Research Article

In vivo Antioxidative and Anti-inflammatory activity of *Trigonelline and Andrographoloide* in Dimethyl Nitrosamine Intoxicated liver Inflammation in Albino rats

Javed Iqbal*[a], Sara Zahid[a], Abida jaffar[b], Fatima Zahid[a]
a- Department of Pharmacy, The University of Lahore, Pakistan.
b- Dera Ghazi Khan Medical College, Dera Ghazi Khan, Lahore, Pakistan.
***Corresponding author's E-mail:** javed.iqbalamd@gmail.com

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ABSTRACT

This study was conducted to evaluate the hepatoprotective and anti-inflammatory activity of *Trigonelline* and *Andrographoloide* in different dose regimen to lessen the oxidative stress against Dimethylnitrosamine (DMN) induced toxicity in albino rats. DMN is a well-known hepatotoxin that is widely used to induce toxic liver injury in a range of laboratory animals. Physiological and biochemical potential of active Trigonelline and Andrographoloide was evaluated by the serum levels of some liver enzymes like , ALT,AST, ALP and total protein (TP) ($p<0.05$) which are demonstrated as a marker of liver injury. Moreover another crucial biochemical parameter MDA ($p<0.05$), one of the products of lipid per oxidation was also evaluated with three antioxidants, GSH, SOD and CAT ($p<0.05$). In conclusion, the combined effect of *Trigonelline* and *Andrographoloide* showed most effective action (ALT=50.42, r= 0.868; AST=42.99, r=0.868; ALP= 181.40, r=0.253 ;TP= 4.51, r= -.607;GSH=5.28, r= -.772; SOD=66.23,r=-.854; MDA=51.40, r=0.840 and CAT=31.95, r= -.670) as compared to individual treatment in DMN induced liver injury in rats with maximum recovery supported by histopathological investigations. The current study elucidated that combined effect of active *Trigonelline* and *Andrographoloide* proved to be potent antioxidative and anti-inflammatory mediators which can reduce the hepatic fibrosis with less side effects.

Keywords: Hyperlipidaemia, antioxidants, free radical, reactive oxygen species, oxidative stress, liver inflammation.

INTRODUCTION

Liver, being a metabolic organ, exhibit various functions to maintain the steady state of the body and most of the metabolic syndromes are linked with the performance of the liver. It is responsible for the production of the substances that metabolize the fats; it converts glucose into glycogen for storage in the body and also stores vitamins and minerals. Cholesterol is also produced by the liver. Various diseases are directly related to the liver including hepatitis, cirrhosis and liver cancer. Alcohol is directly involved in altering the normal metabolism of the liver. Like other substances or molecules are metabolized by the liver but consumption of alcohol over long periods of time show detrimental results. Liver is commonly injured by xenobiotics and toxins and lead to the weakening of the liver and hepatic disorders. Including xenobiotics, the metabolism and excretion of drugs are accomplished by the liver. The drugs given in any disorder have some side effects and may affect the hepatocytes like Tagfur, Cytoxan (anti-cancer drugs), Serzone as well as medicines given for the treatment of diabetes [1]. The interest of investigators has been moved towards the herbs because they are used for functional foods and an important source for the preparation of drugs. Hence, herbal medicines have been used worldwide for the treatment of various diseases including disorders of liver[2]. Many hepatotoxins are used in the laboratory for the damaging of liver in animals. Dimethylnitrosamine is one of the commonly used hepatotoxins. The hepatotoxicity induced by the Dimethylnitrosamine shows the membrane damaging by the process of lipid peroxidation (LPO) and lead to the cell necrosis and the metabolism of the body is harmed[3]. Acute toxicity by dimethylnitrosamine is responsible for the altering of gene expression which has been investigated by microarray studies[4]. The secondary metabolites isolated from plants possess a wide range of therapeutic activities and also serve as model compounds for the synthesis of new drugs. These active compounds have been isolated as a result of detailed phytochemical screening of plant extracts[5]. Antioxidants have always been associated with the health benefits of human beings and much attention has been devoted to the natural antioxidants during last few years. Plants have always been a powerful source of natural antioxidants. *Trigonella Foenum-Graecum* commonly known as Fenugreek or Methi has anti-oxidative, anti-lipidemic, anti-fibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating properties. The alkaloids present in the plant belong to pyridine group and mainly include *Trigonelline, gentianine, carpaine and choline. Trigonelline* being an active constituent of *Trigonella Foenum-Graecum* protect completely against harmful increase in the membrane ratios of cholesterol, phospholipids and sphingomyelin, phosphatidylcholine in rats with carbon tetrachloride induced cirrhosis. It is also used in different parts of the world as anti-diabetic, anti-cancer and anti-microbial. A markable improvement against the hepatic injury was diagnosed by the histological examination of the diseased liver tissue treated with *Trigonelline.* This active constituent also showed to impart same therapeutic effects in albino mice

due to CCL_4 and paracetamol induced hepatic pathological states. This active ingredient has also been reported to exhibit activity against the bacterial, pyretic, ulcerative, malarial, immune suppression and venom infections[6]. Further investigation shows that this compound works as antimicrobial at low concentrations and hold their activity as antifungal at relatively higher concentrations [7].

Another active constituent *Andrographoloide* used against the hepatotoxic activity is the principle active ingredient of *Andrographis Lineata* and is used excessively as compared to the allopathic compounds. Previous research reveals the balanced levels of hepatic biomarkers like SGOT, SGPT, serum transaminase, alkaline phosphatase, liver triglycerides and bilirubin in the rats intoxicated with CCL_4 to induce the hepatic injury after treating with methanolic extract of 100mg/kg of andrographolide[8]. The administration of *Andrographoloide,* and other constituents found in *Andrographis Lineata* like *Andrographoloide* and *neoandrographolide* in the peritoneal cavity at a dose of 100mg/kg of the total body weight for one week showed increase in the antioxidant defence mechanism at cellular level and reduction in the lipid peroxidation. Similar clinical effects at equal doses have been appeared for the aqua-alcoholic extracts. These extracts exhibit significant fall in the oxidative stress in AKR mice suffering from lymphomas. Anti-inflammatory effect is due to the *Andrographoloide* as it decreases the production of free oxygen radicals in WBCs specially neutrophils involved in immune system. Urine samples in rats were examined for higher antioxidant level through the oral administration of 1g/kg body weight of the alcoholic extracts of *Andrographoloide*. Ethanolic extracts of leaves, stem and fruits are also potent for these therapeutic effects[9]. The heart of 10-12 week aged Wistar male albino rat showed increase in the relatively lower levels of glutathione as well as the antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase to some extent. The aqua-ethanolic extracts help to decrease elevated lipid peroxidation occurring due to isoproterenol[10].

In past few years, many researchers investigated the incredible effects of many herbs like *Trigonella Foenum-Graecum* (Trigonelline) and *Andrographis Lineata* (Andrographolide) alone on curing of hepatotoxic damages. However, there was no scientific study on the hepatoprotective role of these herbs in different therapeutic combinations which may possible to have a synergistic role against liver damage. In this connection the present work was designed to evaluate the hepatoprotective role of *Trigonelline* and *Andrographoloide* extracts alone and in different therapeutic combinations to assess the key processes, which are responsible to ameliorate the damaging effect of Dimethylnitrosamine.

MATERIALS AND METHODS

Chemicals

All chemical reagents of analytical grades were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Standardized active compounds of *Trigonelline* and *Andrographoloide* were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Induction of hepatic damage

For induction of liver injury in the rats, single intraperitoneal injection of Dimethylnitrosamine was injected (10mg/kg body weight) to rats (*Rattus norvegecus*) then this hepatocellular injury was determined by liver function tests.[11]

Table 1: Experimental Design

Groups	No. of Rats (n)	Treatments
A	**10**	Control
B	**10**	Dimethylnitrosamine @ 3ml/kg b.w. Per week
C	**10**	Dimethylnitrosamine @ 3ml/kg b.w. Per week + *Trigonelline* @ 200 mg/kg b.w.
D	**10**	Dimethylnitrosamine @ 3ml/kg b.w. Per week + *Andrographoloide* @ 50 mg/kg b.w.
E	**10**	Dimethylnitrosamine @ 3ml/kg b.w. Per week + *Trigonelline* @ 200 mg/kg b.w. + *Andrographoloide* @ 50 mg/kg b.w.

Blood and liver separation

At the end of experiment the rats were sacrificed and blood samples were withdrawn through heart puncturing and collected in glass tubes. Serum were separated by centrifugation for 10 minutes at 3000 rpm and stored at -80 ^{o}C until biochemical analysis. Liver lobes were kept in 10 % formalin for histopathological examination.

Biochemical assays for liver function tests

The estimation of AST, ALT [12] and ALP [13] were estimated by using commercially available Bio Merux and Randox kits. Total protein was determined by Lowry method.

Estimation of serum total protein (TP)

Procedure

45 calorimetric test tubes were taken for each sample of rat serum from each group i.e., Group A to Group E. 2.0ml

reagent was taken in all the tubes by pipette and 40µl of each serum sample was taken in tubes labeled as 1,2,3,4,.....45. Contents of tubes were mixed and then incubated at 37°C for 5 min. After incubation 40µl of standard solution from the kit was added to tube labeled as standard. The content were again mixed for 1.0 minutes and then absorbance of sample and standard (Abs S) were measured after 1,2 and 3 minutes at 546 nm.

Calculation

The concentration of TP was estimated by applying the following formula:

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of sample}}{\text{Abs of standard}} \times \text{Standard concentration}$$

Antioxidative analysis

Estimation of reduced glutathione (GSH-Rx)

Liver GSH was estimated according to the method of Edwards [14]. GSH reacts with Elman,s reagent (5,5-dithio bis (nitro benzoic acid) or DTNB) to produce a chromophore TNB with a maximal absorbance at 412 nm and oxidized glutathione GSSG. The amount of glutathione measured represents the sum of reduced and oxidized glutathione in the sample ([GSH]t=[GSH]+2x[GSSG]. The rate of absorbance change (ΔA412nm /min) is made to be linear for the convenience and consistence of measurement, and is linearly proportional to the total concentration of GSH. The concentration of unknown (sample) is determined by calculating from the linear equation generated from several standards of glutathione.

Estimation of catalase (CAT)

Catalase was assayed according to method of Aebi [15]. The estimation was done spectrophotometricaly following the decrease in absorbance at 230 nm. The liver tissue was homogenized in phosphate buffer (ph 7.0) at 1-4°C and centrifuged at 5000 rpm. The reaction mixture contained 0.01M phosphate buffer (ph 7.0), 2mM H_2O_2 and the enzyme extract. The specific activity of catalase was expressed in terms of units/gram of liver tissue. Absorbance values were compared with a standard curve generated from known grades of catalase.

Estimation of thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation in liver tissue was estimated calorimetrically by measuring Thiobarbituric acid reactive substances (TBARS) by method of Ohkawa [16]. To 0.2 ml of sample 0.2ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA were added. Pyridine mixture was added and the contents were vortexed thoroughly for 2 min after centrifugation at 3000 rpm for 10 min the upper organic layer was taken and its OD was read at 532 nm against an appropriate blank. The levels of lipid peroxides were expressed as millimoles of thiobarbituric acid reactive substances per 100 gram of liver tissue using extinction coefficient of 1.5 $\times 10^{5M\text{-}1cm\text{-}1}$

Estimation of superoxide dismutase (SOD)

Superoxide dismutase activity was measured as modified by kakkar[17]. The assay mixture contained 0.1ml of sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M) 0.1 ml of phenazine methosulphate (186µm), 0.3ml of nitro blue tetrazolium (300µm), 0.2ml of NADH (750µm). Reaction was started by the addition of NADH. After incubation at 300 C for 90 sec, the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. The reaction mixture was stirred vigorously with 4.0 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer was separated. The colour intensity of chromogen in butanol layer was measured at 560nm against n-butanol and concentration of SOD was expressed as units /gm of liver tissue. Absorbance values were compared with standard curve generated from known SOD.

Statistical Analysis

The data was subjected to analysis of variance using a COSTAT computer package (Cohort Software, Berkeley, California). The mean values were compared with the least significance difference test. Pearson Correlation was obtained using SPSS analysis version: 16).

RESULTS

Table 2: Ameliorating effect of *Trigonelline* and *Andrographoloide* on Alanine Amino Transferase (ALT, IU/l) with Dimethylnitrosamine Induced Toxicity in Rats (*Rattus norvegicus*)

GROUPS	TREATMENT DURATION			MEAN±SD LSD=3.62
	IST MONTH	2ND MONTH	3RD MONTH	
A	28.30±6.51	28.55±6.18	31.25±3.06	29.37±05.45 h
B	89.75±9.69	94.01±17.74	112.73±13.97	98.93±17.04 a
C	85.20±8.75	68.71±7.75	39.41±4.89	64.44±20.51 b
D	74.23±6.78	62.14±4.89	41.82±3.21	59.40±14.48 c
E	62.86±2.49	53.94±2.97	34.45±4.02	50.42±12.46 d
MEAN±SD LSD=2.09	58.68±20.89 a	53.43±20.13 b	41.91±26.36 c	

Values are mean ± SD from 10 rats in each group; Values not sharing a common letter differ significantly at p<0.05

Table 3: Ameliorating Effect of *Trigonelline* and *Andrographoloide* on Aspartate Amino Transferase (AST, IU/l) WITH Dimethylnitrosamine Induced Toxicity in Rats (*Rattus norvegicus*)

GROUPS	TREATMENT DURATION			MEAN±SD LSD= 2.89
	IST MONTH	2ND MONTH	3RD MONTH	
A	32.46±3.55	31.25±2.70	30.16±3.03	31.29±3.15e
B	64.16±8.21	75.37±10.36	105.07±7.90	81.53±19.53a
C	61.79±7.97	52.48±6.88	40.19±4.69	51.49±11.05b
D	63.03±4.37	55.24±7.58	37.49±5.25	51.92±12.27b
E	52.95±6.39	43.17±4.56	32.84±2.07	42.99±9.49c
MEAN±SD LSD=1.67	52.09±11.68a	44.88±14.43b	39.95±23.92c	

Values are mean ± SD from 10 rats in each group; Values not sharing a common letter differ significantly at p<0.05

Table 4: Ameliorating Effect of *Trigonelline and Andrographoloide* on Alkaline Phosphatase (ALP, IU/l) WITH Dimethylnitrosamine induced Toxicity in Rats (*Rattus norvegicus*)

GROUPS	TREATMENT DURATION			MEAN±SD LSD=37.59
	IST MONTH	2ND MONTH	3RD MONTH	
A	84.48±9.35	78.80±6.27	81.33±6.22	81.54±7.55d
B	167.92±15.63	157.81±31.71	148.13±29.36	157.95±26.89ab
C	165.56±15.12	147.24±12.63	107.04±10.89	139.95±27.84bc
D	169.73±15.49	152.05±8.91	102.44±18.59	141.41±32.33bc
E	154.64±4.29	142.31±1.97	247.26±378.89	181.40±216.40a
MEAN±SD LSD=21.70	142.65±27.86a	134.83±24.97ab	118.11±130.95b	

Values are mean ± SD from 10 rats in each group; Values not sharing a common letter differ significantly at p<0.05

Table 5: Ameliorating Effect of *Trigonella foenum-graecum*, *Andrographis lineata*and *Eclipta alba* on Total Proteins (TP, mg/dl) with Dimethylnitrosamine induced Toxicity in Rats (*Rattus norvegicus*)

GROUPS	TREATMENT DURATION			MEAN±SD LSD= 0.33
	IST MONTH	2ND MONTH	3RD MONTH	
A	6.27±0.48	6.19±0.53	6.16±0.55	6.21±0.50a
B	4.54±0.64	4.13±0.91	3.20±0.89	3.96±0.98e
C	4.40±0.78	5.20±0.56	5.99±0.42	5.20±0.88c
D	4.39±0.45	5.26±0.56	6.86±0.88	5.50±1.22bc
E	4.21±0.40	4.14±0.45	5.18±0.67	4.51±0.70d
MEAN±SD LSD=0.19	4.67±0.87c	5.07±0.87b	5.75±1.26a	

Values are mean ± SD from 10 rats in each group; Values not sharing a common letter differ significantly at p<0.05

Table 6: Ameliorating effect of *Trigonelline* and *Andrographoloide lineata* on Serum Reduced Glutathione (GSH, µg/ml) with Dimethylnitrosamine induced Toxicity in Rats (*Rattus norvegicus*)

GROUPS	TREATMENT DURATION			MEAN±SD LSD= 0.34
	IST MONTH	2ND MONTH	3RD MONTH	
A	7.72±1.06	7.81±0.85	7.99±0.73	7.84±0.87a
B	3.41±0.66	1.89±0.64	1.91±0.67	2.40±0.96e
C	3.56±0.72	5.70±0.51	5.85±0.46	5.04±1.20d
D	4.14±0.90	5.11±0.65	6.07±0.64	5.11±1.07d
E	4.22±0.60	5.18±0.57	6.45±0.63	5.28±1.09cd
MEAN±SD LSD=0.19	4.44±1.41c	5.34±1.57b	6.16±1.72a	

Values are mean ± SD from 10 rats in each group; Values not sharing a common letter differ significantly at p<0.05

Table 7: Ameliorating Effect of *Trigonelline* and *Andrographoloide* on Serum Malondialdehyde (MDA, nmol/ml) WITH Dimethylnitrosamine induced Toxicity in Rats (*Rattus norvegicus*)

GROUPS	TREATMENT DURATION			MEAN±SD LSD= 3.02
	IST MONTH	2ND MONTH	3RD MONTH	
A	43.76±4.33	41.65±2.40	44.22±4.46	43.21±3.88g
B	71.71±13.90	80.69±11.42	90.11±7.91	80.84±13.35a
C	68.21±7.88	62.52±4.07	54.77±4.46	61.83±7.87b
D	63.03±8.52	51.55±6.65	51.03±9.14	55.20±9.69c
E	54.61±4.46	52.35±4.35	47.23±5.50	51.40±5.60d
MEAN±SD LSD=1.74	55.72±11.35a	53.54±12.30b	50.77±15.43c	

Values are mean ± SD from 10 rats in each group; Values not sharing a common letter differ significantly at p<0.05

Table 8: Ameliorating Effect of *Trigonelline and Andrographoloide* on Serum Superoxide Dismutase (SOD, µg/ml) with Dimethylnitrosamine induced Toxicity in Rats (*Rattus norvegicus*)

GROUPS	TREATMENT DURATION			MEAN±SD LSD= 3.28
	IST MONTH	2ND MONTH	3RD MONTH	
A	77.96±4.37	77.04±6.82	77.69±6.19	77.55±5.74a
B	51.59±11.29	46.31±13.00	37.96±15.59	45.29±14.14f
C	54.43±7.11	58.44±8.95	61.86±14.12	58.24±10.58e
D	59.16±3.97	64.89±3.75	71.37±3.38	65.14±6.20d
E	60.87±3.36	66.76±2.86	71.06±2.74	66.23±5.14cd
MEAN±SD LSD=1.89	62.09±8.58c	66.01±10.33b	69.19±14.10a	

Values are mean ± SD from 10 rats in each group; Values not sharing a common letter differ significantly at p<0.05

The data presented in (Table-2) clearly depicts that Dimethylnitrosamine had a significant effect on ALT level in serum. The administration of Dimethylnitrosamine (3ml/kg.B.wt per week) in rats induced increase (29.0%) in ALT level (98.93IU/l) as compared to normal positive control group (29.37IU/l). However when Dimethylnitrosamine treated rats were orally administered with 200mg/kg of *Trigonelline*, ALT level was significantly lowered (64.44 IU/l) by 65.1% followed by 60% decrease in ALT level (59.40IU/l) by treating with *Andrographoloide* (50mg/kgb.wt.) as compared to Dimethylnitrosamine treated rats. Maximum recovery was obtained by treating the rats with the combination therapy of *Trigonelline* (200mg/kgb.wt.) and *Andrographoloid*e (50mg/kgb.wt.) respectively with a significant decreased ALT level of 50.42IU/l (50%) as compared to Dimethylnitrosamine treated rats showing no significant difference with the positive control group (29.37IU/l).

Table 9: Ameliorating Effect of *Trigonelline* and *Andrographoloide* on serum catlase (CAT, nmol/mol protein) WITH Dimethylnitrosamine induced Toxicity in Rats (*Rattus norvegicus*)

GROUPS	TREATMENT DURATION			MEAN±SD LSD= 1.82
	IST MONTH	2ND MONTH	3RD MONTH	
A	32.85±2.71	33.08±2.20	33.56±3.39	33.16±2.73e
B	17.63±3.00	16.24±5.11	16.06±2.28	16.64±2.51g
C	26.15±5.06	30.16±5.29	27.08±3.90	27.79±4.94f
D	31.05±4.78	33.06±4.20	30.86±3.25	31.66±4.11e
E	27.85±5.46	32.06±4.61	35.95±2.11	31.95±5.34e
MEAN±SD LSD=1.05	30.37±12.56c	33.73±6.46b	42.49±17.07a	

Values are mean ± SD from 10 rats in each group; Values not sharing a common letter differ significantly at p<0.05

Time related variations were also recorded with highly significant results achieved. The lowest value of serum ALT (41.9 IU/l) was recorded in the third month followed by 53.43IU/l and 58.68 IU/l in the second and first month respectively. Treatment and time related interactions were also analyzed. Maximum recovery was recorded in the third month with combination therapy of Trigonelline (200mg/kgb.wt.), and Andrographoloide (50mg/kgb.wt.) respectively with the minimum serum ALT level of 34.45 IU/l.

The data depicted in (Table-3) analyzed the effect of *Trigonelline and*, *Andrographoloide* on serum aspartate transaminase (AST) in Dimethylnitrosamine induced rats. The normal level of serum AST observed in rats was 31.29IU/l. But when rats were treated with Dimethylnitrosamine the normal level of serum AST was elevated significantly to 81.53IU/l which is 38.0% increase as compared to normal control group. Oral administration of *Trigonelline* (200 mg/kg body weight) significantly reduces the serum AST level 51.49IU/l (63.15%) as compared to Dimethylnitrosamine treated rats. Monotherapy of *Andrographoloide* (50mg/kgb.wt.) also revealed approximately the same results with lowered AST level 51.92IU/l (63.68%) as compared to Dimethylnitrosamine treated rats. Maximum recovery was obtained by the combination treatment of *Trigonelline (*200mg/kgb.wt.) and *Andrographoloide* (50mg/kgb.wt.) respectively with the lowest serum AST level of 42.99IU/l (41.49%) as compared to Dimethylnitrosamine treated rats which was approximately similar to normal AST level 31.29IU/l. Time related treatment interactions during the period of three months were also considered with dramatically significant results achieved. The minimum value of serum AST level 39.95 IU/l was recorded in the third month followed by 44.88 IU/l and 52.09 IU/l during the second and first month respectively. Combination treatment of *Trigonelline* (200mg/kgb.wt.) and Andrographoloide (50mg/kgb.wt.) provided maximum recovery in the third month with the minimum serum AST level of 32.84 IU/l.

Data pertaining to alkaline phosphatase (ALP) to study the effect of *Trigonelline and Andrographoloide* on liver biochemical marker ALP (IU/l) in Dimethylnitrosamine treated rats for the induction of hepatotoxicity shown in (Table-4) exhibits highly significant effect on serum ALP level. The induction of Dimethylnitrosamine in rats raised the ALP level to 157.95 IU/l (193.7%) as compared to normal control ALP level 81.54IU/l. The rats were treated with *Trigonelline* (200 mg/kg bodyweight) which showed ALP level 139.95IU/l (88.6% decrease compared with Dimethylnitrosamine treated rats). Maximum recovery with minimum ALP level in serum (181.40IU/l) was obtained by the combination treatment of *Trigonelline* (200mg/kgb.wt.) and *Andrographoloide* (50mg/kgb.wt.) showing 70.6% recovery when compared with rats induced by Dimethylnitrosamine only which was very much non-significant with normal control group (83.54IU/l). Time related treatment interactions during the course of three months revealed that maximum ALP level was restored in the third month 118.11IU/l followed by 134.83IU/l and 142.65IU/l of ALP been recorded in the second and first month respectively. The above discussion clearly illustrates the fact that monotherapy of Trigonelline or *Andrographoloide* have no effect in restoring the serum ALP level indicated by the data. However when Trigonelline and Andrographoloide are used in combination in suitable doses, it proved to be the best remedy to alleviate the hepatotoxicity induced by Dimethylnitrosamine.

The data related to total proteins in serum analyzed the effect of *Trigonelline and, Andrographoloide* on total proteins in Dimethylnitrosamine treated rats

(3ml/kg.B.wt per week) leading to significant decrease in total protein level (Table-5). Only 63.7% decrease in total protein level (3.96mg/dl) was recorded in rats treated with Dimethylnitrosamine as compared to normal control group (Maximum value=6.21mg/dl) indicating lower hepatocyte damage. The lower level of total proteins due to the effect of Dimethylnitrosamine was combated with rats treated with *Trigonelline* (200 mg/kg b.wt) with 76.1% increase in total proteins level (5.20mg/dl) when compared to rats treated with Dimethylnitrosamine. The maximum recovery was recorded in the rats undergoing the combination therapy of Trigonelline (200mg/Kg b.wt.) and Andrographoloide (50mg/Kg b.wt.) with the higher level of total proteins in serum 4.51mg/dl with the recovery rate of 69.7% compared with Dimethylnitrosamine treated rats. Highly significant time related variations were also observed. Optimum values of total proteins (5.75mg/dl) were recorded in the third month followed by 5.07mg/dl and 4.67mg/dl at the second and first month respectively. Treatment and time related interactions also showed significant differences among themselves. Maximum value (5.18mg/dl) of total proteins was recorded with combination treatment of Trigonelline (200 mg/kg body weight) and Andrographoloide (50mg/Kg b.wt.) at the third month of treatment. All the above results clearly concluded that Trigonelline treatment is very effective in curing the liver damage caused by various hepatotoxins but it shows comparatively better results when it is used in combination with Andrographoloide. These results are in complete agreement with the work of Azeem[18] that Trigonelline and, Andrographoloide are used as hepatoprotective drugs which recover the oxidative status by scavenging the superoxide anions and interrupting lipid peroxidation caused by toxic chemicals such as Dimethylnitrosamine.

Data presented in (Table-6) showed that Dimethylnitrosamine had a deleterious effect on serum antioxidative status. The lowest value of glutathione (2.40µg/ml) in rats was reported in group receiving Dimethylnitrosamine (3ml/kg b.wt per week) and showed a significant decrease of 30.6% as compared to normal 7.84µg/ml. The treatment with Trigonelline raises the glutathione level to 5.04µg/ml (47.61%) as compared to Dimethylnitrosamine treated rats. This result was shown to be non-significant with the rats undergoing the treatment with Andrographoloide (50mg/Kg b.wt.) having 46.96% increase in glutathione level as compared to Dimethylnitrosamine treated rats. A significant increase in glutathione level (5.28µg/ml) 43% was recorded in rats undergoing the combination treatment of Trigonelline (200 mg/kg body weight) and Andrographoloide (50mg/Kg b.Wt.) showing maximum recovery rate of 41.37% as compared to Dimethylnitrosamine treated rats. It revealed that Trigonelline and Andrographoloide are the best combination therapy to restore the glutathione level in rats treated with Dimethylnitrosamine. The level of glutathione was also considered with different time durations of three months and the optimum recovery was recorded in the third month by the highest value of glutathione 6.16µg/ml followed by 5.34µg/ml and 4.44µg/ml in the second and first month respectively in rats treated with combination treatment of *Trigonelline* (200 mg/kg body weight) and *Andrographoloide* (50mg/Kg b.wt.). All the above results showed that *Trigonelline* and *Andrographoloide* modulate the oxidative stress through decreasing lipid peroxidation and improve the immunity level in the body from incoming toxicant such as medication, drugs, xenobiotics such as paracetamol and Dimethylnitrosamine etc.

The data presented in (Table-7) was studied to analyse the effect of *Trigonelline* and *Andrographoloide* on serum maliondialdehyde (MDA) in Dimethylnitrosamine treated rats which showed highly significant differences among themselves. The normal MDA level recorded was 43.21nmol/ml in rats of positive control group. This MDA level was highly raised to 187% (80.84 nmol/ml) when rats were orally administered with Dimethylnitrosamine (3ml/kgb.w per week). This abnormal increase in MDA level was controlled in rats by treating them with Trigonelline (200 mg/kg body weight) which lowered the MDA level to 76.4% (61.83nmol/ml) as compared to Dimethylnitrosamine treated rats. Monotherapy of Andrographoloide (50mg/Kg b.wt.) lowered the MDA level to 63.5% (55.20 nmol/ml) as compared to Dimethylnitrosamine treated rats. Maximum recovery was obtained by the combination treatment of Trigonelline (200 mg/kg body weight)and Andrographoloide (50mg/Kg b.wt.) with the MDA level of 51.40 nmol/ml which is very close to normal MDA level 43.21 nmol/ml with the recovery rate of 55% as compared to Dimethylnitrosamine treated rats. When the level of recovery was observed with different time durations of three months, the minimum level of MDA was recorded in the third month 50.77 nmol/ml followed by 53.54 nmol/ml and 55.72 nmol/ml been recorded in the second and first month respectively. Time with the combination treatment of Trigonelline (200mg/Kg b.wt.) and Andrographoloide (50mg/Kg b.wt.) with the minimum MDA level of 47.23 nmol/ml. All the above results fully indicate that co- administration of Trigonelline and Andrographoloide is the best option to control the toxicity of Dimethylnitrosaminein rats. These results are in complete agreement with the previous work of Mousle, Wahsha and Li [19, 20, 21] that Trigonelline and Andrographoloide are used as medicines which modulate the oxidative stress by decreasing lipid peroxidation, enhancement of oxidative stress either in a condition of oxidative insult by xenobiotics like Dimethylnitrosamine or other toxic chemicals with the same kind of effects.

The representation in (Table-8) clearly depicts that Dimethylnitrosamine has a significant effect on the level of superoxide dismutase enzyme in serum. The administration of Dimethylnitrosamine (3ml/kgb.wt per week) in rats induced decrease of 171.2% in SOD level (45.29µg/ml as compared to normal SOD level of

77.55µg/ml. The decreased level of SOD in Dimethylnitrosamine treated rats was controlled by treatment with Trigonelline (200mg/Kg b.wt.) with the SOD level of 58.24µg/ml (77.7% increases as compared to Dimethylnitrosamine treated rats). The monotherapy of *Andrographoloide* increased the SOD level to 69.5% (65.14µg/ml) as compared to Dimethylnitrosamine treated rats. Both these results were non-significant with the results obtained by the combination therapy of Trigonelline (200 mg/kg body weight) and Andrographoloide (50mg/Kg b.wt.) with SOD increase of 66% as compared to Dimethylnitrosamine treated rats. Time related variations were also recorded with highly significant results achieved. The highest value of serum SOD level (69.19µg/ml) was recorded in the third month followed by 66.01µg/ml and 62.09µg/ml in the second and first month respectively. Treatment and time related interactions were also analyzed. Maximum recovery was recorded in the third month with combination therapy of Trigonelline (200mg/Kgb.w.) and Andrographoloide (50mg/Kgb.w.) with the maximum SOD level of 71.06 µg/ml.

The data depicted in (Table-9) analyzed the effect of *Trigonelline* and *Andrographoloide* on serum catalase (CAT) level in Dimethylnitrosamine induced rats. The normal level of serum catalase observed in rats was 33.16 nmol/mol proteins. But when rats were treated with Dimethylnitrosamine (3ml/kgb.wt per week), this normal level of serum catalase was declined significantly to 16.64 nmol/mol protein which is 199.2% decrease as compared to normal control group. Oral administration of Trigonelline (200 mg/kg body weight) significantly raised the catalase level 27.79 nmol/mol protein (59.8%) increase as compared to Dimethylnitrosamine treated rats. Monotherapy of Andrographoloide (50mg/Kg b.wt.) also revealed increased catalase level of 31.66 nmol/mol protein (52.5%) increase as compared to Dimethylnitrosamine treated. Optimum recovery was observed in rats undergoing the combination treatment of Trigonelline (200mg/Kg b.wt.) and Andrographoloide (50mg/Kg b.wt.) with the maximum restored catalase level of 31.95 nmol/mol protein showing recovery rate of 34.5% as compared to Dimethylnitrosamine treated rats. Time dependent changes and interactions during the period of three months were also considered with dramatically significant results achieved. The maximum value of catalase level 42.49 nmol/mol proteins was recorded in the third month followed by 33.73 nmol/mol protein and 30.37 nmol/mol protein during the second and first month respectively. Combination treatment of *Trigonelline* (200mg/Kg b.wt.) and Andrographoloide (50mg/Kg b.wt.) provided maximum recovery in the third month with the maximum catalase level of 35.95 nmol/mol protein. All the above results showed that *Trigonelline* and *Andrographoloide* collectively possess more capacity for protection against Dimethylnitrosamine induced toxicity.

Histopathological Investigation

Effect of *Trigonelline* and *Andrographoloide* individually and in different combinations during Dimethylnitrosamine exposure on hepatic histological images of experimental rat of various groups were examined.

GROUP A= Served As Control

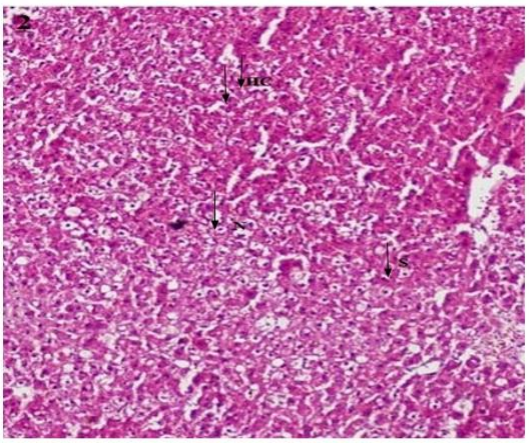


FIGURE: 4.4b Liver section of normal rat showing central vein (CV) with its endothelial lining (thick arrow), normal arrangements of Kupffer cells (KC), normal hepatocytes (HC) architecture with nucleus (N) and normal sinusoid (S). (2): liver section of control rat showing normal hepatic cells (HC), sinusoid (s) and nucleus (N). H & E stain X 200.

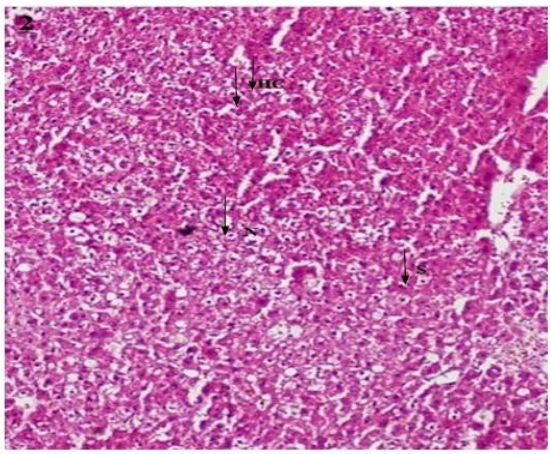
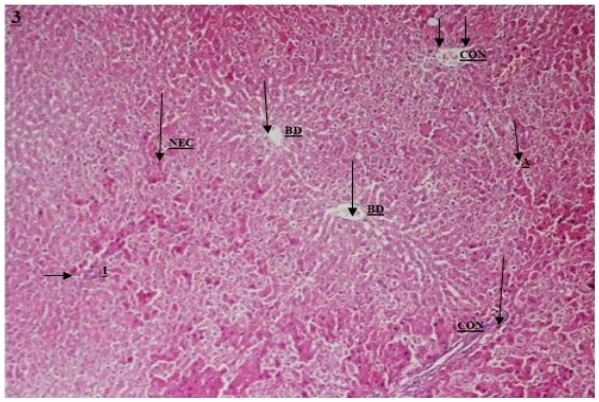
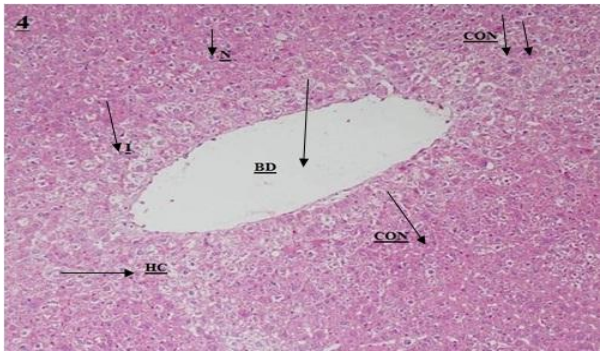


FIGURE: 4.4b Liver section of normal rat showing central vein (CV) with its endothelial lining (thick arrow), normal arrangements of Kupffer cells (KC), normal hepatocytes (HC) architecture with nucleus (N) and normal sinusoid (S). (2): liver section of control rat showing normal hepatic cells (HC), sinusoid (s) and nucleus (N). H & E stain X 200.

GROUP B = Dimethylnitrosamine (3 ml/kg body weight per week)





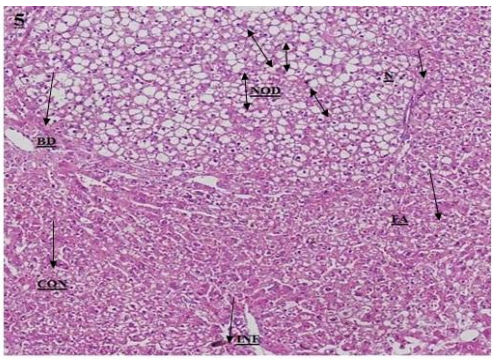


FIGURE: 4.5 Liver transverse section of rat after administration of Dimethylnitrosamine showing massive necrosis (NEC), ballooning degeneration (BD) with fatty degeneration or steatosis, centrilobular hepatic congestion (CON), inflammation (I) and apoptosis (A). (4) Liver section showed severe hepatic lesions (HC), degenerated ballooned (BD) hepatocytes, hepatocytes with open nucleus (N), and inflammatory cells (I) in portal tract. (5) nodular formation with cirrhosis in upper zone showing cirrhotic changes (double head arrows), fatty degeneration (FA), ischemic congestions (CON) and ballooning degeneration (BD) of hepatocytes seen. H & E stain X 200.

GROUP C =Dimethylnitrosamine(3 ml/kg body weight per week) + Trigonelline (200mg/kg body weight)

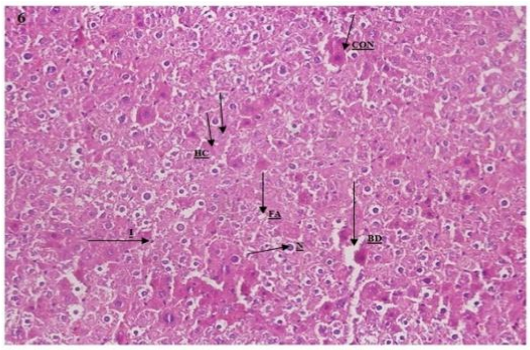


FIGURE: 4.6 Liver transverse section of the rat treated with Dimethylnitrosamine + Trigonella foenum-graecum the section shows lower diameter of hepatocytes (HC), hepatocytes nuclear (N) diameter increase, less ballooning degeneration appear, mild fatty change (FA) and less increase in inflammatory cells in portal tract. H & E stain X 200.

GROUP D = Dimethylnitrosamine(3 ml/kg body weight per week)+Andrographoloide (50mg/kg body weight)

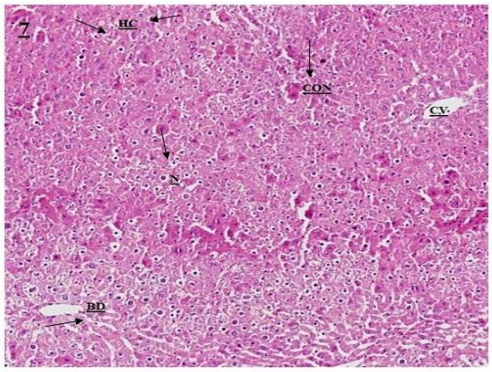


FIGURE: 4.7 Liver section of rat treated with Dimethylnitrosamine + Andrographis lineata showed very less congestions (CON), hepatocytes (HC) with punched out nuclei (N), distinct central vein (CV) and diffuse steatosis or ballooning degeneration (BD) with fatty degeneration. H & E stain X 200.

Group E = Dimethylnitrosamine(3 ml/kg body weight per week)+ Trigonelline (200mg/kg body weight)+ Andrographoloide (50mg/kg body weight)

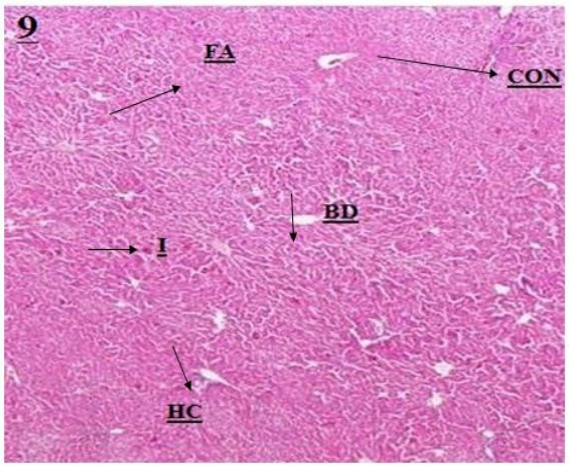


FIGURE: 4.9 Liver transverse section of rat treated with Dimethylnitrosamine +Trigonella foenum-graecum + Andrographis lineata showed very mild fatty change (FA), very mild inflammatory (I) infiltration, regeneration of hepatocytes (HC) and reduced ballooning degeneration appearance. H & E stain X 200.

The histological examination showed normal architecture of liver. Hepatocytes were arranged in trabecules running radiantly from the central vein. Control rat showed normal echo texture, with normal sinusoidal architecture. Normal hexagonal or pentagonal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. They are regular and contained a large spheroidal nucleus. Control rat showed radially arranged hepatic cords around the Central vein and intact sinusoidal pattern. Hepatic sinusoids are normal, with normal diameter of the central vein. Hepatocytes show a normal morphology. Examination of liver rat treated with Dimethylnitrosamine showed that the normal structural organization of the hepatic lobules was impaired and the characteristic cord-like arrangement of the normal liver cells was lost. The central and portal veins were congested. Considerable number of hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization which was so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass cells frequently forming a narrow peripheral rim was left. The nuclei of these cells were entrancing. Central vein and sinusoids between hepatocytes were dilated. Some leucocyte infiltration and fatty deposition was also evident. Ischemic necrosis is seen in isolated areas and Sinusoidal pattern is normal in rest of the regions. Scattered areas of ischemic necrosis are seen, with fatty change. Hepatic nodular formation with cirrhosis was observed in upper zones. Prominent changes in few regions with marked fatty changes like hepatic nodules, showing cirrhotic changes and few cells shows pleomorphism. Liver of animal received Dimethylnitrosamine and Trigonelline showed that most of these histopathological changes were diminished and portal inflammation is mild, limiting plates were intact. In the central vein congestions are prominent. Congestions also present in hepatocytes. No fibrosis occurs. Mostly hepatocytes showed punched out

nuclei. Animals treated with Dimethylnitrosamine + Andrographoloide showed that most of these histopathological changes were diminished but some hepatocytes appeared with vacuolized cytoplasm and kuppfer cells were activated in this group. Hepatic sinusoids are not normal, but normal diameter of the central vein. However early fatty change is present in scattered areas, representing minimal inflammatory changes. Moderate ischemic changes in predominant areas were found. Few sinusoids show minimal dilation. Fatty change is not apparent in this section. Animal treated with Trigonelline + Andrographoloide showed that little sinusoidal dilation is present in isolated regions. Hepatic sinusoids are normal, with normal diameter of the central vein. These histopathological changes were diminished but some hepatocytes appeared with vacuolized cytoplasm and Kuppfer cells were activated. Histopathological changes were diminished and portal inflammation was mild, limiting plates were intact.

DISCUSSION

In the present work experimental animals (*Rattus norvegecus*) were divided into nine groups, ten in each group. The first group was referred as Control group; remaining 8 groups were given Dimethylnitrosamine (3ml/Kg b.wt. per week) in water, twice a week for 6 weeks to induce the hepatic damaging. The Dimethylnitrosamine group was referred as negative control group while remaining groups were treated with Trigonelline (200mg/Kg b.w) and *Andrographolide* (50mg/Kg b.wt) (50mg/Kg b.wt) respectively alone and in different therapeutic. Liver damage score (LDS) could be observed in experimental animals at high values when treated with Dimethylnitrosamine (3ml/kg b.wt. per week) and predict the vascular alterations as well as high degeneration in hepatocytes. As the changes related to Dimethylnitrosamine induced liver injury are in close propinquity to that of viral hepatitis, in the current study Dimethylnitrosamine induced hepatic insult was selected as the experimental model to evaluate the individual as well as combined effect of *Trigonelline* and *Andrographolide* in different dosage and time course of therapy. The mechanism of hepatotoxin action of Dimethylnitrosamine is very complex and is widely accepted mechanism that this hepatotoxin is absorbed in parenchymal cells of the liver and metabolized to convert into free radical by the action of cytochrome P_{450} dependent monooxygenases. These newly produced radicals are covalently attached with biological macromolecules particularly with fatty acids present in the form of phospholipids in plasma membrane. Free radicals initiate the process of lipid peroxidation (LPO) when reacts with polyunsaturated fatty acids (PUFA), in cellular membranes, and lead to the free radical chain reaction process. As a result, peroxidation, chloromethylation and damaging of PUFA take place that totally disturbs the physiological and structural integrity. The lipid peroxidation reaction destroys phospholipids associated polyunsaturated fatty acids which in turn lead to the change in the physiological role of mitochondria, inside the cellular network in the form of endoplasmic reticulum, and most important cellular membrane. Moreover, homeostasis of Ca^{2+} is destroyed leading to the loss of cellular calcium. The catabolic end product of PUFA specially binds to proteins and inhibits the catalytic activity of enzymes. The intoxication of Dimethylnitrosamine leads to hypermethylation of biological macromolecules. Particularly, when t-RNA, r-RNA and m-RNA are formed to produce functional protein, the Dimethylnitrosamine intoxication results in the decreased or inhibition of production of protein molecules. Whereas, when phospholipids bind with protein to form conjugated lipoprotein, the Dimethylnitrosamine intoxication results in the decreased secretion of lipoprotein. A persistent alternation of proliferation and regeneration occur in hepatocytes by consumption of hepatotoxins but absolute results depend on age, treatment time, affected animal state and availability of potentiating agents. Dimethylnitrosamine has been thoroughly studied for its hepatotoxic properties and it also effect on (FRS) free radical scavengers and antioxidants. Liver fibrosis can result in remodeling of hepatic architecture leading to hepatic insufficiency, extracellular matrix proteins and collagen deposition, like phenotype the conversion of hepatic stellate cells (HSC) into myofibroblasts is considered as the central event in fibrogenesis. The final outcome in stimulation of many profibrogenic cytokines takes place, for example (PDGF) platelet derived growth factor, endothelin-1, (TGF-β1) transforming growth factor beta 1 and others. Due to toxicity liver damage then hepatic cells cannot play proper functions in transportation and changes occur in movement of ions of cell membrane leading to cell enzymes escape. Finally in hepatocytes reduced levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) and elevation in serum. Aspartate transaminase (AST) known as aspartate aminotransferase and shows metabolic activity because of its free exchange amino group involve aspartate and glutamate and active enzyme in cell.

In the case of urea cycle aspartate and glutamate perform important steps, particularly responsible for nitrogen excretion and ammonia detoxification. In normal cell metabolism aspartate and glutamate action like second amino acid pool, movement of nitrogen (N) responsible for maintain balance. Compartments of mitochondria and cytosol both have stability after this reaction. Main function is transport of NADH pass into inner membrane of mitochondria after the formation of malate aspartate shuttle an integral part. During hunger most important amino acid alanine secrete from tissue of muscle. In hepatocytes preventing steatosis and maintenance of starvation concentration of blood sugar by is accomplished by alanine transamination, and alanine essential substrate for liver gluconeogenesis. Indication of hepatocytes integrity is elevation of transaminases. Alkaline phosphatase (ALP) present in biliary ducts

cellular lining of liver. Hepatic disorders such as intrahepatic cholestasis and sludge enhance the level of alkaline phosphatase in blood plasma. ALP in decrease level secrete by cells lining the kidney, placenta and intestines. Flow of bile come from hepatocytes along with biliary tract to gallbladder, responsible of suitable concentration of alkaline phosphatase in plasma, hepatic bile system and ALP depends on totally mucosal cells lining. Due to some toxicity organs not play proper work, alkaline phosphatase (ALP) released in the blood and bile not able to excrete this enzyme. Measurement of proper level of bile in small intestine and liver biliary system show integrity by ALP serum level.

Elevation of alkaline phosphatase caused cholestasis from swelling of liver. Mild enhancement of alkaline phosphatase occur by centrilobular lesions but action of these lesions in periportal areas of hepatic lobule excrete high level ALP, result bile flow was impaired. Dramatic high or low level of alkaline phosphatase shows severe conditions of pathophysiological consequences such as periportal fibrosis, inflammation, liver lipidosis, intrahepatic cholestasis and bile sludging. Current results for elevated liver enzymes are in agreement with the previous findings of Yadav *et al*., (2008) that acute Dimethylnitrosamine treatment was responsible for significant damaging of liver tissue as observed by the increased levels of hepatic enzymes and combination therapy of *Trigonelline* and *Andrographolide* showed hepato-protection as shown by the important alterations in hepatic profile[22]. The extracts used in combination showed more protection of liver as compared to either drug alone. Similarly, Priya et al., (2011) documented the increased values of hepatic enzymes and stated the cellular leakage and reduced physiological integrity in hepatic plasma membrane[23]. Moreover, these findings are also in agreement with investigations of Lee *et al*., (2007) and Chaudhary *et al*., (2010) that Trigonelline possess the hepatoprotective activity against wider range of liver damage inducing agents, it is unique largely among other antioxidants and maintains the level of serum ALT that was affected by induction with various toxic agents like Dimethylnitrosamine. Trigonelline and Andrographolide in suitable combined ratio can be used as a drug for the patients of liver disorders as it possesses the great medicinal hepatoprotective property to control the elevated liver biomarkers AST , ALT and ALP levels[24, 25].

Sometimes the cells show their ability to tolerate and repairing mechanism due to damaging by ROS by keeping an appropriate balance between ROS generation and production of antioxidants against ROS. In case of overproduction of ROS, the chemical environment inside the mitochondria is altered because the cellular antioxidative enzymes are unable to neutralize them. Infect the cytochrome C escapes into the cytoplasm because the mitochondrial pore protein that forming the channel through double membrane of mitochondria becomes non-functional and begins the process of apoptosis. The increased levels of oxidants are linked with increased expression of anti-oxidative SOD containing manganese (MnSOD). That is why it has been found that elevated serum MnSOD levels are generally observed in patients with HCC, chronic hepatitis and liver cirrhosis.

The discovery of the role of nitric oxide (NO) and nitric oxide synthase (NOS) family in regulating various physiological processes of the cell has made the phenomenon of oxidative stress more complex than previously realized. NO is a vital ubiquitous molecule which has a very high affinity for superoxide anion radical (O_2^-) as compared with enzymatic and pharmacologic antioxidants. Therefore high levels of peroxynitrite ($ONOO^-$) are particularly followed as a causative agent of oxidative stress. In the normal physiological conditions, neither $O_2^{\cdot-}$ nor NO is proved to be toxic independently because there are efficient antioxidative mechanisms to neutralize them. $O_2^{\cdot-}$ is efficiently neutralized by superoxide dismutases (SOD) and its isoenzymes MnSOD, Cu/Zn-SOD and EC-SOD which are located in mitochondria, cytosol and extracellular compartment respectively. Whereas, NO is removed physiologically from the cell by its rapid diffusion via tissues in to erythrocytes where it reacts with oxyhaemoglobin and quickly gets converted into nitrate. Thus biological half life of NO in vivo is limited to one part of a second. Basically the reaction of NO with O_2^- is continued every time and $ONOO^-$ is produced but when NO is generated in excess, it rapidly combines with O_2^- and produce high levels of peroxynitrite ions ($ONOO^-$) which damages and even kills the cell by overcoming the endogenous SOD because NO is the only biological molecule which possess a very high affinity for O_2. In case of chronic oxidative stress produced by the massive production of $ONOO^-$ such as in hyperglycemia, tobacco smoking, dyslipidemia, prolonged drug use, ordinary antioxidants become ineffective. Various chemotherapeutic strategies are also under consideration to increase the oxidative stress of tumour cells inside the body without affecting the healthy cells. Nathan e Chon was the first who in 1981 employed the glucose oxidase, a potent pro-oxidant agent which acts as H_2O_2 precursor and reported significant decrease in tumour growth with no effects on healthy tissues. Distinct environmental factors or alterations in cellular mitochondria generate high rate of reactive oxygen species (ROS) which results in the increased production of acute intracellular oxidative stress which has recently been related to the progression of hepatocellular carcinoma and chronic liver diseases. Hepatocarcinogenesis involves the down regulation of specific oxidoreductase enzymes comprising the free radical scavenger systems distinctively represented by Catalase (CAT), Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) which is considered the pathological hallmark of HCC. In case of Glutathione Reductase (GSHRx) the rats were treated with suitable doses of Trigonelline at 200 mg/kg and Andrographolide at 50 mg/kg in which the significant results were obtained

by the treatment with Trigonelline at 200 mg/kg body weight with 78.7% recovery as compared to Dimethylnitrosamine treated rats. Maximum recovery was achieved by the combinatorial administration of *Trigonelline* and *Andrographolide* at 50mg/Kg b.Wt. and 50mg/Kg b.w. respectively showing 58.04µ mol/g of GSHRx value.

Table 10: Pearson s' Correlation Coeeficients of different variables in Serum of Rats Under Dimethylnitrosamine Stress Receiveing *Trigonella foenum-graecum* (Trigonelline), *Andrographis lineata* (Andrographolide) and *Eclipta alba* (Wedelolactone) alone and in Different Therapeuti Combinations

VAR.	ALT	AST	ALP	TP	GSH	MDA	CAT	SOD
ALT	1	.868**	.253**	-.607**	-.772**	.840**	-.670**	-.854**
AST		1	.200**	-.624**	-.782**	.816**	-.669**	-.868**
ALP			1	-.191**	-.241**	.200**	-.196**	-.235**
T.P				1	.637**	-.496**	.465**	.605**
BIL.					-.761**	.687**	-.567**	-.762**
ALB.					.774**	-.653**	.540**	.724**
GSH					1	-.694**	.551**	.817**
MDA						1	-.647**	-.816**
CAT							1	.654**
SOD								1

Increased levels of TBARS (80.84 nmol/g vs 43.21 nmol/g) and decreased level of SOD (45.29µg/mg vs 76.75µg/mg), CAT (16.64µg/mg vs 33.16µg/mg) in our study suggested that excessive lipid peroxidation results in tissue damage and failure of antioxidative defence line to prevent the excessive formation of ROS. Administration of Andrographoloide and Trigonelline helped to recover all these cellular changes by increasing the enzymatic antioxidants (SOD, CAT) as well as non-enzymatic antioxidant (GSH) and reducing the TBARS levels in the serum. 100% recovery for GSH, CAT and TBARS, and 96% recovery for SOD were recorded in group F rats receiving combined therapy. The results show that Trigonelline and Andrographolide ameliorate/scavenge the ROS to overcome the oxidative damage caused by using Dimethylnitrosamine for artificially induced hepatic injury and Trigonelline and Andrographolide are used as medicines which modulate the oxidative stress by decreasing lipid peroxidation, enhancement of oxidative stress either in a condition of oxidative insult by xenobiotics like Dimethylnitrosamine or other toxic chemicals with the same kind of effects. Combination treatment of Trigonelline @ 200mg/kg, and Andrographolide @ 50mg/kg provided maximum recovery in the third month with the maximum catalase level of 66.09mg/dl in liver. These results are in complete agreement with Pradeep *et al.*, 2007 that Trigonelline and Andrographolide are used as a medicine for improving the antioxidative status and inhibit lipid peroxidation either in a condition of oxidative insult in response to incoming xenobiotics or other chemicals with the same kind of effects in liver[26].

The time course studies were conducted to evaluate the correlation between healing process and time span. A continuous decreasing trend in ALT (47.64mg/dl, 43.85mg/dl and 34.35mg/dl), AST (48.84mg/dl, 38.88mg/dl, and 27.86mg/dl) and ALP (146.03mg/dl, 133.93mg/dl and 109.56mg/dl) values was recorded after 1st, 2nd and 3rd week in group F rats. This reflects the convincing recovery of ALT (39%, 46% and 91%), AST (49%, 75% and 100%) and ALP (27%, 30% and 65%) towards normalcy after 1st, 2nd and 3rd week respectively as described in Table 10. The time course studies revealed that healing process by Trigonelline and Andrographoloide is directly proportional to a specific time period for serum enzymes. The time course recovery of serum enzymes, SOD, CAT, GSH and TBARS, show a similar behavior as maximum recovery of these chemical attributes was observed after 3rd week of the experiment. A number of histopathological abnormalities are evident in rats receiving Dimethylnitrosamine as hepatotoxin such as cellular necrosis, dilated hepatic sinusoids, degenerated hepatocytes, apoptotic bodies, binucleated cells, focal necrosis, diffuse kupffer cells, and steatosis. Portal area infiltrated by mononuclear inflammatory cellular exudates mainly containing lymphocytes. The results of the current study demonstrated that combined therapy of *Trigonelline* and *Andrographolide* may help to recover the necroinflammatory lesions induced by the ingestion of Dimethylnitrosamine. The previous work of Shaikh *et al.* (2013) and Shaker *et al.* (2010) clearly demonstrated that Trigonelline has anti-inflammatory potential and alter histopathological changes induced by Dimethylnitrosamine, such as necrosis, fatty changes, ballooning, and inflammatory infiltration of lymphocytes around the central veins[27, 28].

CONCLUSION

In conclusion, the potent active ingredients *Trigonelline* and *Andrographoloide* showed a protective role against Dimethylnitrosamine induced toxicity in the form of mono as well as combined therapy against multiple biochemical markers in serum by ameliorating the enzyme activities, anti-lipid peroxidation against ROS through anti-oxidants and particularly hepatic profile. The anticipating results in the present study revealed that the combination therapy/treatment by using the *Trigonelline* and *Andrographoloide* may be fruitful and prosperous for treating the various form of hepatic disorders.

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REFERENCES

1. Pradhan SC, Girish C, Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine, Indian J Med Res, 124, 2006, 491-504.
2. Zarban A and Masood Z, Evaluation of antioxidant properties of silymarin and its potential to inhibit peroxyl radicals in vitro, Pak. J. Pharm. Sci, 21 (3), 2008, 249-254.
3. Radha KD and Yogesh KC, Herbal medicines for liver dis-eases, Digestive Diseases and Sciences, 50(10), 2005, 1807-1812.
4. Harries HM, Fletcher ST, Duggan CM and Baker VA, The use of genomics technology to investigate gene expression changes in cultured human liver cells, Toxicol. In Vitro, 15 2001, 399-405.
5. Dhanabal PS, Elango K, Suresh B, Protective and Therapeutic Effects of the Indian Medicinal Plant Pterocarpus santalinus on D-Galactosamine-induced Liver Damage, Asian Journal of Traditional Medicines, 16, 200, 124-132.
6. Chopra S, Ahmad FJ, Khar RK, Motwani SK, Mehdi S, Iqbal Z, Talegaonkar S, "Validated analysis of the biomarker trigonelline", Anal. Chim. Acta, 577, 2006, 46-51.
7. Chang HM and But PPH, Pharmacology and Applications of Chinese Materia Medica, English translation by Shem Chang-Shing Yeung, Sih Cheng-Yao and Lai-Ling Wang (Chinese Medicinal Material Research Centre, "e Chinese University of Hong Kong), Singapore: World Scientific Publishing Co. Pte. Ltd, 2, 1987, 918-928
8. Bhatnagar SS, Santapau H, Desa JD, Biological activity of Indian medicinal plants. I. Antibacterial, antitubercular and antifungal action, Indian J Med Res, 49, 1961, 799-813
9. Sheeja K, Guruvayoorappan C, Kuttan G, Antiangiogenic activity of Andrographis paniculata extract and andrographolide, Int Immunopharmacol, 7, 2007, 211-221.
10. Shi MD, Lin HH, Lee YC, Inhibition of cell-cycle progression in human colorectal carcinoma Lovo cells by andrographolide, Chem Biol Interact, 174, 2008, 201-210.
11. Amirtharaj GJ, Thangaraj KR, Archana K, Raghupathy V, Ashish G, Eapen CE, Venkatraman A, Pulimood AB, Balasubramanian KA, Ramachandran A, Acute liver injury induced by low dose dimethylnitrosamine alters mediator of hepatic vascular flow, Toxicological Reports, 1, 2014, 707-717.
12. Rietman S, Frankle S, A Colorimetric Method for Determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases, Am. J. Clin. Path, 28. 1957,412-415.
13. Ochoa EA. Serum alkaline phosphatase using phenolphthalein monophosphate in a 12- channel automated system, Clinical Biochemistry, 2, 1968, 71-80.
14. Edwards MJ, Keller BJ, Kauffman FC, Thurman RG, Toxicol, Appl. Pharmacol, 119, 1963, 275-279.
15. Aebi H. Methods in enzymatic analysis, 3rd Ed. New York Academic Press, 1974, 674-684.
16. Ohkawa H, Onishi N, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal. Biochem, 95, 1979, 351-358.
17. Kakkar P, Das B, Viswanathan PN, A modified spectrophotometric assay of superoxide dismutase, Ind. J. Biochem. Biophys, 21, 1984, 131-132.
18. Azeem A, A Khalaf, MM Eldin, SM Mekawy and AM Ahmed, Comparative study on the protective effect of some antioxidant against ccl4 hepatotoxicity in rat. Egyptian journal of nature toxin, 6(1), 2009, 59-82.
19. Mousl HM, Al-Qarawi AA, Ali BH, Abdel-Rehman HA, Moougy SAE, Effect of Lead Exposure on the Erythrocytic Antioxidant levels in goats, Med. A, 49, 2002, 531-534.
20. Washa M and Al-jassabi S, The Role of Silymarin in the protection of Mice liver damage against Microcystin-LR Toxicity, Jordan Journal of Biological Sciences, 2, 2009, 63-68.
21. Li W, Xu X and Zhang H, Secondary metabolites from Andrographis paniculata, Chemical and Pharmaceutical Bulletin (Tokyo), 55(3), 2007, 455-458.
22. Yadav NP, Pal A, Shanker K, Bawankule DU, Gupta AK, Darokar MP and Khanuja SP, Synergistic effect of silymarin and standardized extract of Phyllanthus amarus against CCl_4-induced hepatotoxicity in Rattus norvegecus, Phytomedicine, 15(12), 2008, 1053-1061.

23. Priya V, Jananie RK and Vijayalakshmi K, "GC/MS determination of bioactive components of Trigonella foenum grecum", J. Chem. Pharm. Res, 3(5), 2011, 35-40.

24. Lee CK, Park KK, Lim SS, Park JHY and Chung WY, Effects of the Licorice Extract against Tumor Growth and Cisplatin Induced Toxicity in a Mouse, Xenograft Model of Colon Cancer. Biol Pharm Bull, 30(11), 2007, 2191-2195.

25. Chaudhary GD, Kamboj P, Singh I and Kalia AN, Herbs as liver saver, Indian journal of nature products and resources, 1(4), 2010, 397-408.

26. Pradhan SC and Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine, Indian J Med Res, 124, 2006, 491-504.

27. Shaikh RS, Syed FS and Tayade DT, "Phytochemical analysis of leaves of Trigonella foenum- graecum L. from anjangaon, amravati region of maharashtra state", Int. J. Pharm Pharm. Sci. Res, 3(3), 2013, 94-96.

28. Shaker E, H Mahmoud H and Mnaa S, Silymarin, the antioxidant component and Silybum marianum extracts prevent liver damage, Food and Chemical Toxicology, 48, 2010, 803-806.

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