. Group : 02 (Positive control)



D) Hematoxlyin and Eosin 80X



E) Hematoxlyin and Eosin 80X



F) Masson'strichrome 160X

Figure 16. Histology of rat sciatic nerve of positive control group: (D) light microscopyof transverse section showing small nerve funicles with reduced fiber density, (E)scattered fibers with axonal swelling and degeneration. (F) Transverse section of special stain for collagen highlights axonal degeneration with dilated axons.

Table 1. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on SGOT level in ethanol treated rats.

SI. No.	Group	nKat/L
1.	Normal control (Vehicle)	2233.197*** ± 103.368
2.	Positive control (PC)	3847 ± 345.470
3.	PC + T_4 0.5 mg/kg	2944.755* ± 216.493
4.	PC + T_4 1.0 mg/kg	2511*** ± 115.673

Values are represented as mean ± SEM (n=6). *** P<0.001 Vs PC,*p< 0.05 Vs PC group by using one Way ANOVA followed by Dunnett's multiple comparisons.

Table 2. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on SGPT level in ethanol treated rats.

SI. No.	Group	nKat/L
1.	Normal control (Vehicle)	956.525*** ± 33.143
2.	Positive control (PC)	1526.277 ± 133.681
3.	PC + T_4 0.5 mg/kg	1039.069** ± 77.084
4.	PC + T_4 1.0 mg/kg	1111.889** ± 31.855

Values are represented as mean ± SEM (n=6). *** P<0.001 Vs PC, **P< 0.01 Vs PC group by using one Way ANOVA followed byDunnett's multiple comparisons.

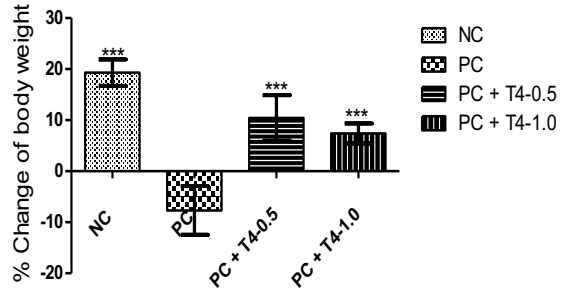


Figure 1. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on % body weight change in ethanol treated rats.

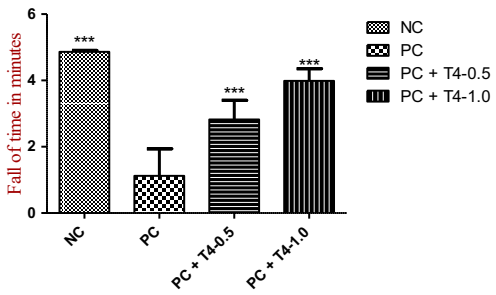


Figure 2. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on motor co-ordination in ethanol treated rats.

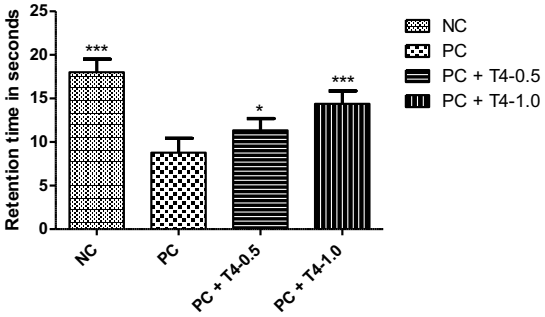


Figure 3. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on thermal hyperalgesiain ethanol treated rats.

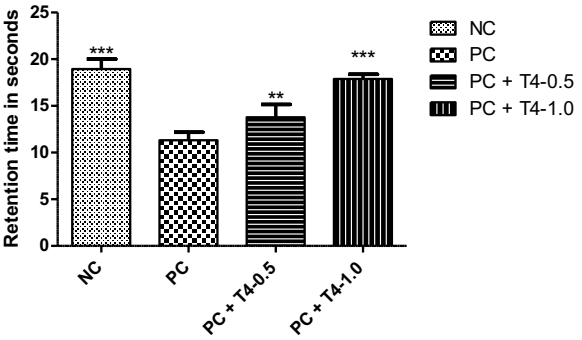


Figure 4. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on cold hyperalgesia in ethanol treated rats.

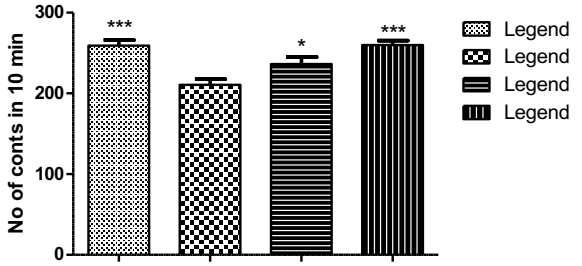


Figure 5. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on locomotor activity in ethanol treated rats.

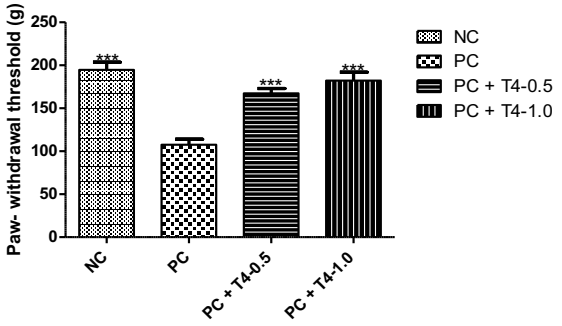


Figure 6. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on mechanical nociceptive thresholdsin ethanol treated rats.

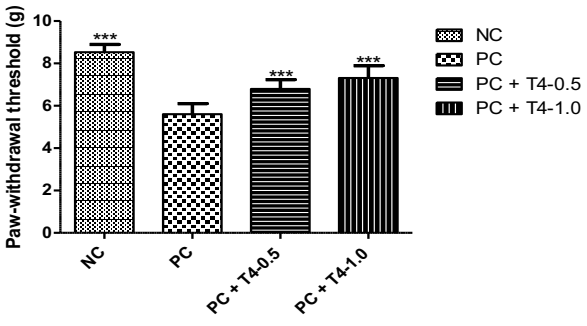


Figure 7. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on mechanical nociceptive threshold (mechanical hyperalgesia) in ethanol treated rats.

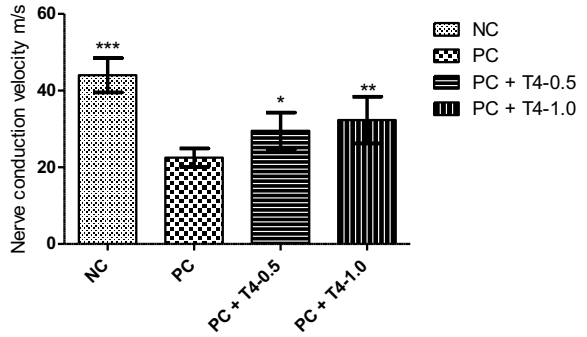


Figure 8. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on nerve conduction velocity in ethanol treated rats

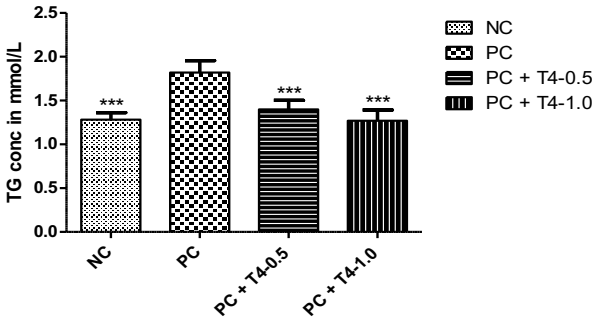


Figure 9. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on TG level in ethanol treated rats.

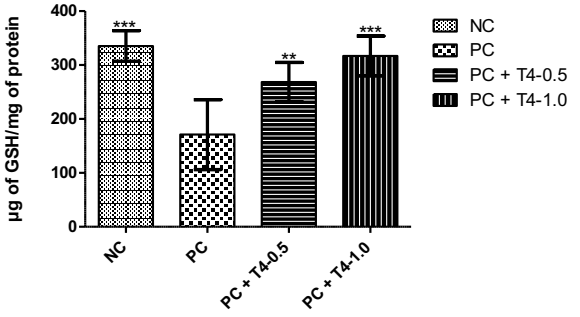


Figure 10. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on GSH level in liver of ethanol treated rats.

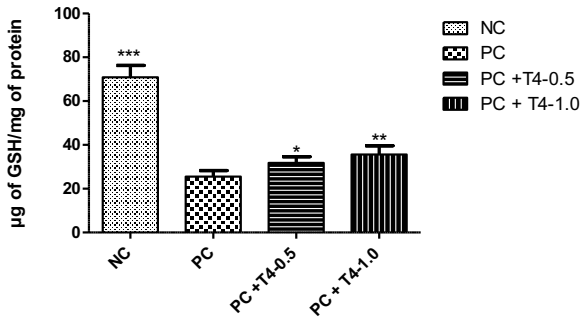


Figure 11. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on GSH level in sciatic nerve of ethanol treated rats.

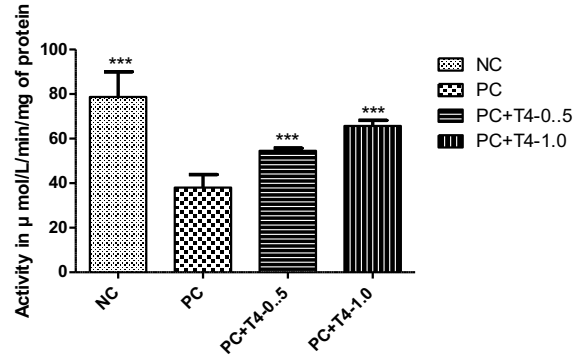


Figure 12. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on catalase activity in liver of ethanol treated rats.

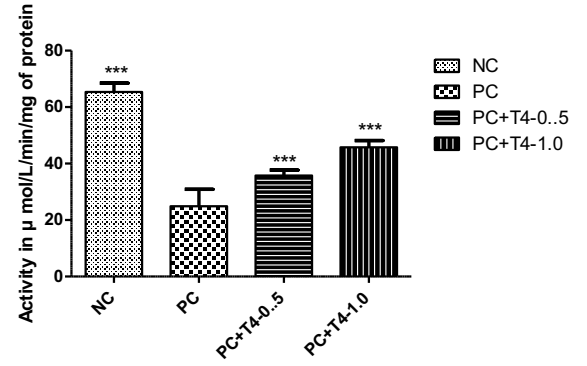


Figure 13. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on catalase activity in sciatic nerve of ethanol treated rats.

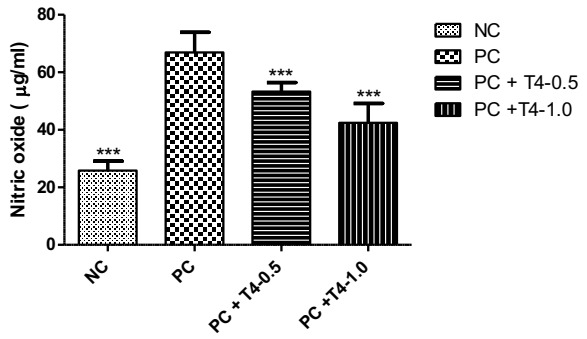


Figure 14. Effect of treatment of two different dose of T_4 (0.5mg/kg, 1.0mg/kg) on NO level in nerve homogenate of ethanol treated rats.

4. DISCUSSION

The neurologic effects of ethanol consumption are complex, affecting on both the central and peripheral nervous system. In the peripheral nervous system, it produces a small-fiber dying back painful neuropathy [14]. In recent years, ethanol consumption in Asian countries has increased with an associated rise in the rates of alcohol-related health problems. Thus, understanding the basis of clinical expression of ethanol-induced neuropathy is an issue of growing importance, and adequate treatment of this symptom may require therapeutic strategies [15].

Rats chronically fed ethanol exhibited mechanical and thermal hyperalgesia and tactile allodynia all of which are symptoms frequently occurring in patients with painful PN. Ethanol-induced hyperalgesia became evident at 6 weeks and kept on progressively increasing till the end of the study, i.e., 10 weeks [16, 10]. These results are consistent with the previous reports demonstrating neuropathic-pain-like state in the rats following chronic alcohol consumption. It is reported PKC and PKA are both known to be important in nociceptor function and in mediating other effects of alcohol.PKC was found to contribute to the enhanced nociception. This secondary messenger dependence differs from that for the enhanced nociception produced by hyperalgesic inflammatory mediators, to which both PKA and PKC contribute. It is reported that MEK/ERK signaling in inflammatory pain in ethanol induced PN [10]. It is also reported that 5 weeks ethanol treatment resulted in significantly decreased mechanical nociceptive threshold along with microglial activation in the spinal cord of rats and also reported that increased concentration of metabotropic glutamate receptor in chronic ethanol treated rats that leads to nociceptive processing, inflammatory pain and hyperalgesia.

In this study administration of ethanol (10g/kg, p.o twice a day for 10 weeks) in rats exhibited decreased % of body weight, thermal& cold hyperalgesia, decreased motor co-ordination, locomotor activity, reduced nerve conduction velocity and decreased anti-oxidant, NO levels all of which are symptoms frequently occurring in patients with PN. All these data suggested that PN was successfully induced in rats and this model can be used for the screening of potential neuroprotective drugs for the treatment of alcohol induced PN.

In the present study treatment with two different concentrations of T_4(0.5 & 1mg/kg s.c thrice weekly for 10 weeks) significantly alleviated alcoholic induced PN in rats. Rats treated with T_4 exhibited less decrease in % of body weight, decreased thermal & cold hyperalgesia, improved motor co-ordination, locomotor activity, nerve conduction velocity and improved the antioxidant and NO level compared with ethanol treated rats.

T_4 treatment showed improved behavioral, electrophysiological, biochemical, liver function test and histological features in ethanol treated rats. However, T_4 has mild protective effect on the fiber loss induced by ethanol. Studies published on T_4 so far indicated that thyroid hormone has pluripotent roles of regulations on various system like immune response, these studies of influences of exogenous T_4in human and animals on

immune system have yielded conflicting results. Thyroid hormones are critically involved in cell growth and development, neuronal and glial cell differentiation [3, 17,4, 18, 19]. All these mechanisms may preventing the neuronal damage occurred by the ethanol. Administration of T_4in protective study shown evidence of prevented inflammation, axonal swelling, unremarkable epineurium, vessels were normal in morphology and presence of regenerating clusters compare to ethanol treated rats. T_4treated rats exhibited reversed fiber loss as it was evidenced by presence of regenerating clusters. The beneficial effect of T_4 could be due to its anti-oxidant and probably by anti-apoptotic activity [20, 21].

5. CONCLUSION

The present study involved the evaluation of neuroprotective activity of two different concentration of T_4 in alcohol induced PN in rats by assessing behavioural, electrophy- siological, biochemical, liver function testand histopathology.From this we conclude that T_4 improved motor co-ordination, mechanical nociceptive threshold, hyperalgesia, nerve conduction velocity, locomotor activity,antioxidant activity, nitric oxide level and also improved liver function activity. Two different concentrationof T_4 have shown neuroprotective effect in alcohol induced PN in rats. T_4 1.0 mg/kg exhibited better significant effect compared to T_4 0.5 mg/kg.

Authors

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Declaration of interest

The authors do not have any conflict of interest between them to declare

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