RESEARCH ARTICLE

Asian Journal of Biochemical and Pharmaceutical Research

Evaluation of Hepatoprotective Efficacy of Polyherbal Formulation Amlycure DS on Anti-Tubercular Drug Induced Hepatotoxicity

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Abstract: Amlycure DS is a polyherbal formulation containing unique synergistic mix of herbs in optimal therapeutic concentrations as its constituents claiming liver protective and corrective properties. In the present study, the Amlycure DS was evaluated for its protective effect in ameliorating the hepatotoxicity associated with INH administration in experimental animal albino rats. Isoniazid- a chemically isonicotinyl hydrazine (INH), is used to prevent and treat tuberculosis disease. It is noted that while metabolism of Isoniazid, acetyl hydrazine is produced, which exerts adverse effects on the liver. The present findings demonstrated the hepatoprotective efficacy of both polyherbal liquid dosage form (ADS-RF) and polyherbo-mineral solid dosage form (ADS-ND) of Amlycure DS led to significant amelioration of toxin-induced changes in the biochemical parameters and histological hepatic damage at two dose levels, comparable to standard drug Silymarin.

Key Words: Isoniazid, Polyherbal formulation, Amlycure DS, Silymarin, Hepatoprotective activity, Metabolic systems

INTRODUCTION:

The basic metabolic system of the human body is controlled and managed by two vital organs-liver and kidney [1, 2]. The liver and kidney both actively metabolize many drugs, hormones and xenobiotics and protect our metabolic systems. Among major functions of liver are removal of damaged red blood cells from the blood in co-ordination with spleen, production of bile, clotting factors, stores vitamins, minerals, protein, fats and glucose from diet [3, 4]. The most important task of the liver is to filter toxic substances from the body, like chemotherapeutic drugs, antitubercular drugs, antibiotics and toxicants. The liver metabolizes virtually every drug or toxin introduced in the body. Metabolism of drugs or toxins occurs in 2 phases [5]. In the phase 1 reaction, the drug is made polar by oxidation or hydroxylation. The cytochrome P450 enzymes catalyze phase 1 reactions. All drugs may not undergo this step, and some may directly undergo the phase 2 reaction [6, 7]. Phase 2 reactions may involve conjugation with a moiety (i.e., acetate, amino acid, sulfate, glutathione, glucuronic acid) [8]. Subsequently, drugs with high molecular weight may be excreted in bile and the kidneys excrete the smaller water soluble molecules. Most of the intermediate products are transient and highly reactive. Several reactions may result in the formation of intermediate metabolites that are far more toxic than the parent substrate and may result in liver injury. And when disruptions in normal functions of liver and kidney arise, metabolic disorders occur. In simpler terms, if accumulation of toxins in body fluids is faster than their metabolizing ability by liver, hepatic damage and hepatic disease takes place.

In central control of metabolism of drugs and their toxic intermediates, P450 enzyme plays critical role. For example, P450 enzyme activity increases several folds during metabolism of chemical agents like

phenobarbital, phenytoin, carbamazepine, primidone, ethanol, glucocorticoids, rifampin, griseofulvin, quinine and omeprazole, whereas in contrast, P450 enzyme activity ceases during metabolism of isoniazid, amiodarone, cimetidine, erythromycin, ketoconazole, metronidazole, sulfonamides and quinidine [9].

Among several useful drugs, Isoniazid- a chemically isonicotinyl hydrazine (INH), is used to prevent and treat tuberculosis disease. It is noted that while metabolism of Isoniazid, acetyl hydrazine is produced, which leaves adverse effects on the liver. In turn, transient elevations in liver enzyme aminotransferases is noted that play role to overt hepatitis. Actually, N-acetyltransferase (NAT2) enzyme metabolizes isoniazid to acetyl isoniazid, which in turn is hydrolyzed to toxin acetyl hydrazine [10]. Acetyl hydrazine is further metabolized by CYP 2E1 and in down chain several hepatotoxic derivatives are produced which severely damage liver. One of the mechanism is that metabolic oxidation of acetyl hydrazine leads to formation of reactive acylating species which bind covalently to microsomal protein and liver cell micromolecules, hence acetyl hydrazine and hydrazine act as acylating agents and their conjugations cause hepatocyte injury [11].

There is no rational therapy available for preventing metabolic disorders associated with side effects of modern medicine administration. However, one of the oldest and richest healthcare systems available to human kind that has the potential to protect human metabolism integrity is- The Indian system of medicine-Ayurveda which has Outstanding Contribution to Modern Therapeutics. Most common herbs used as ingredients as per Indigenous Ayurvedic Sciences for the treatment of metabolic disorders, liver diseases, renal toner, anti diabetic, anti obesity, anti cholesteremic, as well as in immune modulating formulations are Andrographis paniculata, Aloe vera, Azadirachta indica, Achillea millefolium, Boerhaavia diffusa, Cichorium intybus, Capparis spinosa, Curcuma longa, Cissampelos pareira, Eclipta alba, Emblica officinalis, Embelia ribes, Fumaria officinalis, Glycyrrhiza glabra, Lawsonia alba, Ocimum sanctum, Phyllanthus niruri, Picrorhiza kurroa, Plumbago zeylanica,, Raphanus sativus, Rheum emodi, Solanum nigrum, Terminalia chebula, Tinospora cordifolia, Tephrosia purpurea, Terminalia arjuna, Tecomella undulata and Tamarix gallica etc. [12- 32].

About 600 commercial herbal formulations with claimed hepatoprotective activity are marketed all over the world. These formulations contain a number of herbal constituents claiming choleretic, bitter, carminative, anti-inflammatory, anti-microbial, anti-viral and regenerative effects on hepatocytes [33]. Few of the famous Indian commercial formulations manufactured by pharmaceutical companies utilizing above herbs in different combinations as part of their claimed hepatoprotective formulations are Amlycure DS (Aimil), Liv-52 (Himalaya Drug Company), Livergen (Standard Pharmaceuticals), Livokin (Herbo-med), Octogen (Plethico Pharmaceuticals Ltd.), Hepjaun (S.G. Phytopharma Pvt. Ltd.), Hepin (Anglo French Drugs industries Ltd.), New Livfit (Charak), Livomyn (Alembic), Stimuliv (Franco-Indian Pharmaceuticals), Tefroliv (TTK Pharma Pvt. Ltd.,) and Adliv (Albert David) [34-36].

After comparisons of the herbal constituents for all the above mentioned herbal formulation it was found that Amlycure DS possess the broad coverage of herbs as its constituents claiming widest pharmacological properties, and are available in optimal concentrations and having the good apportioned. Amlycure DS comprising primarily of Andrographis paniculata, Tinospora cordifolia, Phyllanthus niruri, Boerhaavia diffusa, Eclipta alba, Ocimum sanctum, Cichorium intybus, Solanum nigrum, Berberis lyceum, Plumbago zeylanica, Glycyrrhiza glabra, Foeniculum vulgare, Rheum emodi, Picrorhiza kurroa etc. Notably the herbs of Amlycure DS are described in ayurvedic literature for their usage in various disorders of liver as hepatoprotectants. Several of above mentioned herbs have shown significant activity against HBV in clinical and experimental studies also [37-38].

Hence, it was hypothetically worthwhile to carry out the present study with the aim to carry out preliminary phytochemical screening and to explore the hepatoprotective effects of Amlycure DS on the bases of biochemical and histopathological parameters with an objective to ascertain whether such a potential herbomix (Amlycure DS) is effective in ameliorating or preventing the hepatotoxicity associated with INH administration in experimental animal rat.

MATERIALS AND METHODS:

Chemicals and drugs: Isoniazid was procured from HiMedia Laboratories Pvt. Ltd, Mumbai, India and all other chemicals used for the study were of analytical grade; Silymarin, from Microlabs, India Ltd.

Amlycure DS soluble dosage form and syrup are licensed ayurvedic polyherbal formulations, procured from Aimil Pharmaceuticals (India) Ltd, New Delhi India. The solid dosage form (SDF) comprises of aqueous extracts of different medicinal plants and also the mineral based ayurvedic formulations like PunernavadiMandoor, ShwetParpati, Arkshar, ShankhBhasam where as the syrup comprises of aqueous extracts in rich concentration in a sugar base palatable liquid. The concentrations of active constituents of both the formulation as per label claim were almost equal per unit doses. The dosages from label claims were converted from human to rats, using the standard factor as per minimum and maximum recommended dosage [39].

Animals: Healthy adult Wistar albino rats (200-250g) of either sex between 6-8 months of age were used for investigation and were procured from Panacea Biotec Ltd, Lalru (140501), India. They were housed in group of not more than 4 in the each polypropylene cage; maintained under standard conditions (12:12h light: dark cycle; 25±3ºC; 40-60% humidity) and fed with standard rat pellet diet (Sanjay biological museum, Amritsar, Punjab) and water ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) [Protocol no.: IAEC/2012/III/0019(PCL-M)] of the Institute under the guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment & Forests, New Delhi.

Experimental study protocol:

Preliminary Phytochemical screening: Preliminary phytochemical screening [40] was carried out to detect the presence of Flavanoids, Glycosides, Terpenes, Alkaloids, Saponins, Tannins, Carbohydrates, Proteins, Amino acids, Steroids etc. Amlycure DS (ADS-ND) was firstly crushed and boiled in water (pH 5) then filtered out; this filtrate was used for the phytochemical screening. Amlycure DS (ADS-RF) liquid was first decolorized with the charcoal to remove the colour then filtered out. The tests were carried out with filtrate.

Induction of hepatotoxicity: Hepatotoxicity in Albino Wistar rats was produced by isoniazid (250mg/kg, p.o. for 14 days) [41]. Silymarin was used as the reference standard (63mg/kg/p.o.) for the treatment. The treatment was extended to 40 days but induction of the toxicant stopped at 14 days.

Experimental design: In experiment, totally 35 rats were used. The rats were divided into 7 groups of five animals each.

Group I: Normal control group administered saline 5ml/kg, p.o .

Group II: Negative control group administered hepatotoxin drug Isoniazid, 250mg/kg/, p.o. for 14 days

Group III: Standard control treated group administered hepatotoxin drug Isoniazid, 250mg/kg/, p.o. for 14 days along with Silymarin (63mg/kg/p.o.) for 40 days.

Group IV: Treated group administered hepatotoxin drug Isoniazid, 250mg/kg/, p.o. for 14 days along with ADS-RF- I (1402mg/kg, p.o.) for 40 days.

Group V: Treated group administered hepatotoxin drug Isoniazid, 250mg/kg/, p.o. for 14 days along with ADS-RF-II (5608mg/kg, p.o.) for 40 days.

Group VI: Treated group administered hepatotoxin drug Isoniazid, 250mg/kg/, p.o. for 14 days along with ADS-ND-I (95mg/kg, p.o.) for 40 days.

Group VII: Treated group administered hepatotoxin drug Isoniazid, 250mg/kg/, p.o. for 14 days along with ADS-ND (285mg/kg, p.o.) for 40 days.

Animals were fasted overnight, given Silymarin (63 mg/kg/p.o.), ADS-RF-I (1402 mg/kg p.o.), ADS-RFII (5608 mg/kg p.o.), ADS-ND-I (95 mg/kg p.o.), ADS -ND-II (285 mg/kg p.o.), daily for 40 days to the third,

fourth, fifth, sixth and seventh group of animals respectively along with hepatotoxin drug Isoniazid (250mg/kg, p.o) administered for 14 days. At 15th day 1ml Blood was collected from all animals by retro-orbital bleeding for the evaluation of serum parameters like Aspartate transaminase (AST) [42], Alanine transaminase (ALT) [43], Alkaline phosphatase (ALP) [44], lactate dehydrogenase (LDH) [45], Bilirubin (Total and Direct) [46] and In-vivo antioxidant parameter as Thiobarbituric Acid Reactive Substances (TBARS) [47].

Histopathological study of liver: Liver tissues were removed, washed with cold saline and preserved in 10% formalin and subsequently dehydrated in graded alcohol and utilized for the histopathological studies. After embedding in paraffin 4μM sections were cut and mounted on aminosilane coated glass slides. After drying overnight, paraffin sections were warmed for 1 h at 15ºC. Sections were then deparaffinised in four changes xylene, 10 min each, followed by one change on 100% ethanol for one minute. The slides were rehydrated by one min changes each of 100%, 95%, 75%, and 50% ethanol then held in distilled water. After staining with

Hematoxylin for 4 min, slides were rinsed in distilled water, decolorized with lithium carbonate solution and rinsed with distilled water again. Following a one min wash in 70% ethanol, slides were stained with Eosin for one min and then dehydrated. Cover slips were mounted with Premont. The tissues were examined by light microscopy at 45X for degeneration, fatty changes or necrotic changes as evidence of hepatotoxicity.

Statistical analysis: All the data were expressed as mean ± SEM. Statistical significance was tested using one way ANOVA followed by using Bonferroni post-tests. Statistical significance was determined at $P < 0.05$ (significant), $P < 0.01$ (very significant) and $P < 0.001$ (most significant) accordingly.

RESULTS AND DISCUSSION:

Among therapeutic agents, herbs appear as richest source of hepatoprotective molecules that offer protection to liver from damage. The biological functions hepatoprotective molecules perform are antioxidant, free radical scavenging, anti-inflammatory, antiviral, choleretic and stimulation hepatic regeneration. The antioxidant molecule helps to prevent hepatic diseases initiated by free radicals especially reactive oxygen species (ROS). Oxidative stress or oxidative damage has been declared to be involved in the development of several chronic hepatic diseases. The reaction between ROS with biomolecules like lipids, protein and DNA may lead to increased risk of chronic hepatic diseases. Due to that, treatment or prevention strategy can be done by retaining balance between ROS and antioxidants [48].

In the present study, rat group treated with hepatotoxic anti-tubercular drug-Isoniazid for 14 days, showed significantly elevated levels of ALT, ALP, AST, LDH, total & direct bilirubin and hepatic lipid peroxidation compared to saline control group, hepatoprotectant Silymarin and tested herbal formulation Amlycure DS forms ADS-RF & ADS-ND different doses (Table 1 & Table 2). The mineral based SDF of Amlycure DS (ADS-ND) have shown the most significant lowering of liver enzyme levels against Isoniazid intoxications and similar results are with ADS-RF (syrup), both comparable to Silymarin.

Phytochemical groups like alkaloids, carbohydrates, tannins and flavonoids were detected in significant proportions in extractives forms ADS-RF & ADS-ND of tested ayurvedic polyherbal formulation Amlycure DS. The herbs used in Amlycure DS are known for high contents of secondary metabolites like flavanoids, phenols, coumarins, curcuminoids, lignans, essential oil and terpernoids [49]. The hepatoprotective activity in Amlycure DS is probably due to the presence of Phytochemicals such as andrographolide and neoandrographolide from Andrographis paniculata, picroside and kutkoside from Picrorrhiza kurroa, phyllanthin and glycyrrhizin from Phyllanthus niruri, glycyrrhizin from Glycyrrhiza glabra [50-52] are known to act as potent hepatoprotective agents. The above phytochemicals have proven antioxidant, antiviral, antifibrotic or anticarcinogenic properties and are future prospects for further development as hepatoprotective drugs [50]. Another compound curcumin, a main phytochemical of

Curcuma longa has known good antioxidant activity and inhibits lipid peroxidation as evidenced from in vivo studies on role of cytochrome P450 (CYP) against CCl4 induced hepatotoxicity. Curcumin suppressed the CCl4 induced CYP isoenzyme inactivation. The role of curcumin on hepatic diseases by restoring lipid profile and marker enzymes is also reported [53].

In present study, histopathological sections of Isoniazid induced hepatotoxicity comprised of piecemeal necrosis, apoptosis, focal inflammation, portal inflammation and fibrosis in the liver tissue (fig 9). After Isoniazid administration, hepatocellular disintegration, vacuolation in the centrilobular region [54], Kupffer cell hyperplasia with congestion of hepatocytes and micro-vesicular fatty infiltration with special stains have also been observed in earlier reports [55]. The Amlycure DS administration via extractives forms ADS-RF & ADS-ND significantly prevented above histological degenerative changes in the liver and showed significant dose dependent hepatocellular integrity protective activity (fig 10-14), much comparable to standard hepatoprotective drug Silymarin, much being used in medical practice.

CONCLUSION:

Patients with long term diseases or disorder like diabetes mellitus, oncology under chemotherapy, tuberculosis, hormone replacement therapy etc. are unusually susceptible for fatty degeneration of liver. Based on present study we conclude that modern drugs have side effects observed in the form of metabolic disorders leading to liver malfunctions, hepatocyte injury and kidney diseases, however, if some natural hepatoprotectants herbs based ayurvedic formulations like Amlycure DS is co-administered with toxic drug to such patients, liver metabolism shall rejuvenate with considerable protection to hepatic cells. The hepatoprotective role of ADS-RF & ADS-ND forms of Amlycure DS might be due to available anti-oxidants tannins and flavonoids and also due to anti-inflammatory action. Various potential mechanisms are explained for action of ayurvedic formulations in preventing the oxidative stress induced damage and in protecting histological integrety. Some ayurvedic formulations like punarnavadi mandoor, shankh bhasam and shwet parpati have already been established for their potent therapeutic effects in liver disorder. In present study, ADS-RF & ADS-ND have significant protective action and therapeutic activity as compared to standard drug Silymarin against Isoniazid induced hepatotoxicity. ADS-ND at optimal dose showed optimum effect and ADS-RF showed good effects as compared to the standard drug Silymarin treated rat group in several aspect. Therefore, superior efficacy of Amlycure DS as hepatoprotective polyherbal formulation was found than standard hepatoprotective drug Silymarin.

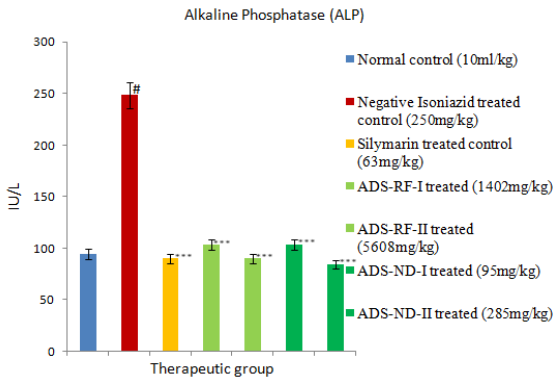

Fig. 1: Effect of ADS-RF and ADS-ND on Alkaline Phosphatase (ALP) in Isoniazid Induced Hepatotoxicity. Amlycure DS (ADS-RF-II and ADS-ND-II) showed most significant hepatoprotective activity as compared to negative (Isoniazid) treated control and quite comparable to standard drug Silymarin.

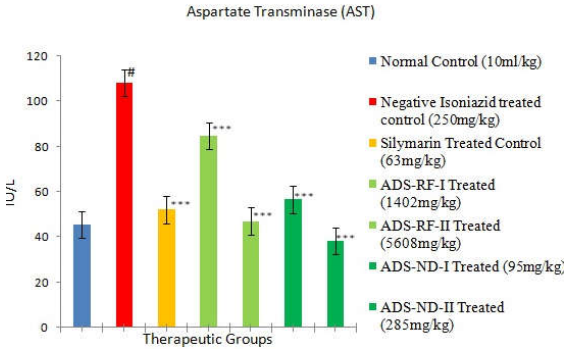

Fig. 2: Effect of ADS-RF and ADS-ND on Aspartate Transaminase (AST) in Isoniazid Induced Hepatotoxicity. Amlycure DS (ADS-RF-II and ADS-ND-II) showed most significant hepatoprotective activity as compared to negative (Isoniazid) treated control and quite comparable to standard drug Silymarin.

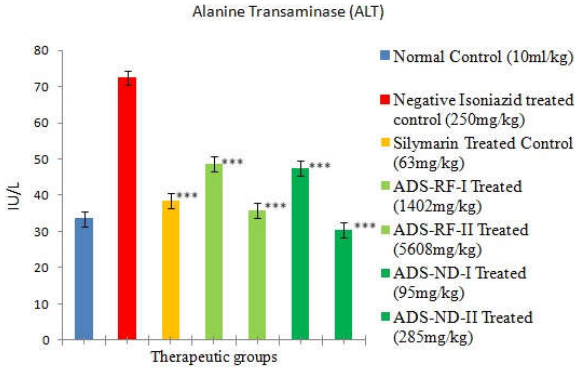

Fig. 3: Effect of ADS-RF and ADS-ND on Alanine Transaminase (ALT) in Isoniazid Induced Hepatotoxicity. Amlycure DS (ADS-RF-II and ADS-ND-II) showed most significant hepatoprotective activity as compared to negative (Isoniazid) treated control and quite comparable to standard drug Silymarin.

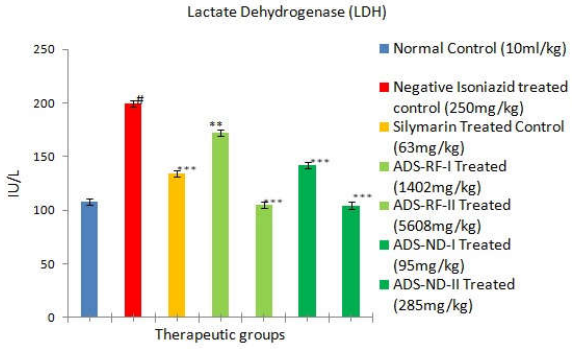

Fig. 4: Effect of ADS-RF and ADS-ND on Lactate Dehydrogenase (LDH) in Isoniazid Induced Hepatotoxicity. Amlycure DS (ADS-RF-II and ADS-ND-II) showed most significant hepatoprotective activity as compared to negative (Isoniazid) treated control and quite comparable to standard drug Silymarin.

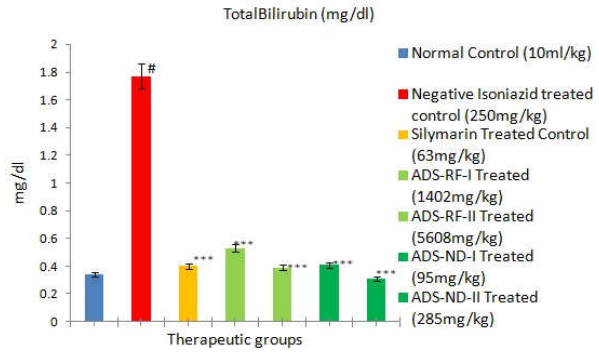

Fig. 5: Effect of ADS-RF and ADS-ND on Total Bilirubin in Isoniazid Induced Hepatotoxicity. Amlycure DS (ADS-RF-II and ADS-ND-II) showed most significant hepatoprotective activity as compared to negative (Isoniazid) treated control and quite comparable to standard drug Silymarin.

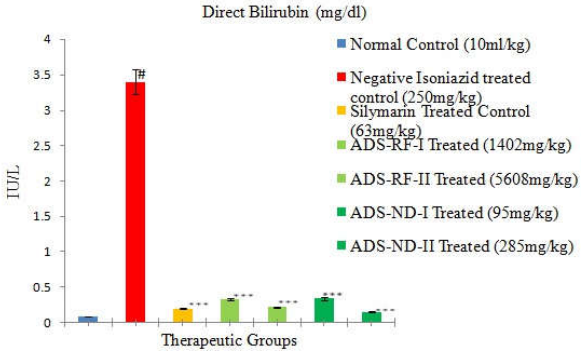

Fig. 6: Effect of ADS-RF and ADS-ND on Direct Bilirubin in Isoniazid Induced Hepatotoxicity. Amlycure DS (ADS-RF-II and ADS-ND-II) showed most significant hepatoprotective activity as compared to negative (Isoniazid) treated control and quite comparable to standard drug Silymarin.

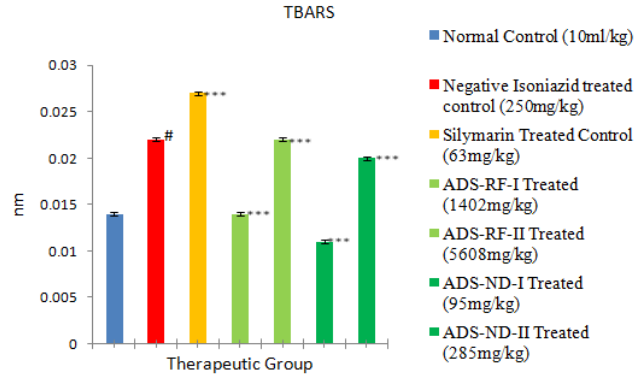

Fig. 7: Effect of Amlycure DS (ADS-RF & ADS-ND) on Anti-oxidant lipid peroxidation activity measured by Thiobarbituric acid reactive (TBARS). Negative control group showed very high level of oxidative stress as comparable to normal control. Amlycure DS (ADS-RF-II and ADS-ND-II) optimum dose of treated and silymarin group effectively present the oxidative stress with their most significant anti-oxidative (lipid peroxidation) activity with TBRAS essay values being comparable with that of normal group.

Histological study:

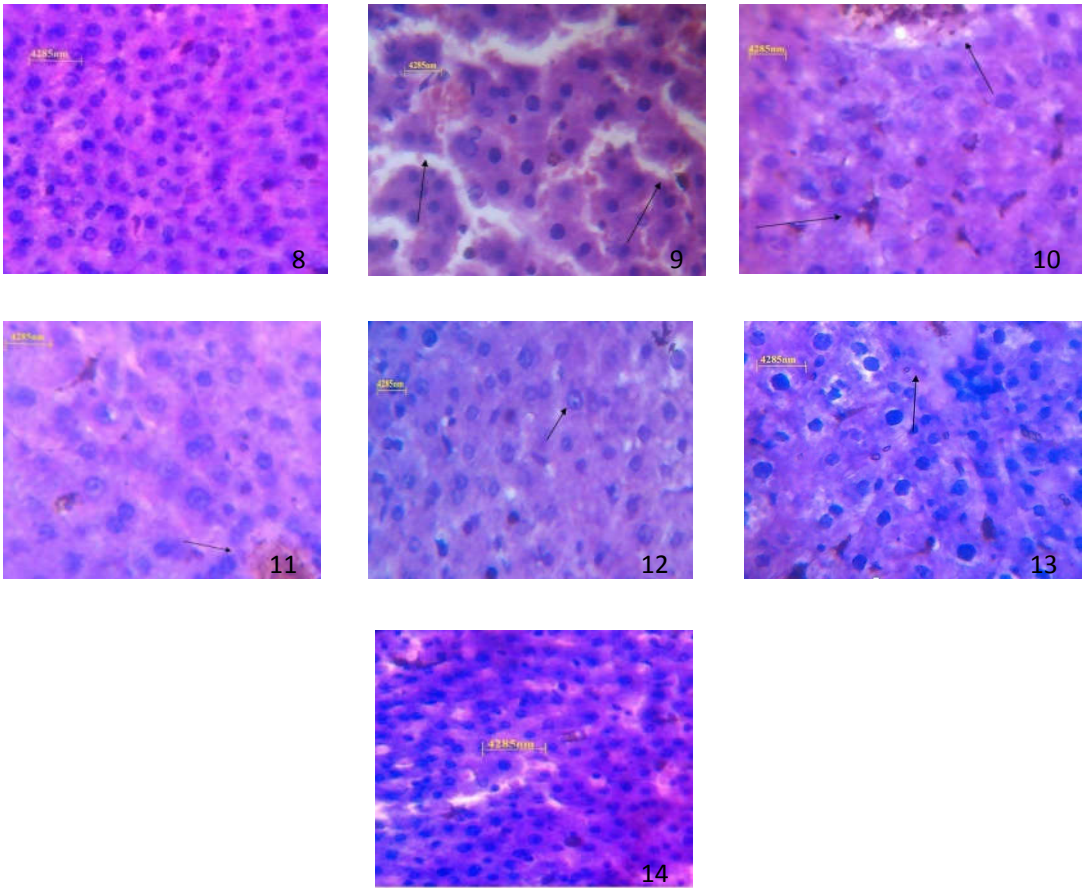

Fig. 8 Normal Control treated group I: Hepatocytes showed the normal lobular architecture of the liver with hepatocyte arranged in single cords.

Fig. 9 Toxicant Isoniazid ((anti-tubercular drugs)– treated group II: Hepatocytes showed granular degeneration, Cell injury in centrilobular zone, vacuolation of cytoplasm as a feature of ballooning degeneration, apoptosis, dropout necrosis, bridge necrosis and inflammation.

Fig.10 Toxicant Isoniazid along with Standard drug Silymarin treated group III: Hepatocytes showed minimal inflammation with less granular degeneration and piecemeal necrosis.

Fig. 11 Toxicant Isoniazid along with ADS-RF-I treated group IV: Hepatocytes showed minimal inflammation with less granular degeneration and piecemeal necrosis.

Fig. 12 Toxicant Isoniazid along with ADS-RF-II treated group V: Hepatocytes showed well brought out hepatic cell with well preserved cytoplasm and cellular boundaries.

Fig. 13 Toxicant Isoniazid along with ADS-ND-I treated group VI: Hepatocytes showed minimal inflammation with less granular degeneration and piecemeal necrosis.

Fig. 14 Toxicant Isoniazid along with ADS-ND-II treated group VII: Hepatocytes showed well brought out hepatic cell with well preserved cytoplasm and cellular boundaries.

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