INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-8, Issue-4, Oct-Dec-2017 Coden IJABFP-CAS-USA Copyrights@2017

Received: 14th Aug-2017 *Revised: 24th Sep- 2017* *Accepted: 25th Oct-2017*

DOI: 10.21276/Ijabpt, http://dx.doi.org/10.21276/ijabpt *Research article*

HEPATOPROTECTIVE EFFECT OF *VITEX NEGUNDO* LEAF ON ISOPROTERENOL-INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

Mohammed Siddig Younis[1], E. Maruthi Prasad*[2], S. Manjunatha[2], Lakshmi Devi Kodidhela[2]

[1]Department of Medical Laboratory Technology, College of Medical Applied Science, Taibah University, Almadina Almonawwarah, Saudi Arabia

[2]Department of Biochemistry, Sri Krishnadevaraya University, Anantapuramu- 515003, India

ABSTRACT: The role of traditional medicine in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine. With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions. Isoproterenol (ISO) induced hepatotoxicity serves as a well standardized model for studying certain physiological and pathological events i.e, changes in lipids, enzymes and hormones during the course of liver toxicity. Recently, herbal drugs, which are non-toxic and naturally occurring, are gaining much significance in the treatment of many diseases. *Vitex negundo* is warehouse of assorted bioactive constituents or phytochemicals which find ample use in the pharmaceutical industry. The aim of present study is to evaluate the hepatoprotective effect of *V.negundo* against ISO-induced oxidative stress. Adding up to this liver markers, lipid profile, electrolytes and liver histopathology were examined to assess the protective effect of *V.negundo* in ISO-induced hepatotoxicity. Our results showed protective effect against ISO-induced hepatic injury. Further studies are needed to evaluate the identification of bioactive constituents from the *V. negundo* for its hepatoprotecive activity.

Key words: *Vitex Negund*, Leaf, Isoproterenol, Hepatoprotective

*Corresponding author: E. Maruthi Prasad, [2]Department of Biochemistry, Sri Krishnadevaraya University, Anantapuramu- 515003, India, E-mail emaruthip@gmail.com

Copyright: ©2017 E. Maruthi Prasad. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

INTRODUCTION

The liver consists of many different cell types. Broadly, they can be classified as parenchymal cells (hepatocytes) and non-parenchymal cells (NPCs). The hepatocytes form the structural basis of the liver and make up the majority of the mass of the liver (Godoy et al., 2013). The NPCs include other various cell types such as Kupffer cells, sinusoidal endothelial cells, stellate cells, periportal broblasts and hepatic dendritic cells. Isoproterenol (4-[1-hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,2-diol) is a synthetic catecholamine and beta-adrenergic agonist which causes severe oxidative stress in resulting infarct like necrosis (Klahr *et al,* 1973). ISO-induced hepatotoxicity also serves as a well standardized model for studying certain physiological and pathological events i.e., changes in lipids, enzymes and hormones during the course of toxicity. It also alters the membrane permeability, liver tissue integrity, Ca2+ overload and insufficient oxygen utilization. The liver is particularly sensitive to oxygen and the integrity of the liver cell depends on an adequate oxygen supply. It has been reported to exhibit many metabolic and morphological aberrations in the liver tissue of experimental animals similar to that of human liver toxicity by a multiple step mechanism (Ganesan *et al,* 2007).

The primary disturbance of ISO-induced toxicity has been reported to enhance adenylate cyclase activity resulting in increased cAMP formation, which in turn leads to increased lipid accumulation in the liver (Gøtzsche, 1983). It is also well known to generate free radicals and to stimulate lipid peroxidation in the cell damage, has received radical and terminate the chain reaction before vital molecules are damaged. The present study is designed to evaluate the alterations in the liver tissue lipid profile, marker enzymes, tissue electrolytes and histopathological studies. This revealed the protective role of *V. negundo* against ISO-induced hepatotoxicity in wistar rats.

MATERIALS AND METHODS:

Chemicals: ISO was purchased from Sigma-Aldrich (USA). All other chemicals were of analytical grade and were supplied by Sisco Research Laboratories (Mumbai, India).

All the rat liver tissues (kept in liquid nitrogen (-196^0 C) and maintained at the same temperature) were collected from Department of Biochemistry, S.K.University. Liver tissue were homogenized and used for the studies of liver markers, lipid profile and electrolytes.

1. Control rats
2. VNEXT treated rats (300mg/kgbw orally)
3. ISO administered (85mg/kgbw, s.c., on 39th & 40th day)
4. VNEXT pretreated + ISO administered rats

Liver tissue homogenate cholesterol and triglycerides were estimated using Accurex enzymatic diagnostic kit and HDL was estimated by using Autozyme cholesterol diagnostic kit. The homogenate was centrifuged at 2500g and the clear supernatant solution was used for the estimation of liver tissue marker enzymes.

Total Cholesterol (TC) (Allain *et al*, 1974): Cholesterol standard and water blank were also treated in a similar manner. After incubation, absorbance was read at 510nm and values are expressed as mg/dL.

Triglycerides (TG) (Foosati *et al*, 1982)**:** Triglyceride standard and water blank were also treated in a similar manner. After incubation, absorbance of the standard and liver tissue homogenate was read at 510nm against and values are expressed as mg/dL.

High Density Lipoprotein cholesterol (HDL) (Assmann, 1983)**:** The colour developed was read at 510nm against a blank and a standard (50 mg%) was run simultaneously. Values were expressed in mg/dL.

Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL):

VLDL & LDL were calculated using the Friedewald *et al* (1972) formula as follows.

VLDL=TG/5

LDL=Total Cholesterol-TG/5-HDL

Aspartate aminotransferase (AST) (Bayoumi *et al*, 1976)**:**

α- keto glutarate + L-aspartate ↔ L-glutamate + oxaloacetate – pyruvate

The activity of glutamate oxaloacetate transaminase is expressed as IU/L (@ 340nm) of liver homogenate.

Alanine aminotransferase (ALT) (Bayoumi *et al*,1976)**:**

α-ketoglutarate +L-alanine ↔ L-glutamate+ pyruvate.

The activity of glutamate oxaloacetate transaminase is expressed as IU/L (@ 340nm) of liver homogenate.

Alkaline phosphatase (ALP): This is based on the kinetic method and the absorbance was taken at 405nm` The activity of ALP was expressed as a U/L.

Sodium: Liver tissue homogenate sample was analyzed for sodium by magnesium uranyl acetate using the method of Trinder (1951).

Potassium: The liver tissue homogenate sample was analyzed for potassium by the method of Jacobs and Hoffmann (1931).

Calcium: Liver tissue homogenate calcium was estimated by OCPC method as described by Dycus and Lewis, (1957).

Histopathological studies: The liver tissues were cut in to small pieces and preserved in 10% buffer formalin for histomorphological examination (Raghuramulu *et al*, 1983).

Statistical analysis: All the results were expressed as mean ± SD of a six individual observations. Duncan's Multiple Range (DMR) test was performed to know the level of significance among all the experimental groups.

RESULTS AND DISCUSSION

Effect of VNEXT on liver marker enzymes:

AST, ALT and ALP are decreased in their activities in ISO administered rats when compared to control rats. Pretreatment with VNEXT showed significant increase in their activities when compared to ISO administered rats and were maintained near to normal levels. These observations are in agreement with earlier findings of Shreesh et al, 2008 (Fig. 1).

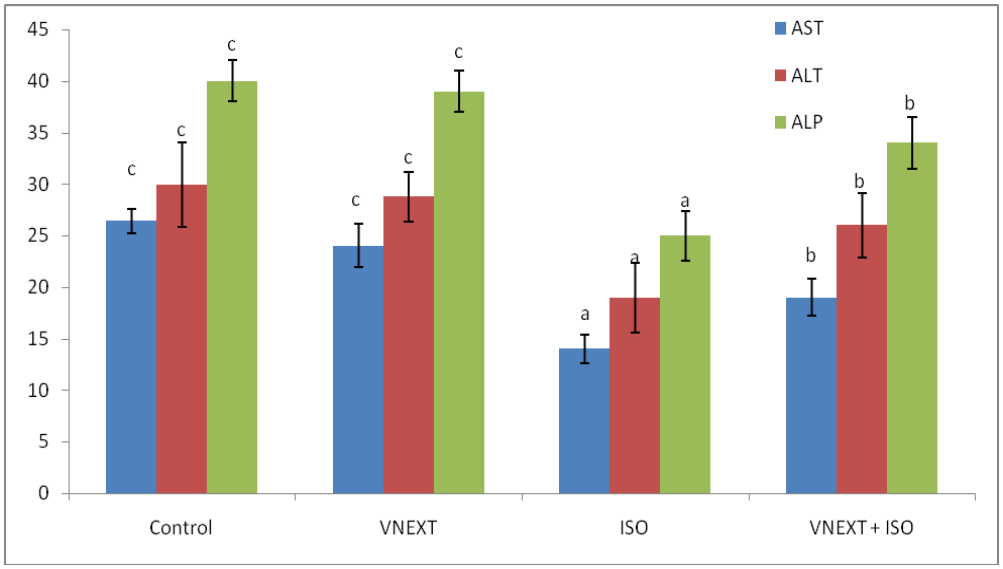
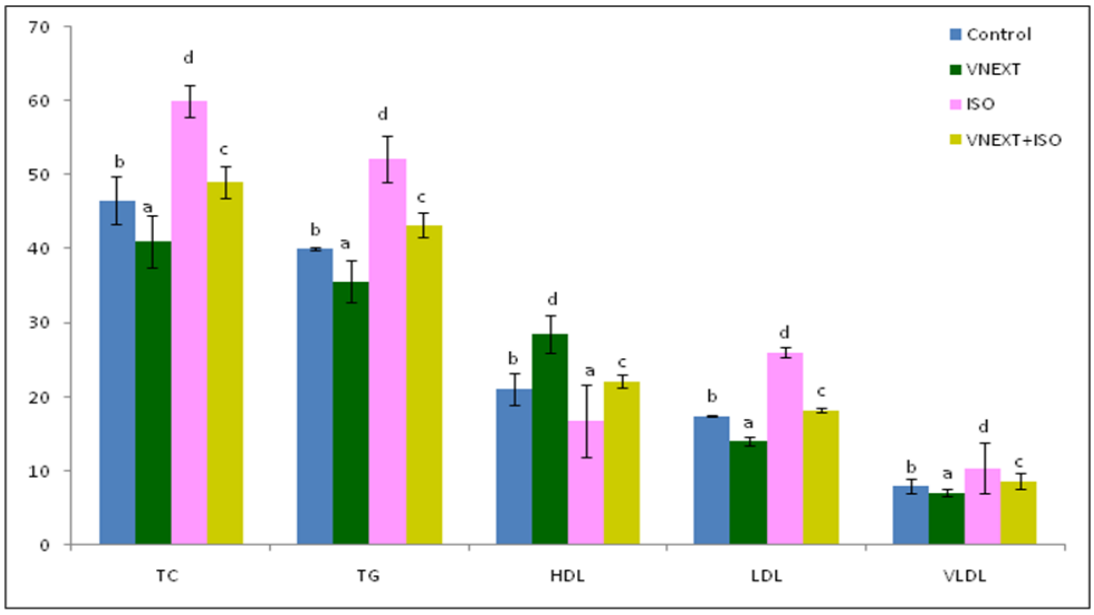


Fig. 1: Effect of VNEXT on liver marker enzymes

Effect of VNEXT on liver tissue lipid profile:

In liver tissue the levels of TG, TC, LDL and VLDL were significantly increased where as HDL decreased significantly in the ISO administered rats as compared to normal control rats.

Rats pretreated with VNEXT showed a significant decrease in the levels of liver tissue TG, TC, LDL and VLDL and a significance decrease in the level of HDL as compared to ISO administered rats (Libby et al, 2000) (Fig. 2).

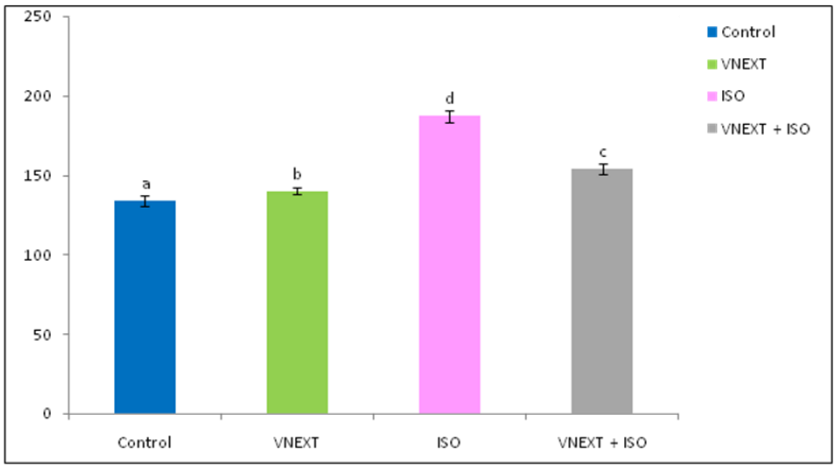


Values are mean ± S.D. Values that do not share a common superscript (a, b, c and d) differ significantly from each other ($p<0.05$, Duncan's multiple range test).

Fig. 2: Effect of VNEXT on liver tissue lipid profile

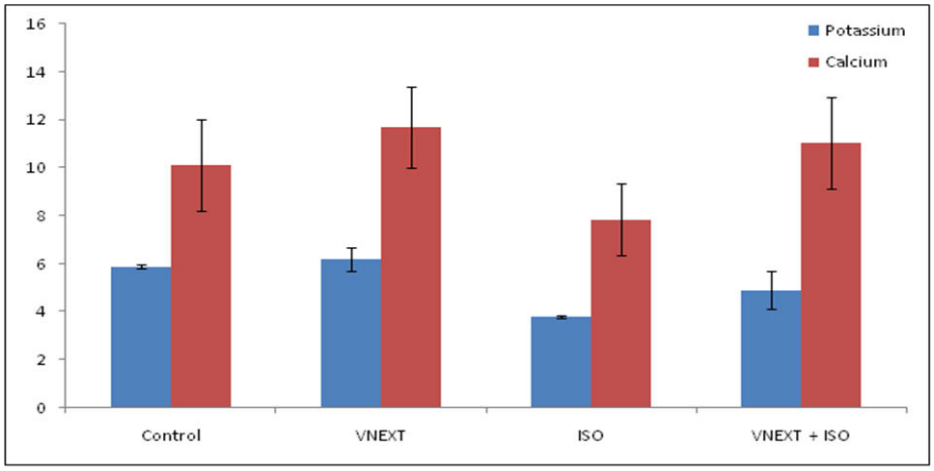
Effect of VNEXT on liver tissue electrolytes (Na, K+& Ca2+):

ISO administered rats showed a significant increase in liver tissue levels of sodium and a significant decrease in potassium and calcium when compared to control rats. The rats pretreated with VNEXT showed a significant decrease in sodium and a significant increase in potassium and calcium levels when compared to ISO administered rats. Electrolytes and minerals play a major role in metabolism, as well as most cellular activities. They hold fluids in compartments of the body and maintain normal acid-base balance (Damodara et al, 2007) (Fig. 3 & 4).



Values are mean ± S.D. Values that do not share a common superscript (a, b, c and d) differ significantly from each other ($p<0.05$, Duncan's multiple range test).

Fig. 3: Effect of VNEXT on sodium of control and experimental rats

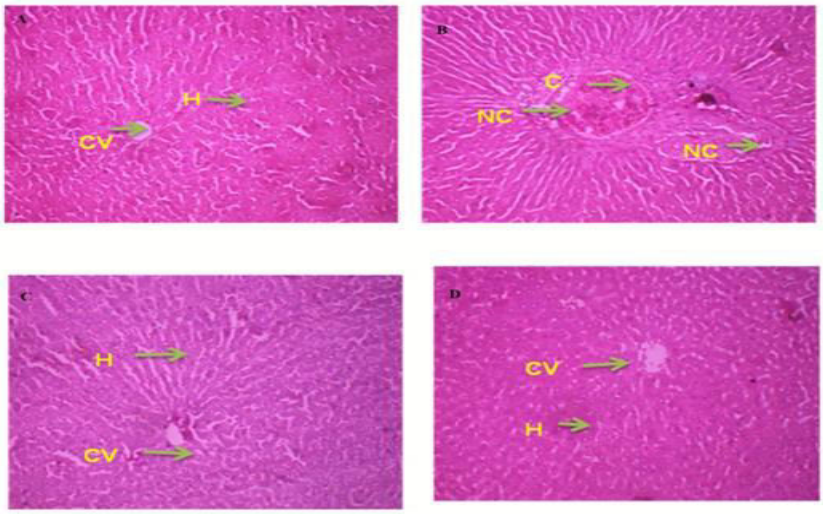


Values are mean ± S.D. Values that do not share a common superscript (a, b, c and d) differ significantly from each other ($p<0.05$, Duncan's multiple range test).

Fig. 4: Effect of VNEXT on potassium & calcium of control and experimental rats.

Histological studies of liver tissue of control and experimental rats:

The histological observations of the liver sections are in agreement with the biochemical changes. Control rats showed normal architecture, VNEXT treated group showed near normal architecture with slight sinusoidal dilatations, ISO administered group showed inflammatory necrosis and fibrosis, pretreated showed near normal hepatic architecture. Restoration of normal architecture in pretreated group indicates the hepatoprotective effect of VNEXT.



A-Control group, B- ISO group (85 mg/kgbw), C- VNEXT + ISO (300 mg/kgbw), and D- VNEXT treated

SUMMARY & CONCLUSION

The present study revealed the effects of ISO in ISO-induced hepatotoxicity on liver tissue and also protective effect of ethanol extract of *V.negundo* leaves in these conditions in albino wistar rats. In order to evaluate the protective effect of ethanolic extract of *V.negundo* leaves on marker enzymes, lipid profile, electrolytes such as K+,Ca2+ and Na+ and histology of liver tissue were studied. Hyperlipidemia is one of the major factors responsible for the occurrence of hepatotoxicity. It is postulated that ISO induces MI by its lipolytic action and increasing circulating lipids and lipoprotein levels as well as hepatic and cardiac lipids.

In the present studies also the TC, TG and lipoproteins such as VLDL and LDL were increased where as HDL was decreased. VNEXT pretreatment for a period of 40 days significantly ameliorated these lipid levels, which might be due to the presence of saponins, flavonids, terpenes and glycosides. Rats pretreated with VNEXT exhibited decrease in the positive inotropic effect induced by ISO administration. VNEXT increased liver tissue K+ and Ca2+ levels and reduced Na+ levels compared to ISO administered rats. This could be due to presence of alkaloids, saponins and Ca2+ antagonist in VNEXT. Decreased levels of liver tissue marker enzyme in ISO induced hepatotoxicity can be attributed to the damage of liver structural integrity, because these are cytoplasmic in location and are released into circulation after cellular damage. Even mild changes in their levels may have led to the leakage of these enzymes from liver tissue into the blood stream, but VNEXT administration might have minimized the effect of ISO and would have prevented the damage, there by maintaining the values at near normal in pretreated rats when compared with ISO administered rats. ALT (in cytoplasm) and AST (in cell cytoplasm and mitochondria) occur in much higher concentration in liver than elsewhere and consequently their decrease reflects hepatic damage more specifically.

VNEXT pretreated rats showed near and normal architecture where as ISO administered rats showed necrotic architecture compared to control rats. ISO administered rats showed sinusoids adjacent to the terminal hepatic veins are dilated, and hepatocytes show nuclear vacuolation. In the case of extract pretreated rats near-normal hepatic architecture is seen, which is attributed to protective effect of VNEXT. The above mentioned hepatoprotective effects of VNEXT are mainly attributed to its phytochemical constituents and active principles.

REFERENCES

Allain, Charles C., Lucy S. Poon, Cicely SG Chan, W. F. P. C. Richmond, and Paul C. Fu. (1974). "Enzymatic determination of total serum cholesterol." Clinical chemistry 20, no. 4: 470-475.

Assmann, Gerd, Hilko Schriewer, Gl Schmitz, and E. O. Hägele. (1983). "Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl2." Clinical Chemistry 29, no. 12: 2026-2030.

Friedewald, William T., Robert I. Levy, and Donald S. Fredrickson. (1972)."Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge." Clinical chemistry 18, no. 6: 499-502.

Bayoumi, Riad A., and Sidney B. Rosalki. (1976). "Evaluation of methods of coenzyme activation of erythrocyte enzymes for detection of deficiency of vitamins B1, B2, and B6." Clinical chemistry 22, no. 3: 327-335.

Dycus, Augustus M., and Lewis Knudson. (1957). "The role of the velamen of the aerial roots of orchids." Botanical Gazette 119, no. 2: 78-87.

Foosati P, Prencipe L, (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry, 28: 2077-2080.

Ganesan, Balaraman, Rangasamy Rajesh, Rangasamy Anandan, and Nanjappan Dhandapani. (2007). "Biochemical studies on the protective effect of betaine on mitochondrial function in experimentally induced myocardial infarction in rats." Journal of health science 53, no. 6: 671-681.

Godoy, Patricio, Nicola J. Hewitt, Ute Albrecht, Melvin E. Andersen, Nariman Ansari, Sudin Bhattacharya, Johannes Georg Bode (2013). "Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME." Archives of toxicology 87, no. 8: 1315-1530.

Gøtzsche, Ole. (1983)."The adrenergic β-receptor adenylate cyclase system in heart and lymphocytes from streptozotocin-diabetic rats: in vivo and in vitro evidence for a desensitized myocardial β-receptor." Diabetes 32, no. 12: 1110-1116.

Jacobs, H. R. D., and William S. Hoffman. (1931). "A new colorimetric method for the estimation of potassium." Journal of Biological Chemistry 93: 685-691.

Kareem, Mohammed Abdul, Saayi Krushna Gadhamsetty, Althaf Hussain Shaik, E. Maruthi Prasad, and Lakshmi Devi Kodidhela. (2013). "Protective effect of nutmeg aqueous extract against experimentally-induced hepatotoxicity and oxidative stress in rats." Journal of Ayurveda and integrative medicine 4, no. 4: 216.

Klahr, Saulo, Tewfik Nawar, and Anton C. Schoolwerth. (1973). "Effects of catecholamines on ammoniagenesis and gluconeogenesis by renal cortex in vitro." Biochimica et Biophysica Acta (BBA)-General Subjects 304, no. 1: 161-168.

Trinder, P. (1951)."A rapid method for the determination of sodium in serum." Analyst 76, no. 907: 596-599.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com