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Amelioration of Withaferin-A on the alterations of carbohydrate metabolic enzymes: an experimental study using a rat model of Hepatocellular carcinoma

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Abstract
The purpose of this study was to investigate the Amelioration of Withaferin –A on the alterations of carbohydrate metabolic enzymes on N–Nitrosodiethylamine induced hepatocellular carcinoma in Wistar rats. Hepatocellular carcinoma (HCC) belongs to the group of epithelial cancers and represents with a frequency of about 85 % the most common primary liver cancer. The estimated incidence may be located between 500.000-1.000.000 new cases per year and is characterized by a wide geographic variation; it ranges from less than 10 cases / 100.000 in the USA and Western Europe to 50 - 150 cases / 100.000 in parts of Africa and Asia. The most prominent etiological factors associated with hepatocellular carcinoma are chronic hepatitis B and C viral infections, chronic ethanol abuse and intake of aflatoxin B1- contaminated food. Hepatocellular carcinoma was induced in rats by N–Nitrosodiethylamine at the dosage of 0.01% of DEN was dissolved in drinking water and administered to experimental animals daily for 15 weeks after conformation of hepatocellular carcinoma treated with Withaferin-A for 21 days. The levels of carbohydrate enzymes were significantly decreased were markedly increased in Withaferin-A treated animals when compared with cancer bearing animals. Moreover, the levels of carbohydrate metabolic enzymes a good indicators of restoring the liver architecture, were also reversed in liver damage subjects after treatment with the herbal compounds preparation. So, from the obtained results it is concluded that a Withaferin-A is capable of restoring the liver architecture and can also increase the carbohydrate metabolic activities in hepatocellular carcinoma rats.

Keywords: Hepatocellular carcinoma; N–Nitrosodiethylamine; Withaferin-A

1. Introduction

Liver cancer is the sixth most common cancer in worldwide but because of very poor prognosis, it is the third most common cause of death from cancer and 82% of these cases are in developing countries (Parkin et al., 1999). Hepatocellular carcinoma **(**HCC) is a type of cancer that arises from hepatocytes, the major cell type of the liver. Hepatocellular cancer formations are multifunctional process and possible mechanisms leading to those diseases have not been clarified yet. Evidences suggest that the free radical damage as an important contributor to the disease. Liver is the most common site for metastasis from a variety of organs such as lung, breast, colon and rectum. About 80% of the people with HCC have cirrhosis, chronic infection with hepatitis B virus (HBV) and Hepatitis C virus (HCV) also increases the risk of HCC. N–

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Nitrosodiethylamine (DEN) is one of the most important environmental carcinogen in N-Nitrosamine class, which primarily induces tumour in the liver. It also causes oxidative damage in the DNA of the rat liver, a target organ. HCC's limited treatment remedy and the poor prognosis emphasize the importance in developing an effective chemoprevention for this disease. In addition, oxidative stress has recently been suggested to participate in both the metabolism (activation and detoxification) and the carcinogenic actions of nitrosoamines, including DEN (Bartsch*et al*., 2006).

Many medicinal plants have an advantage in drug discovery based on their use by humans for more than thousands of years. The bioactive compounds obtained from these plants are normally non-toxic or less toxic to humans .Withaniasomnifera (WS)is a well-known Indian medicinal plant commonly known as Ashwagandha belonging to the family Solanaceae. It is widely used in many ayurvedic preparations all over the world. Several reports have demonstrated the anti-tumor activity (Sbohat et al., 1967), anti-arthritic (Sethi et al., 1970), anti-pyretic and anti-inflammatory (Budhiraja and Sudhir, 1987) activity of this plant. The root extract has shown to have immunomodulatory activity (Agarwal et al., 1999), antistress effect (Archana and Namasivayam, 1999) and induce iNOS expression (Iuvone et al., 2003). The major active compounds of the methanolic root extract of WSis a rich source of steroidal lactones called Withaferin-A (WFA) and also known as withanolides(Ganzera et al., 2003), glycosides and many different alkaloids. Similarly the methanolic leaf extracts also include a variety of withanolides and very few studies have investigated the molecular mechanisms of the effect of root extracts. In previously many reports revealed that it is used in treating various forms of cancer, the antitumor and radiosensitizing effects of WithaferinA (WFA) have been studied. This withaferin-A from roots are reported to have an antiproliferative activity (Jayaprakasam et al., 2003) and possess an inhibitory effect on the cyclooxygenase-2 (COX-2) enzyme (Jayaprakasam and Nair 2003). However, despite the observation of diverse medicinal activities attributed to this plant, very few biochemical studies have been investigated the molecular mechanisms of the effect of root extract of Withaferin-A (WFA) on hepatocellular carcinoma in experimental rats.

The aim of this present work was to assess the activity of mitochondrial enzymes like Isocitrate dehydrogenase (ICDH), Malate Dehydrogenase (MDH), Succinate dehydrogenase (SDH) and also to investigate the effect of root powder of Withaferin-A on some carbohydrate metabolic enzymes and the liver marker enzymes such as AST (aspartate transaminase), ALT (alanine transaminase) and ALP (alkaline phosphatase) in liver tissue in control and N-nitrosodiethylamine (DEN) treated experimental animals. The purpose of this paper is to evaluate the therapeutic efficacy of Withaferin-A.

2. MATERIALS AND METHODS

2.1. ANIMALS

Male albino rats of Wistar strain (130 ± 20 g) were used throughout the study. The animals were purchased from king institute, chennai, Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu, India and maintained in a controlled environmental condition of temperature and humidity on alternatively 12 h light/dark cycles. All animals were fed with standard pellet diet (Gold Mohor rat feed, Ms.Hindustan Lever Ltd., Mumbai) and water *ad libitum*. Experimental animals were handled according to the University and institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (CPCSEA Reg. No. 765/PO/Re/S/03/CPCSEA).

2.2. Induction of Hepatocellular carcinoma (HCC)

N-Nitrsodiethylamine (DEN) was used as a carcinogen for the present investigation. 0.01% of DEN was dissolved in drinking water and administered to experimental animals daily for 15 weeks (Ramakrishnan G.,*et al*, 2007). After the conformation of the formation of liver cancer (beginning of 13^{th} week) by histopathology, the animals were treatments carried out.

2.3.Collection of plant materials

For the experimental study Withaniasomnifera root powder was purchased under the trade name "AswagandhaChurnam" (Specimen No:A029) from "Indian Medical Practitioners Co-operative Pharmacy and stores Ltd" (IMPCOPS), Thiruvanmiyur, Chennai, India and the crude alcoholic extract was prepared by the method of Suffness and Douros (1979) as described earlier. Withaferin-A was isolated from the alcoholic extract by fractionation and chromatographic separation as described by PankajBandhoria et al 2006**.**

Extraction and isolation of withaferin-A from withaniasomnifera root

Withaferin-A (WFA) was extracted and isolated from commercially available withaniasomnifera root powder (under the trade name "AswagandhaChurnam" Specimen No: A029 from "Indian Medical Practitioners Co-operative Pharmacy and stores Ltd" (IMPCOPS), Thiruvanmiyur, Chennai, India) by fractionation and chromatographic separation as described by PankajBandhoria et al 2006**.** Air dried root powder (1000 g) of WithaniaSomnifera was extracted in 95% v/v ethanol in a hermetically closed glass vessel for 4 days at 37^{o}C under occasional shaking. The ethanolic extract was then filtered through a Whatman filter paper #4 and evaporated in a rotary evaporator under reduced pressure at 60^{o} C and stored. A syrupy solution was diluted with water, and the resulting suspension was extracted sequentially with $CHCl_3$, EtOAc and 1-BuOH, in that order. The chloroform fractions were subjected to column chromatography over silica gel (60 – 120 mesh) and the elution was carried in increasing polarity with $CHCl_3$, 2% MeOH in $CHCl_3$, 5% MeOH in $CHCl_3$ and MeOH. Fractions that were eluted in 5% MeOH in $CHCl_3$ were pooled and concentrated; the residue after crystallization from EtOAc yielded withaferin A (300 mg), mp 252 – 253°C. The final product, withaferin-A, a steroidal lactone (4β, 27 dihydroxy-1- oxo-5β, 6β, epoxy with a 2-24 dienolide) was obtained as a creamy

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white crystalline substance, which had R_f = 0.4 and molecular weight 470. The identity of the isolated withaferin-A was done by mass spectral analysis and its identity was confirmed by comparing with the authentic withaferin-A, purchased from Sigma Aldrich, USA. The yield and purity of the isolated withaferin-A was found to be 0.11% and > 90% respectively. For experimental studies, Withaferin-A obtained was first dissolved in a few drops of absolute ethanol followed by dilution with normal saline containing 0.5% carboxymethyl cellulose (CMC).

Isolation and Characterization of Withaferin-A (WFA) using spectral evidence:

A biologically active fraction was obtained from the 5% MeOH in $CHCl_3$ extract by column chromatography. This fraction consisted of five components as observed on a TLC plate. The major active constituent of Withaferin-A was isolated by column chromatography. The compound-3 was characterized by 1HNMR, IR, UVR and Mass spectroscopy. The 1HNMR spectrum of Compound-3 has been compared with the 1H NMR spectra of a known withanolide-withaferin A. The drug was prepared freshly before use. It is soluble in water. It was dissolved in a few drops of ethanol and a homogeneous suspension was made with normal saline containing 0.5% carboxymethyl cellulose (CMC) and used for the experimental study.

2.6. Experimental design:

The animals were divided into six groups and each group consisted of six animals. withaferin A dissolved in vehicle solution (0.5% CMC) and were administered orally using an intragastric tube for a period of three weeks.

Group I - Control animals treated with normal saline containing 0.5% CMC (vehicle) orally throughout the experimental period.

Group II – Animals were induced hepatocellular carcinoma by providing 0.01% DEN through drinking water for 15 weeks.

Group III - Animals were treated with Withaferin-A at the dosage of 50mg/kg body.wt, dissolved in 0.5% CMC orally for 21 days before administration of DEN as in GroupII.

Group IV - Hepatocellular carcinoma induced animals treated with Withaferin-A at a dosage similar to group III for 21 days, i.e. after the administration of DEN for 15 weeks

Group V -Animals treated with Withaferin-A (as in Group III) alone for 21 days.

2.7. Sample collection

At the end of the experimental period of 18 weeks, all animals were deprived of food overnight and sacrificed by cervical decapitation. Liver was immediately dissected out and washed in ice-cold saline to remove the blood. Tissues was minced and homogenized (10% w/v) with 0.1M Tris-HCl buffer (pH 7.4) in ice cold condition. The homogenates were centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatants were separated and were used for the following assays like liver marker enzymes, glycolysis and gluconeogenesis metabolic key

enzymes assays were determined in the serum and liver tissues of experimental and control animals.

2.8 Mitochondrial Isolation:

After the animals were killed by cervical decapitation, the liver tissues were immediately excised, weighed and homogenized in Tris–HCl buffer 0.01 M (pH 7.4). These homogenates were taken for the isolation of mitochondria. The mitochondria were isolated by the method of Johnson and Lardy (1967). A 10% homogenate was prepared in 0.25 M sucrose and centrifuged at 600x*g*for 10 min. The supernatant fraction was decanted and then again centrifuged at 15000x*g*for 10 min. The resultant mitochondrial pellet was then washed and resuspended in 0.25 M sucrose and used for further analyses. The purity of the mitochondria was estimated by assaying the activity of succinnate dehydrogenase (SDH).

2.9 Biochemical analysis

2.9.1. Assay of Mitochondrial Enzymes:

Isocitrate dehydrogenase (ICDH) activity was assayed according to the method of King (1965). Succinate dehydrogenase (SDH) was assayed according to the method of Slater and Bonner (1952). Malate dehydrogenase (MDH) (L-malate: NAD oxidoreductase) was assayed by the method of Mehler*et al.,* (1948). Na+,K+-ATPase was estimated by the method of Bonting. The activity of Ca2+-ATPase was assayed according to the method of Hjerten and Pan. The activity of Mg2+-ATPase was assayed by the method of Ohnishi et al. The inorganic phosphorus was estimated according to the method of Fiske and Subbarow. Protein content was estimated by the method of Lowry et al. The level of lipid peroxides was assayed by the method of Ohkawa et al.

3. Results

3.1. Changes in the body and tumor weight

Table 1 shows the evaluation of body andtumor weight in control and experimental rats after 14days treatment. Food and water intake were elevated whereas the body weight significantly decreased and increased tumor weight in hepatocellular carcinoma rats compared with normal rats. In hepatocellular carcinoma rats treated with Withaferin-A, significantly increased the body weight and decreased tumor weight as well as the food and water intake when compared to hepatocellular carcinoma control rats.

3.2. Liver marker enzymes level

Figure: 1 shows the effect of Withaferin-A on the levels of marker enzymes in the liver of control and experimental rats. The levels of ALP, ACP and LDH were found to be significantly

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increased in the liver cancer animals when compared with the control animals. On administration of Withaferin-A, the levels of these marker enzymes were found to be significantly reduced in treated animals when compared to cancer induced group. Similarly, the levels of AST, ALT, γ-GT and 5'Nucleotidase were found to be significantly increased in cancer bearing group when compared to the control group. On the administration of Withaferin-A there was a significant decrease in the levels of these enzymes in treated animals when compared to cancer induced group. However, there found to be no significant difference in the activities of these marker enzymes between the control animals (Group-I) and drug control animals treated with Withaferin-A (Group-V).

3.3. Mitochondrial enzymes

Mitochondrial integrity is most vital for cellular functioning. Alterations in the activities of enzymes involved in TCA cycle reflects the extent of damage occurred in this organelles during cancer condition.

Figure 2depicts the effect of withaferin-A on the activities of mitochondrial enzymes in the liver of control and experimental animals. Group II (HCC bearing) animals showed a significant ($p<0.001$) decrease in the activities of mitochondrial enzymes such as ICDH, α-KGDH, SDH and MDH when compared with control (Group I) animals. Withaferin-A treatment resulted in a significant ($p<0.001$) increase in the enzyme activities in Group III ($p<0.001$) and Group IV ($p<0.001$, $p<0.05$) animals when compared to Group II animals. There was no significant difference between withaferin-A alone treated (Group V) animals and control animals (group I)

3.4. Assay of membrane bound ATPases in liver homogenate

Figure 3shows the effect of Withaferin-A on the activities of ATPases in liver tissues of control and experimental animals. The decrease ($P < 0.05$) in the activities of Na+/K+ ATPase, Mg2+-ATPase and an increase ($P < 0.05$) in Ca2+- ATPase activity seen in cancer induced Group II animals when compared with control animals were significantly increased ($P < 0.05$) and decreased ($P < 0.05$), respectively, in Group III and Group IV animals treated with Withaferin-A when compared with Group II animals. There was no significant difference in the activities of ATPases between Group V animals and controls.

3.5. Lipid Peroxidation

Figure 4 depict the effect of Withaferin-A on the levels of lipid peroxidation in the liver of control and experimental animals. There found to be a significant ($p<0.001$) increase in the levels of lipid peroxidation in the liver of cancer induced group II animals when compared with the control group (Group-I). Withaferin-A (Group-III) treatment caused a significant ($p<0.001$) decrease in their levels when compared with cancer induced animals. Cancer induced group treated with Withaferin-A (Group-IV) showed a much more significant ($p<0.001$) decrease in the levels of lipid peroxidation when compared with the cancer-induced group. However, there was

found to be no significant difference in the levels of the lipid peroxidation between the control animals and drug control animals treated with Withaferin-A (Group-V).

4. Discussion

Biochemical tumor markers are used to screen particular tumor condition for diagnosis, prognosis and assessing the response to therapy. These enzymes are unique and the rise in their activities is shown to be in good with the number of transformed cells in cancer conditions . The role of transaminase in biological system is well known. Elevated aminotransferase activities were observed in cancer bearing animals. Transaminase becomes gradually more pronounced towards the terminus, which indicates the severity of an advanced cancer condition. Increased levels of transaminase activity are also seen in hepatocellular carcinoma (Rocchi et al, 1997). The activities of ALT and AST in liver were normalized after treatment with Withaferin-A.

The enzymes ALP and ACP are membrane bound enzymes and its alteration is likely affect the membrane permeability. The ALP and ACP levels are elevated in cancer induced animals (Patel et al, 1994). The activities of these enzymes were brought back to near normal on treatment with Withaferin-A. 5'- Nucleotidase has been reported to be altered in the sera of patients with solid tumors. 5'- Nucleotidase has been increased significantly hepatocellular carcinoma induced animals. On treatment with Withaferin-A, the enzyme activity got decreased significantly. LDH is a cytoplasmic enzyme which catalyses the oxidation of lactate to pyruvate and vice versa. LDH is a marker for membrane integrity and is a regulator of many biochemical reactions in the body tissues and fluids (Mano et al, 1989). There is a marked increase in the enzyme activity of LDH in hepatocellular carcinoma animals. This is due to enhance glycolysis during the growth of tumor. The activity of LDH was brought back to normal on treatment with Withaferin-A.

Mitochondria are considered as the major source of cellular ROS (Halliwell and Gutteridge, 1999) may promote cellular growth and proliferation and contribute to cancer development. Tumor cell mitochondria can differ structurally and functionally from those of normal cells, but clear evidence in favors of this suggestion is lacking. There is however evidence that some chemical carcinogen primarily attacks mitochondria which is the ''molecular clocks'' in eukaryotes (Pederson, 1978). ICDH refers to the NADP+ dependent enzyme, which in several tissues has dual localization being in part of cytoplasmic and in part of mitochondria. SDH is a marker enzyme in TCA cycle and succinate, phosphate and ATP promote its activity. Being a regulatory enzyme its property is altered when it is solubilised. Availability of oxalate is controlled by another chief enzyme in TCA cycle is MDH, which converts malate to oxaloacetate. The result of the present study strongly indicates that the antioxidant Withaferin-A promises therapeutic and possible prophylactic applications. This result also holds potential relevance in terms of cancer chemotherapy in experimental lung cancer.

The decreased activities of TCA cycle marker enzymes such as ICDH,SDH,α-KG and MDH were observed in DEN administered HCC bearing animals. Decreased activities of these

enzymes could be due to the alteration in cancer morphology, ultrastructure and ability of mitochondria to undergo metabolic changes when compared with normal cells , and also the number of mitochondria is drastically reduced in hepatoma cells (Thirunavukkarasu et al., 2001). Administration of Withaferin-A increased the activities of the mitochondrial enzymes such as ICDH, SDH, α-KG and MDH and render protection against DEN induced toxicity which suggests that Withaferin-A is efficient in maintaining the mitochondrial membrane integrity.

Oxidative stress, especially lipid peroxidation is known to be involved in carcinogenesis (Trush and Kensler, 1991). Increased levels of LPO products play a role in the early phases of tumor growth (Rice Evans and Burdon, 1993). LPO products have shown to cause profound alteration in the function and structural organization of cell membrane, including membrane permeability, inactivation of membrane bound enzymes and loss of essential fatty acids (Thirunavukkarasu et al, 2001). The LPO levels of hepatocellular bearing animals were elevated. On Withaferin-A supplementation showed near normal level of LPO. This shows that Withaferin-A inhibits LPO.

LPO is regarded as one of the basic mechanisms of cellular damage caused by free radicals. The DEN is a very effective carcinogen in interacting with membrane lipids and consequently inducing free radical formation . Free radicals react with lipids causing peroxidation, resulting in the release of products such as malondialdehyde, hydrogen peroxide and hydroxyl radicals. An increase in lipid peroxides indicates serious damage to cell membranes, inhibition of several enzymes, cellular function and cell death (Thirunavukkarasu et al, 2001). Withaferin-A significantly reduced the membrane and plasma lipid peroxides in cancer bearing animals. There is extensive evidence that supplementation of Withaferin-A can enhance antioxidant enzymes and other seleno proteins [26]. The antioxidant enzymes may reduce the carcinogen-DNA interaction by providing a large nucleophilic pool for the electrophilic carcinogens. In malignancy, the cell membrane plays a crucial role in the stimulation and control of cell adhesiveness, mortality and proliferation in a much-damaged condition. The protection of membranes is of potential importance in the treatment of disease processes. The membrane bound enzymes such as Na+/K+-ATPase, Mg2+-ATPase and Ca2+-ATPase are responsible for the transport of sodium/potassium, magnesium and calcium ions across the cell membranes at the expense of ATP by hydrolysis. The activities of all the three ATPases in erythrocyte membrane, hepatoma and surrounding tissues have been found to be inhibited in carcinoma bearing animals. These findings are similar to those reported by Verna and Frati. Generally, in hepatoma,membranes forming tertiary capillaries are reduced and dilated or constricted with no microvilli; such typical capillaries contain no ATPases. Ohnishi et al. cited that the activity of Mg2+-ATPase in normal liver cell is 140% higher than that in hepatoma cells.
The membrane bound enzymes such as Na+/K+-ATPase, Mg2+-ATPase and Ca2+ -ATPase are responsible for the transport of sodium/potassium, magnesium and calcium ions across the cell membranes at the expense of ATP by hydrolysis. The activities of the ATPases in erythrocyte mem-brane, lung and liver tissues have been found to be inhibited in carcinoma bearing animals.

These finding are similar to those reported in various cancers. The decreased activities of Na+/K+and Mg2+-ATPase in lung cancer bearing animals may be due to increased LPO which occur in cancer conditions. We have also observed increased levels of LPO in cancer animals. Peroxidation of membrane lipids initiates the loss of membrane integrity and membrane bound enzyme activities, which in turn leads to a disruption in cellular homeostasis. Abnormal lipid peroxides affect membrane bound ATPases activities and their levels were decreased due to the excessive production of thiobarbituric acid reactive substances. Ca2+ -ATPase, the enzyme responsible for active calcium transport, is extremely sensitive to hydroperoxides and this may lead to its inhibition. The impairment in this enzyme may be due to the peroxidative stress, which may act on the sulphydryl groups present in the active sites of the Ca2+-ATPase (Thirunavukkarasu et al, 2001).

5. Conclusion

As outlined above, results from various studies indicate ashwagandha possesses many qualities, including anti-inflammatory, antitumor, and immunomodulatory properties. Although the results from this review are quite promising for the use of ashwagandha as a multi-purpose medicinal agent, several limitations currently exist in the current literature. While ashwagandha has been used successfully in Ayurvedic medicine for centuries, more clinical trials should be conducted to support its therapeutic use. It is also important to recognize that WA may be effective not only in isolation, but may actually have a potentiating effect when given in Combination with other herbs or drugs.

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Table.1. Changes in the body and tumor weight

Particulars	Group-I Control	Group-II DEN Induced	Group-III Pretreated	Group-IV Post treated	Group-V (Withaferin A)
Initial Body Weight (gms)	140 ±13.91	171±17.22	154±15.43	151 ±15.61	159±16.21
Final Body Weight (gms)	195.05±19.2	151.02±15.2	172.05±16.81	177.1±17.8	192.12±19.1
Tumor Weight (gms)	5.52±0.53	7.85±0.71a*	6.82±0.63b*	5.49±13.91b*c*	5.42±0.54

Each value is expressed as mean ±SD for six rats in each group.

a- Group II compared with group I;

b- Group II compared with group III and IV;

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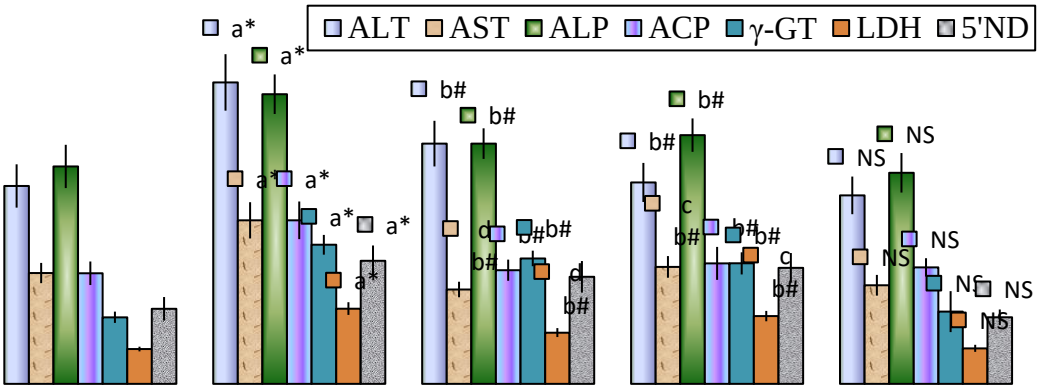
c- Group II as compared with group V
Statistical significance- *p<0.001, @p<0.01, #p<0.05.

Figure.1. Effect of Withaferin-A on the levels of marker enzymes in the liver of control and experimental rats



Each value is expressed as mean ± SD for six rats in each group.
Units – ALT, AST and LDH: µmoles of pyruvate liberated/min/mg protein
ALP and ACP: µmoles of phenol liberated/min/mg protein
5'ND – nmoles of Pi liberated/min/mg protein
a - as compared with group I; b - as compared with group II; c - as compared with group III
Statistical significance - *p<0.001, #p<0.01, @p<0.05, NS-Not significant.

Figure 2. Effect of withaferin-A on the activities of mitochondrial enzymes in the liver of control and experimental animals

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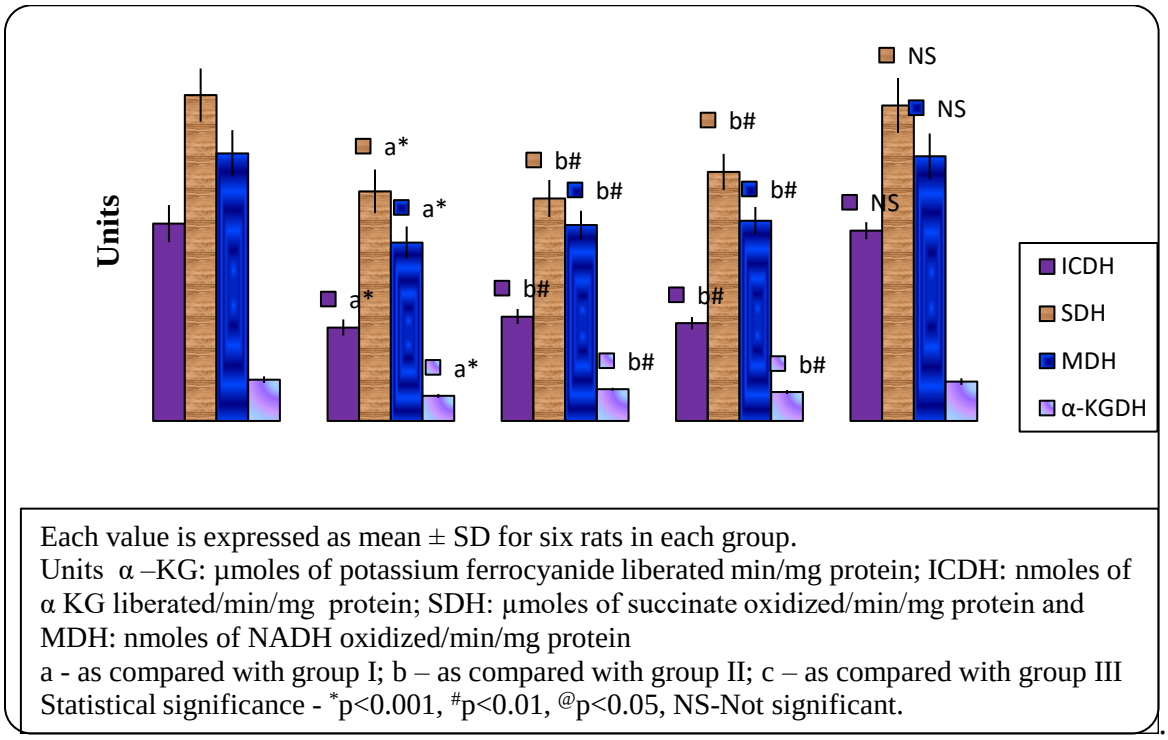
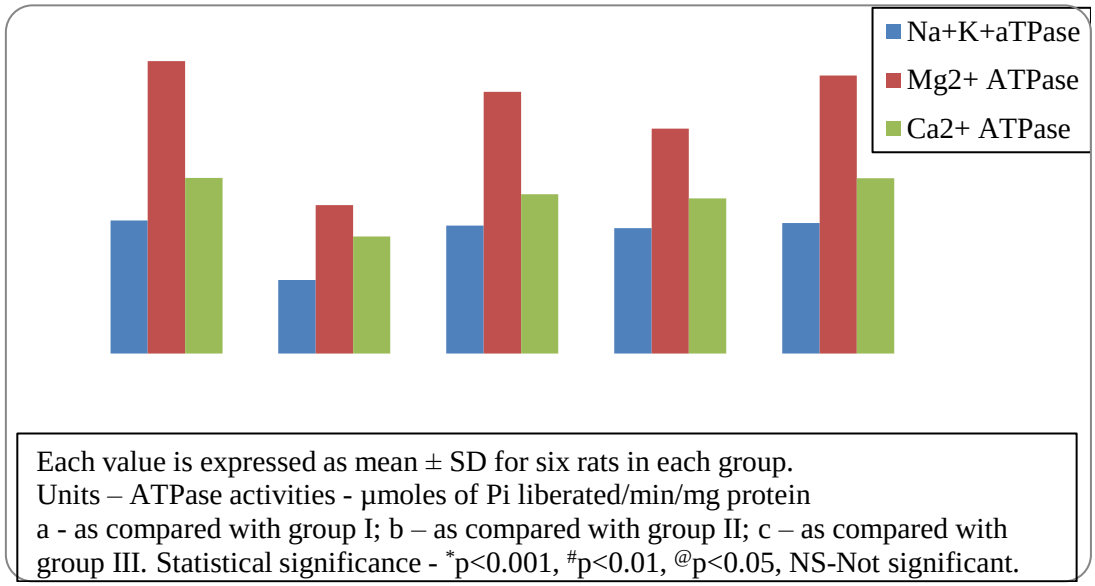


Each value is expressed as mean ± SD for six rats in each group.
Units α –KG: µmoles of potassium ferrocyanide liberated min/mg protein; ICDH: nmoles of α KG liberated/min/mg protein; SDH: µmoles of succinate oxidized/min/mg protein and MDH: nmoles of NADH oxidized/min/mg protein
a - as compared with group I; b – as compared with group II; c – as compared with group III
Statistical significance - $^{*}p<0.001$, $^{\#}p<0.01$, $^{@}p<0.05$, NS-Not significant.

Figure.3. Effect of Withaferin-A on ATPase levels in liver of control and experimental animals



Each value is expressed as mean ± SD for six rats in each group.
Units – ATPase activities - µmoles of Pi liberated/min/mg protein
a - as compared with group I; b – as compared with group II; c – as compared with group III. Statistical significance - $^{*}p<0.001$, $^{\#}p<0.01$, $^{@}p<0.05$, NS-Not significant.

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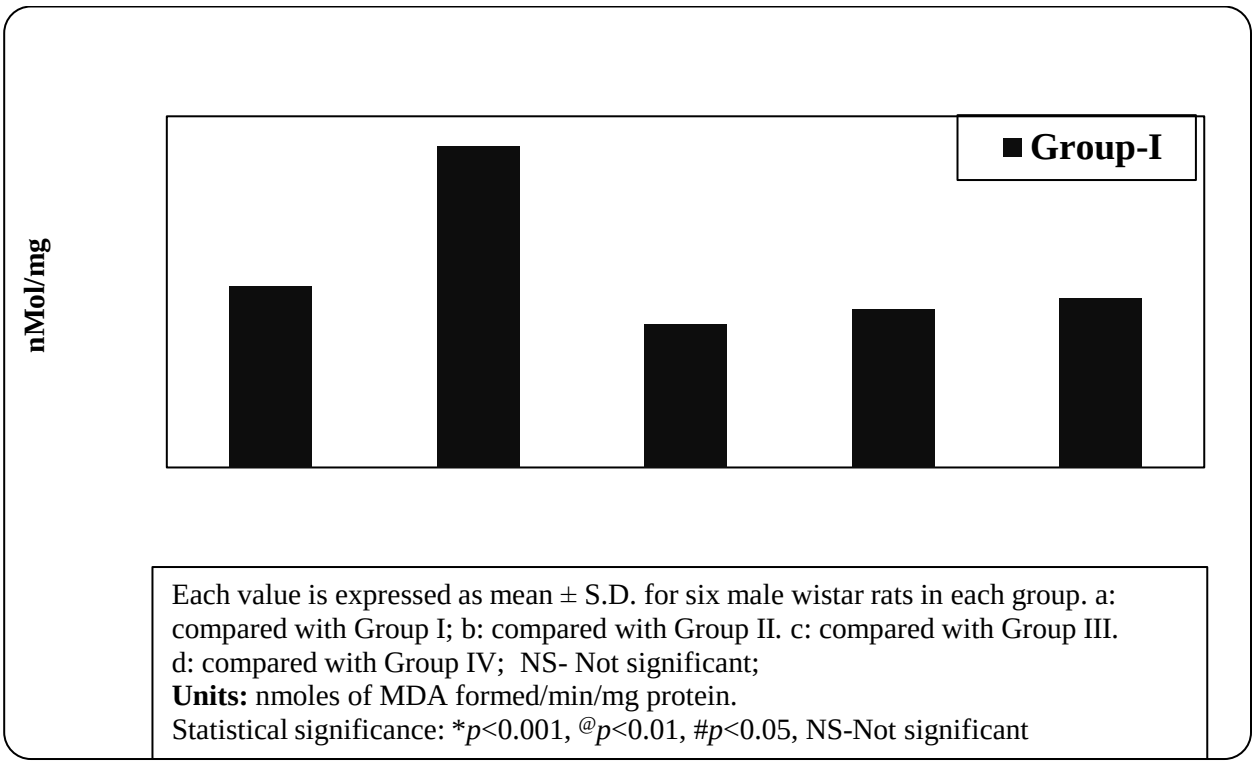
Figure.4. Effect of Withaferin-A on the levels of lipid peroxidation in the liver of control and experimental animals



Each value is expressed as mean ± S.D. for six male wistar rats in each group. a: compared with Group I; b: compared with Group II. c: compared with Group III. d: compared with Group IV; NS- Not significant;
Units: nmoles of MDA formed/min/mg protein.
Statistical significance: **p*<0.001, @*p*<0.01, #*p*<0.05, NS-Not significant