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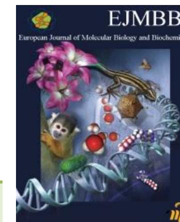

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MODULATORY EFFECT OF LOTUS (*Nelumbo nucifera*) IN ALCOHOL INDUCED LIVER INJURY IN ALBINO RATS

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ABSTRACT

Liver injury and cirrhosis are related to prolonged alcohol consumption. Acute ethanol administration causes fatty liver and hyper-trigylceridemia. The objective of the present study is to elucidate the hepatoprotective and antioxidant properties of *N. nucifera* (bwtp.o) against 30% alcohol intoxication in albino rats. Biochemical protocols were performed in liver tissue homogenates for TBARS, SOD, Catalase GSH, Vitamin C and E, iron, copper, cytochrome P450 and Na^+/K^+/ATPase. The results showed a significant reduction in SOD, Catalase and GSH of alcohol intoxicated groups. Ratsfed with crude powder of *N. nucifera* (1gm/Kg bwtp.o) for 21 days as a treatment period significantly increased the enzymes compared to the control groups. The results suggest that *N. nucifera* exhibit most significant ($p < 0.001$) hepatoprotective and superior antioxidant activity.

INTRODUCTION

Liver disease in many countries is a leading cause of death. Liver injury and cirrhosis are related to prolonged alcohol consumption. Acute ethanol administration causes fatty liver and hyper-trigylceridemia. Unfortunately, most of the corticosteroids and immunosuppressive agents used in the management of liver diseases are intolerable and sometimes cause harmful side effects. This in turn diverts the minds of the people to focus on the use of medicinal plants in therapeutic care. Most of the scientific investigations on animal and clinical studies support the use of herbal medicines in the treatment of liver ailments [1–6]. *N. nucifera* (Nymphaeaceae) provides an effective contribution in Indian medical systems like Ayurveda and Siddha for treatment of liver diseases. *N. nucifera* is an aquatic herb commonly found in large lakes and temple ponds in all over India. The leaves, roots, seeds, flowers and fruits have medicinal values and several parts as raw materials to produce cosmetics [7].

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The seeds of lotus decrease blood lipids induced by a high-fat diet in rats [8]. It also inhibits platelet aggregation [9]; anti fertility effect [10] and free radical scavenger [11]. The lotus rhizome showed the isolation of active fraction of amino acid tryptophan that reduced the blood glucose level [12] and CNS - depressant effect [13] in hyperglycemic mice. Stalk extracts of *N.nucifera* showed antipyretic effect [14]. The lotus plumuleis a potent antioxidant [15] and it is recognized as a cooling food with anti-inflammatory activities [16]. The leaves are used in the treatment of bleeding disorders [17]. The embryo of *N. nucifera* extract increases the heart rate and contraction [18]. The flower extract of *N.nucifera* possess hypoglycemic and hypolipidemic activities [19].

Considering the diverse medicinal properties of *N. nucifera*, the present study was undertaken to evaluate the hepatoprotective and antioxidant effect of *N. nucifera* in alcohol induced liver injury in experimental albino rats.

MATERIALS AND METHODS

Plant material

The fresh flowers of *N. nucifera* were collected from Thanjavur district of Tamil Nadu, India. The flowers were washed with distilled water to remove dirt and soil. The petals of the flowers were carefully collected; shade

dried and kept in an air-dried oven at 45^{0}C for 48 hours. Then the air-dried flower petals was milled into powder, sealed in a plastic bottle, and stored at – 20^{0}C until use.

Selection of animals

In this experiment twenty four healthy male albino wistar rats, 3 months of age, weighing 150g – 190g were selected for acclimation for a period of two weeks in laboratory animal house and maintained under standard conditions of temperature 27±2^{0}C, relative humidity of 60± 5% and 12:12 hour light : dark cycle prior to experimentation. The animals were fed with standard pellet diet and the food was withdrawn 24 hours before the experiment, water was allowed ad libitum. All the experiments were performed in the morning hours. The chemicals used were of the analytical grade purchased from SD's fine chemicals Ltd, Mumbai, India and the standard Orogastric cannula was used for oral drug administration.

Experiment protocol

The experimental animals were divided into four groups (G1, G2, G3, and G4) each contains six animals. G1 control rats received physiological saline and free asses to standard feed and water for 40 days. G2, G3 and G4 rats were intoxicated with 30% ethyl alcohol (1ml/kg bwtp.o) once in a day for 40 days. At the end of chronic exposure to alcohol, G3 animals were treated with aqueous suspension of *N. nucifera* (1g/Kg b wtp.o) suspended in physiological saline were administered intragastrically twice a day for 21 days. G4 rats were treated with standard Silymarin at a dose (0.1g/Kg bwt) twice a day for 21 days. The experiments were performed as per the guidelines outlined in the Institutional Ethical Committee Clearance Protocols entitled "Principles of Laboratory Animal Care".

Biochemical assays

At the end of the drug treatment period all the animals were anesthetized by light chloroform and blood samples were collected from dorsal aorta by heparinized syringe in a vacutainer tubes. Plasma and serum samples were separated by centrifugation at 3000g for 20 minutes and were preserved at –20^{0}C for biochemical assays.

The animals were sacrificed by cervical decapitation, the perfused liver of each animal was dissected out and washed with isotonic solution, and their wet weight was recorded. The liver homogenate was prepared by using phosphate buffer solution. The homogenate was centrifuged and the supernatant was collected for biochemical analysis. The parameters analyzed from serum, plasma and liver homogenate was represented in following table 1.

Histopathological studies

The liver was immediately excised after autopsy and tissues were fixed in 10% formalin solution for a period of 24 hours. These tissues were processed and embedded in paraffin wax and cut into five-micrometer thick sections using a rotatory microtome. The sections were stained with haemotoxylin – eosin for microscopic observations of liver histology in control, alcohol intoxicated and drug treated rats.

Statistical analysis

All the results are expressed as mean ± SD. Statistical significance was compared between the treatment and control groups by Student's t – test. Results with $p < 0.001$ were considered statistically significant.

RESULTS

Alcohol induced hepatotoxicity

The chronic administration of alcohol in rats causes severe necrosis and liver cirrhosis observed by significant increase in the level of lipid peroxides. The condition was reversed and maintained to normal levels in rats treated with aqueous suspension of *N. nucifera* ($P < 0.001$). The variations of TBARS in all the experimental groups are presented in the figure 1.

The induction of alcohol decreased the levels of reduced GSH, SOD, Catalase, Vitamin E, Vitamin C and membrane Na^+/K^+/ ATPase. All the listed enzymes and vitamins were reverted by *N. nucifera* ($P < 0.001$) by increasing their activity and maintained near to control groups. The analytical results of these antioxidant enzymes and vitamins were presented in the figures 2 – 6.

The increased activities of iron, copper, and Cytochrome P450 in alcohol toxicity were significantly ($P < 0.001$) reduced and minimized to normal levels in *N. nucifera* treated groups. The results are shown in the figures 8 and 9.

Histopathology

Histopathological observations support the results of enzyme assays. Figure 10 reveals the normal micro section of the liver with intact liver cell.

Figures 11a and 11b showed the liver of alcohol intoxicated rats with massive fatty changes, gross necrosis and broad infiltration of lymphocytes and kupffer cells around the central vein and loss of cellular boundaries in low and high power views.

The histological pattern of the liver of rats treated with the crude powder (subsequently given alcohol) of *N. nucifera* (Figure 12) showed more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the normal and Silymarin treated groups.

The figure 13 shows the histological pattern of the liver of experimental animals treated with standard Silymarin. The results confirmed the hepatoprotective and antioxidant nature of *N.nucifera* compared with standard and silymarin treated groups.

Table 1. Biochemical parameters analyzed in liver homogenate, plasma and serum

Biochemical parameters	References
Thiobarbituric acid reactive substances (TBARS)	(20, 21)
Reduced glutathione (GSH)	(22)
Superoxide dismutase (SOD)	(23, 24)
Catalase (CAT)	(25)
Vitamin E	(26)
Plasma Ascorbic acid (Vitamin C)	(27, 28)
Iron (Serum)	(29)
Copper (Serum)	(29)
Cytochrome P450	(30)
Na^+/K^+/ATPase	(31)

Fig 1. Effect of *N. nucifera* in TBARS level

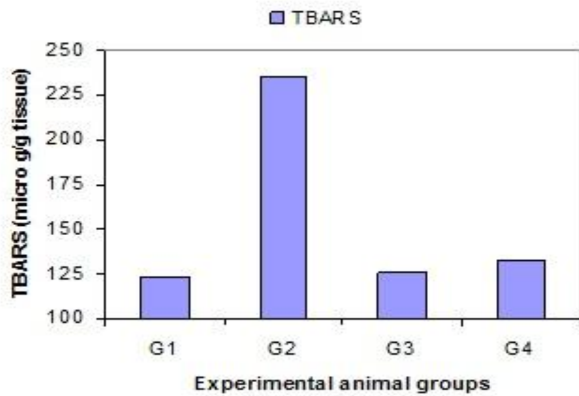


Fig 2. Effect of *N. nucifera* in GSH level in plasma and liver

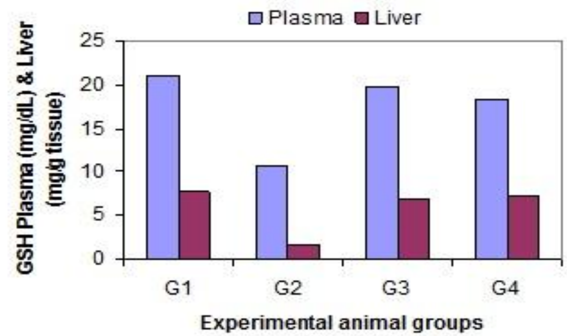


Fig 3. Effect of *N. nucifera* in SOD level

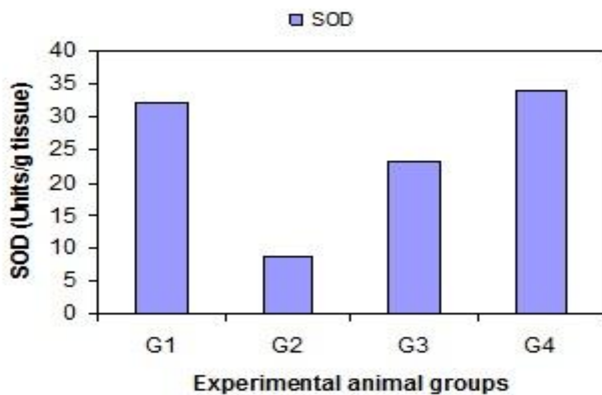


Fig 4. Effect of *N. nucifera* in CAT level

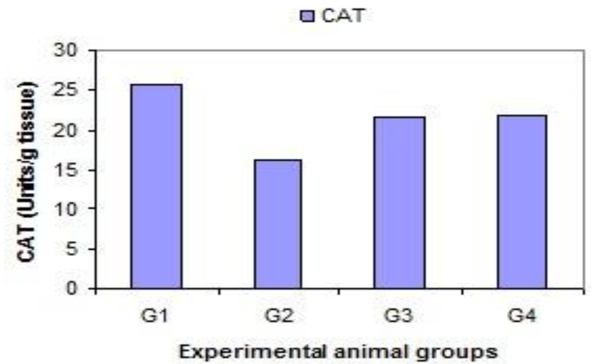


Fig 5. Effect of *N. nucifera* in vitamin E in plasma and liver

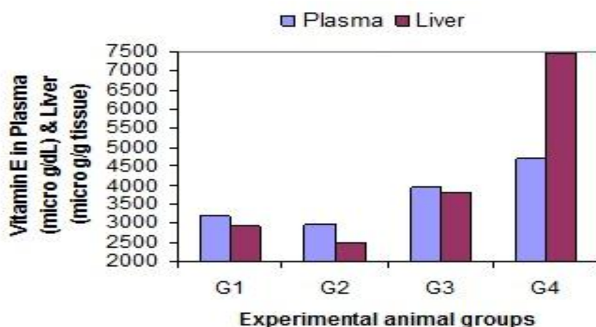


Fig 6. Effect of *N. nucifera* in vitamin C level in plasma

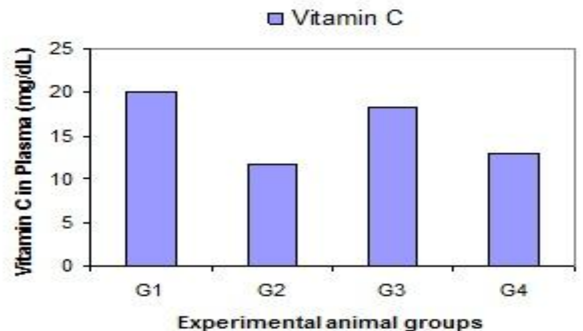


Fig 7. Effect of *N. nucifera* in Na+/K+/ATPase level in liver

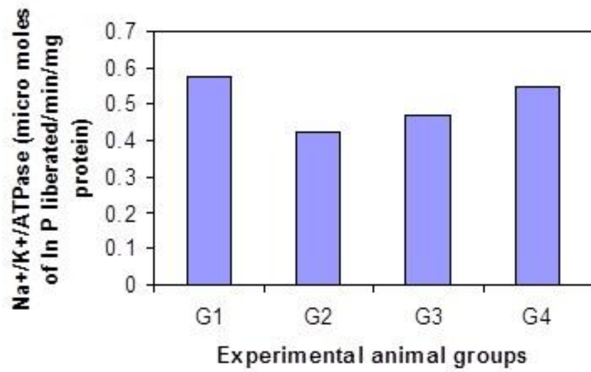


Fig 8. Effect of *N. nucifera* in serum Fe and Cu level.

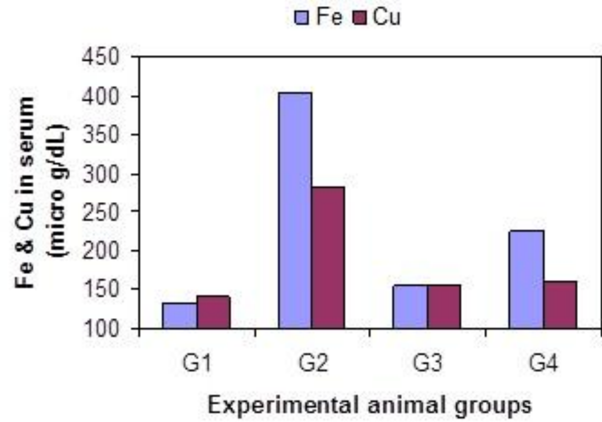


Fig. 9. Effect of *N. nucifera* in Cytochrome P450 in liver

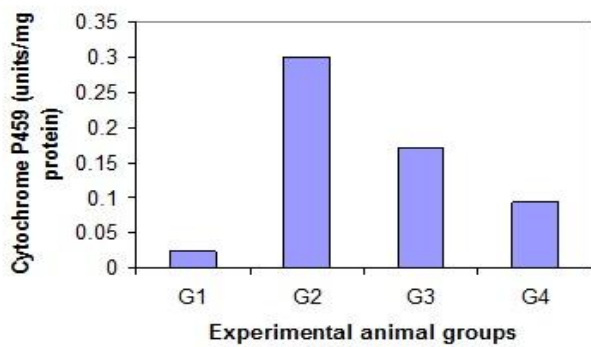


Fig 10. Normal hepatocytes arranged in a radical fashion around a central venule

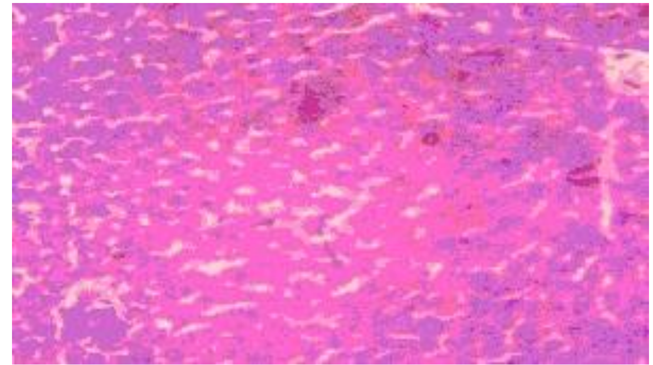


Fig 11a. Low power view of the section of ethanol induced changes in liver

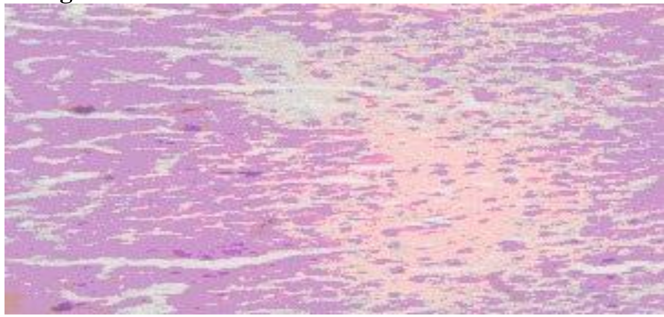


Fig 11b. High power view of the section of ethanol induced changes in liver

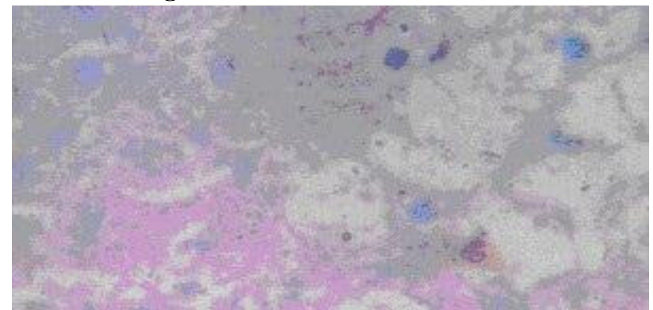


Fig 12. Amelioration of hepatocytes in *N. nucifera* treated rats

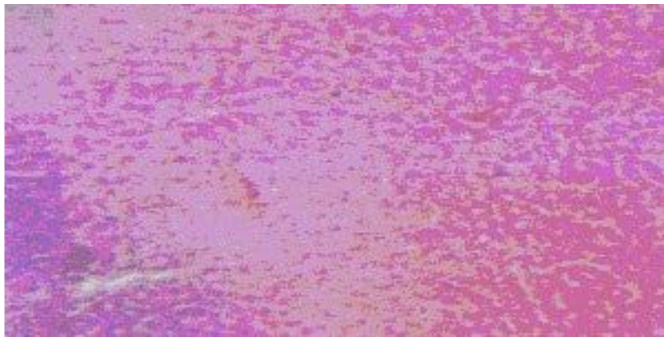
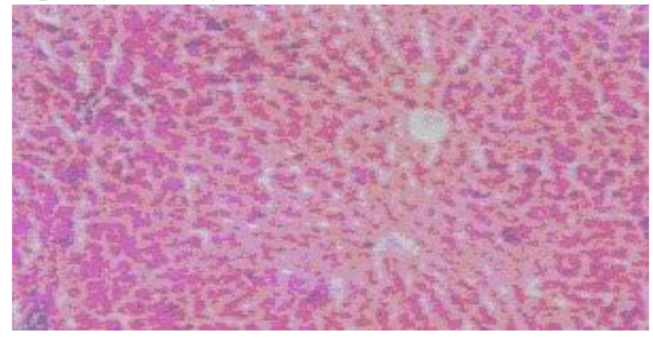


Fig 13. Few sparse steatotic cells amidst regenerating hepatocytes in Silymarin treated rats



DISCUSSION

The present study embodies the hepatotoxicity by 30% ethyl alcohol to evaluate the hepatoprotective and antioxidant activity of *N. nucifera* against the toxicity encountered by the alcohol. Ethanol is capable of generating free radicals in the tissues by increasing the malondialdehyde levels and impairing the antioxidative defense systems [32]. In the present study the increase in TBARS is an indication of oxidative stress induced by ethanol. Accumulation of malondialdehyde alters the cellular metabolism of hepatic and extra hepatic tissues which leads to whole cell deformity and cell death [33]. *N. nucifera* acts as free radical scavenger by decreasing levels of TBARS near to control and standard drug treated groups.

Glutathione (GSH) serves as an important source of reducing equivalents and it is highly protective against oxidative stress in every cell. The excess intake of ethanol develops cirrhosis and liver disease [34]. A marked reduction in glutathione pool occurs in liver after ethanol intoxication which is partly due to the binding of acetaldehyde with cysteine/ glutathione [35, 36]. This statement support our findings with a reduction in glutathione after intoxicated with alcohol. This condition was effectively counter balanced by *N.nucifera* which significantly increased the glutathione level near to standard and control groups.

Superoxide dismutase (SOD) is the major attractive metalloprotein in the antioxidant family. The increased synthesis of superoxide dismutase against superoxide anion radical ($O_2^{\cdot -}$) production is an adaptive response of cell to synthesis SOD through the stimulation of gene transcription [37]. The levels of SOD were decreased in G2, G3 and G4 rats intoxicated with ethanol. SOD and catalase levels are decreased in rats orally fed with alcohol [38]. Ethanol induction increases the generation of superoxides that stimulate the primary defender SOD in neutralizing the oxy radicals in the liver cells. Similar results are observed in our study by reduction of SOD in alcohol intoxicated groups. The natural antioxidant systems are depleted in scavenging the superoxide radicals which furtherdecreased level of superoxide dismutase creating an oxidative stress. SOD levels are significantly increased by *N. nucifera* compared with standard and control groups indicate its positive effect by elevating free radical scavenging activity.

Catalase (CAT) allows the destruction of H_2O_2 out of water and oxygen. It catalyzes the detoxification of alcohols and phenols [39]. The activity of catalase was decreased in all ethanol intoxicated groups. The depletion of catalase is due to the increased generation of peroxy radicals released in the alcohol metabolism. The administration of aqueous suspension of *N. nucifera*i ncreased the catalase activity near to the control and standard groups indicate their protective action against oxidative stress.

Vitamin E can repair oxidizing radicals directly and prevent the chain propagation step during lipid peroxidation [40]. The ascorbic acid (Vitamin C) is closely related to the antioxidant properties, favors integrity and fluidity of membranes [41] controlling the oxidizing reactions of fatty acids, thus keeping cellular respiration and avoiding cell death [42]. A decreased in nonenzymatic antioxidant such as GSH, vitamin C and vitamin E was observed in the liver of alcohol treated rats. The reduced form of glutathione (GSH) is required for the regeneration of vitamin C, which is necessary for the regeneration of Vitamin E [43]. The reduced levels of GSH in alcohol treated rats simultaneously reduced the endogenous production of vitamin C and Vitamin E. Treatment with *N nucifera*as a supplementary drug for 21 days exerts its beneficial effect in increasing the concentration of vitamins compared to standard and control groups. Our results are in good agreement with ethanolic extract of Salviasahendica increase the GSH, vitamin C and E contents on liver and kidney tissues of alcohol treated rats [44].

Serum iron and copper levels were significantly increased in ethanol intoxicated rats compared with the control groups. Dietary supplement of *N. nucifera* decreased the unbound iron and copper through the chelating properties of flavonoids and exhibit its antioxidant capacity.

The level of cytochrome P450 was increased in alcohol treated rats. The induction could increase the generation of prooxidant free radical species ($OH^{\cdot}$), thereby decreasing GSH and α - tocopherol levels leading to augmented oxidative stress [45]. Further administration of *N. nucifera* prevents stimulation of cytochrome P450. Earlier scientific reports stated that flavonoids inhibit cytochrome P450 [46]. The beneficial effect of *N.nucifera* might be due to the presence of flavonoids.

The decrease in liver Na^+/K^+/ATPase activity was observed in ethanol intoxicated rats. Dietary supplement of N.nucifera maintained the hepatic regeneration and membrane stabilization by reversal of Na^+/K^+/ATPase towards control and standard groups reflect the point as it is a promising hepato protectant and effective antioxidant.

CONCLUSIONS

The evaluation of these biochemical parameters isused as marker to assess the hepatoprotective and antioxidant property *N. nucifera*. It prevents the malondialdelyde formation and control the rate of lipid peroxidation. *N. nucifera* act as a detoxificant in stabilization of cytochrome P450 and a good liver protectant by their cellular regeneration capacity. Hence, the present study states that *N. nucifera* has equivalent therapeutic value compared to the standard drug Silymarin. However, further detailed studies are needed to isolate the active phytochemical component that exactly provides the complete protection against liver diseases.

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