Pharmacological Evaluation Of Ibuprofen With Methotrexate In DENA Induced Hepatocellular Carcinoma

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Abstract
This research was an attempt to examine the pharmacological role of ibuprofen and methotrexate combination against hepatic cancer in Wistar rats. One time DENA (200 mg/kg i.p.) was used for generation of liver cancer in wistar rats. The total rats were separated into five different groups. DENA received rats were given with unadulterated ibuprofen (40 mg/kg, p.o.) and methotrexate (5mg/kg, i.p.) for the duration of 18th weeks. The procedure was ended by the 18th week. Results were determined by the serum parameters and liver homogenate parameters. Additional, this combination of ibuprofen and methotrexate possesses the significant liver cells protective effect against DENA-induced hepatocellular carcinoma (HCC) by adjusting the liver enzymes and other biochemicals. The above results declared that ibuprofen and methotrexate combination has highly effective treatment of hepatocellular carcinoma.

Key Points: Methotrexate; Diethyl nitrosamine; alpha-feto protein; ibuprofen; hepatoprotective

Introduction
Hepatocellular carcinoma (HCC) is a key malignant cell of the liver. HCC is now the third most important cause of cancer deaths globally. The occurrence of HCC is maximum in Asia and Africa, where the widespread high occurrence of hepatitis powerfully predisposes to the expansion of persistent liver disease and following growth of HCC.[1]
More than the long-ago 12 years, the occurrence of liver cancer has more than trippled, from 2.8 to 6.8 per 100,000 populations. The mortality rate has equally improved from 3.1 to 5.1 per 100,000 populations more than long-ago 4 years[2].

High dangerous causes
The hazard of HCC, the majority general type of liver cancer, is advanced in public with lasting hepatic diseases. It's also elevated if the hepatic cell is scarred by infection with hepatitis. Hepatocellular carcinoma is more universal in community who consume huge amounts of ethanol and who have a buildup of fat molecules in the hepatic cells[3].

MATERIALS ALONG WITH METHODS:
Animal: Wistar rats (male)
Number of animals in each group: 6
Total number of groups: 5
Study duration: 8 weeks
Ibuprofen Dose: 40 mg/kg/day; p.o.
Methotrexate Dose: 5 mg/kg body weight per week; two divided doses, i.p
DENA Dose: 200 milligram per kilogram; intra peritoneal (i.p. single dose)

Groups: Albino Wistar male/female rats were separated into five groups and every group contains six rats.
Group-I Ordinary control (NC) rats were given with normal diet and were not given any treatment during the research study.
Group-II DENA (Diethyl nitrosamine) control (DC)
Group-III DENA + MTX control (DM)
Group-IV DENA + MTX + Ibuprofen control (DMI)
Group-V DENA + Ibuprofen control (DI)

Induction of HCC: HCC were induced by the i.p. route, 200mg/kg DENA (chemical carcinogen).
Statistical study: The outcomes were showed as mean ± S.E.M. arithmetical difference was experienced by using one-way analysis of variance (ANOVA) followed by Dunnette's multiple judgment test. A difference in the mean p value <0.05 was considered as statistically significant.

EVALUATION PARAMETERS:
Body weight:
Insertion criteria: Normal and fit animals weighing between 140-280 grams in favor of rats were incorporated in this research.
Exclusion criteria: The wistar rats which do not come the above weight between 140-280 grams were excluded from research.
Serum profile: Following parameters were required to be examined:
- Serum Glutamic Pyruvate Transaminase (SGPT)
- Serum Glutamic Oxaloacetate Transaminase (SGOT)
- Alkaline Phosphatase (ALP)
- High Density Lipoprotein (HDL)
- Total Cholesterol (TC)
- Triglycerides (TG)
- Uric Acid
- Urea
- Bilirubin
- Alpha Feto Protein (AFP)

Blood profile: Following parameters were required to be examined:
- Hemoglobin (Hb)
- Total leukocyte (TLC)
- Differential leukocyte count (DLC)
- Erythrocyte sedimentation rate (ESR)

Antioxidant: Following parameters were required to be examined:
- Superoxide dismutase (SOD)
- Catalase
- Lipid per oxidation (LPO)
- Glutathione (GPX)

STATISTICAL STUDY:
Every value were showed as Mean ± SEM from 6 animals in each groups. Outcomes were subjected to statistical analysis using one-way analysis of variance called ANOVA. $p<0.05$ were measured as statistically significant.

RESULTS
Table: 1 Effect of ibuprofen and methotrexate combination on serum cholesterol, triglycerides and high density lipoproteins in DENA induced HCC.

Groups	Cholesterol	TG	HDL
NC	75.16 ± 6.17	65.89 ± 6.32	58.15 ± 6.29
DC	169.05 ± 12.33^{x}	147.04 ± 12.31^{x}	19.22 ± 1.32^{x}
DENA + DM	102.90 ± 10.78^{b}	80.39 ± 6.21^{b}	37.60 ± 2.94^{a}
DENA + DMI	79.53 ± 6.28^{c}	67.92 ± 6.82^{c}	53.05 ± 4.17^{c}
DENA + DI	166.75 ± 12.05	148.21 ± 12.51	56.77± 5.32

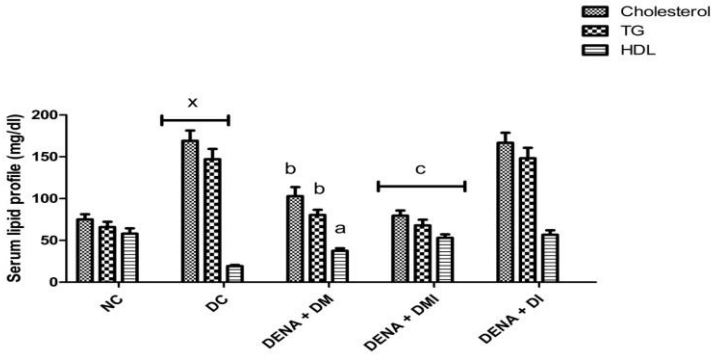
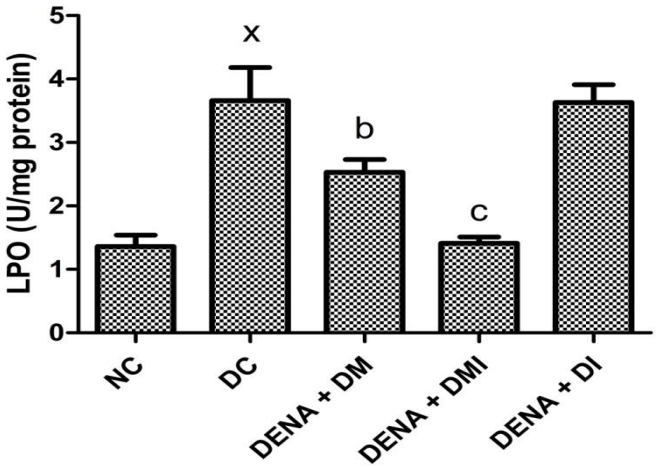
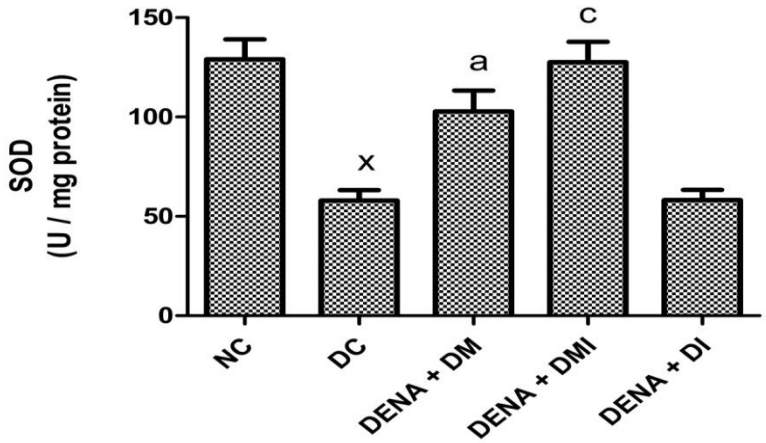
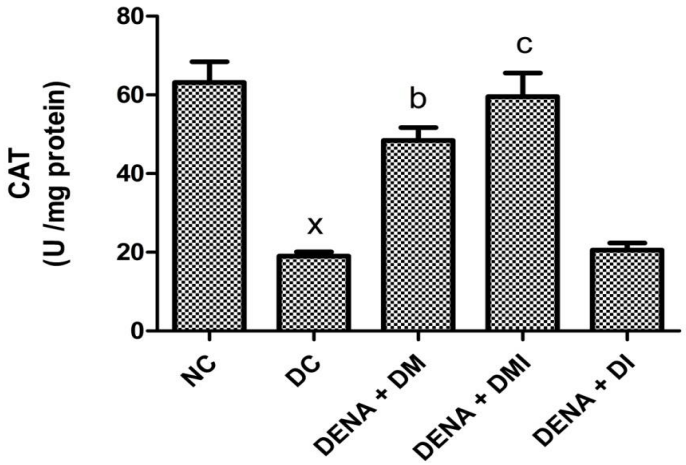


Table: 2 Effect of ibuprofen and methotrexate combination on antioxidant enzymatic activities in DENA induced hepatocellular carcinoma

Groups	LPO U /mg protein	SOD U/ mg protein	CAT U /mg protein	GPX mmol/mg tissue
NC	1.36 ± 0.18	129.05 ± 10.05	63.17 ± 5.27	8.16 ± 0.67
DC	3.66 ± 0.52[x]	57.93 ± 5.27 [x]	19.06 ± 1.04 [x]	2.68 ± 0.18 [x]
DENA + DM	2.53 ± 0.20 [b]	102.76 ± 10.55[a]	48.41 ± 3.28[b]	6.74 ± 0.25 [b]
DENA + DMI	1.41 ± 0.10 [c]	127.61 ± 10.14 [c]	59.55 ± 5.99[c]	8.05 ± 0.61 [c]
DENA + DI	3.63 ± 0.28	58.15 ± 5.21	20.59 ± 1.78	2.74± 0.94







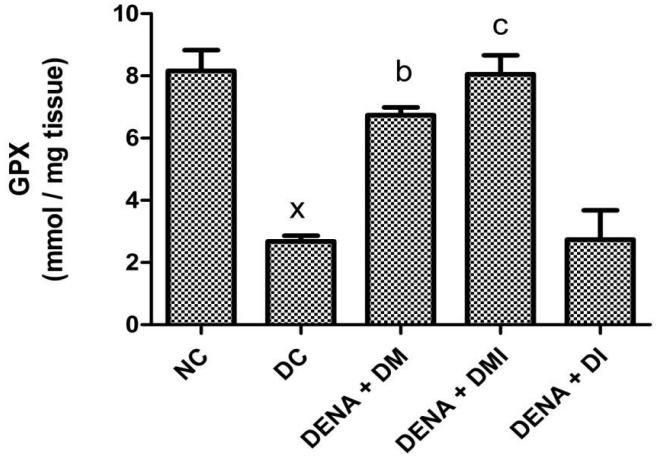
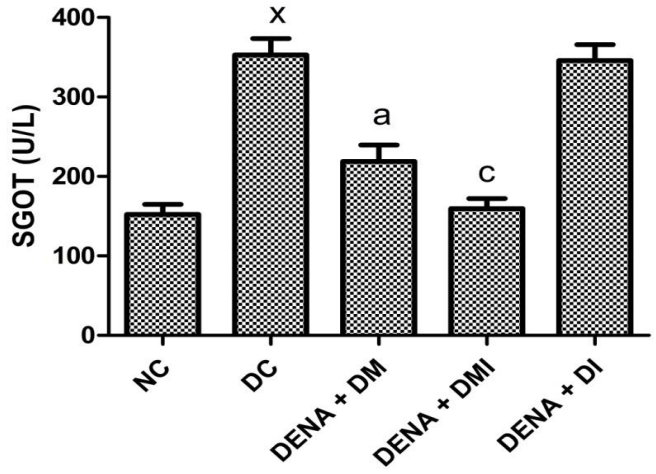
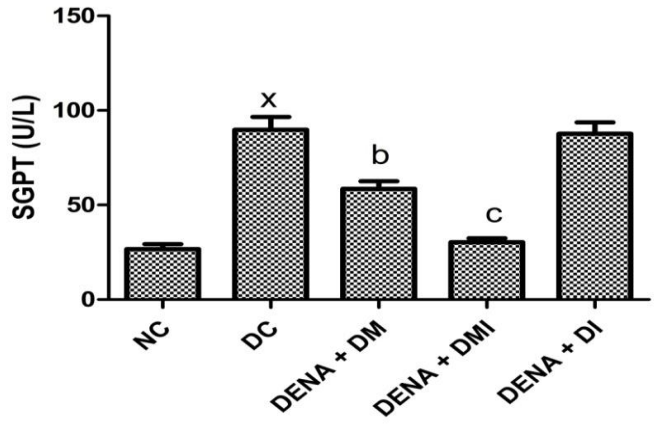


Table:3 Effect of ibuprofen and methotrexate combination on liver function parameters in DENA inducedhepatocellular carcinoma

Groups	SGOT(U/L)	SGPT (U/L)	ALP (U/L)
NC	152.16 ± 12.83	26.64 ± 2.71	268.58 ± 20.32
DC	352.75 ± 20.73 [x]	89.79 ± 6.78 [x]	496.07 ± 32.17 [x]
DENA + DM	218.68 ± 20.95 [a]	58.52 ± 4.06 [b]	352.17 ± 32.84 [b]
DENA + DMI	159.53 ± 12.55 [c]	30.28 ± 2.17 [c]	276.06 ± 20.05 [c]
DENA + DI	345.76 ± 20.06	87.63 ± 6.06	490.47 ± 36.27





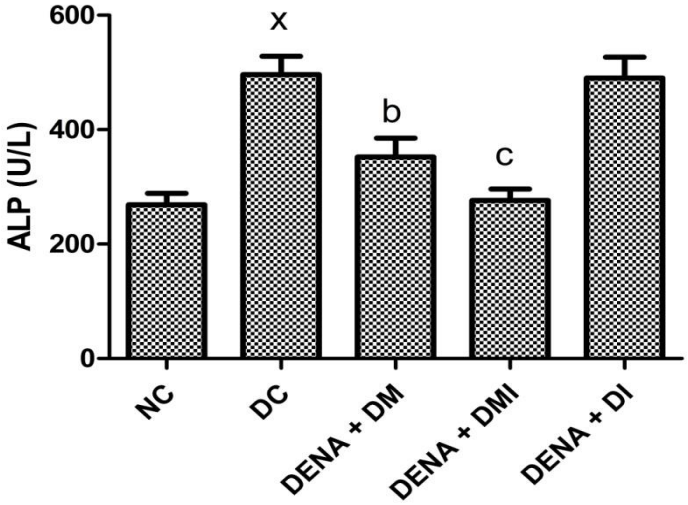
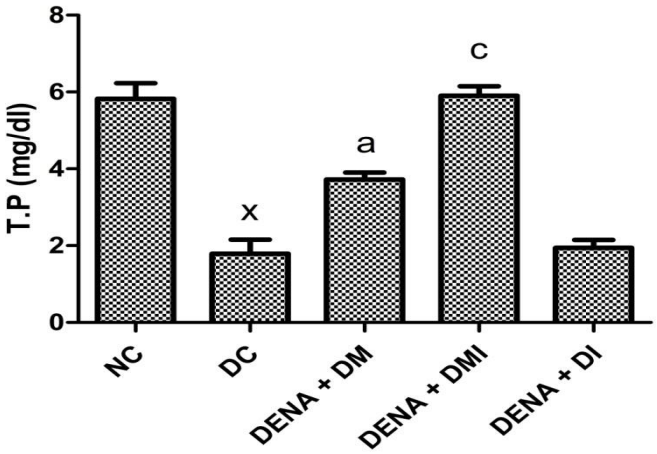
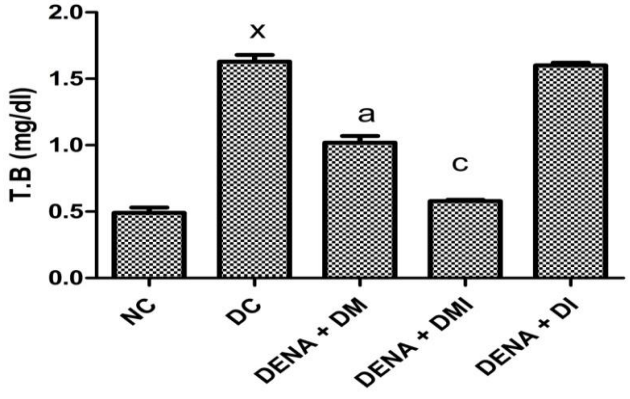


Table:4 Effect of ibuprofen and methotrexate combination on liver function parameters in DENA inducedhepatocellular carcinoma.

Groups	T.P (mg/dl)	T.B (mg/dl)	AFP (mg/dl)
NC	5.82 ± 0.41	0.49 ± 0.04	18.26 ± 1.21
DC	1.79 ± 0.37 [x]	1.63 ± 0.05 [x]	84.07 ± 5.48 [x]
DENA + DM	3.72 ± 0.18 [a]	1.02 ± 0.05 [a]	49.10 ± 2.19 [b]
DENA + DMI	5.90 ± 0.25 [c]	0.58 ± 0.01 [c]	25.64 ± 1.28[c]
DENA + DI	1.94 ± 0.21	1.60 ± 0.02	80.95 ± 5.06





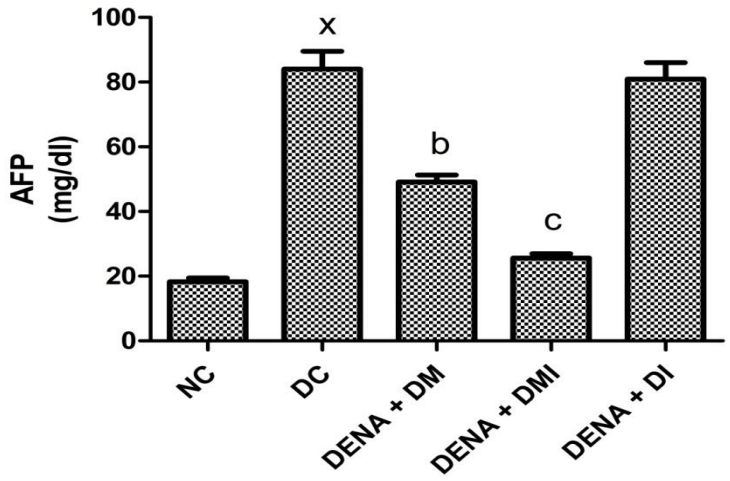
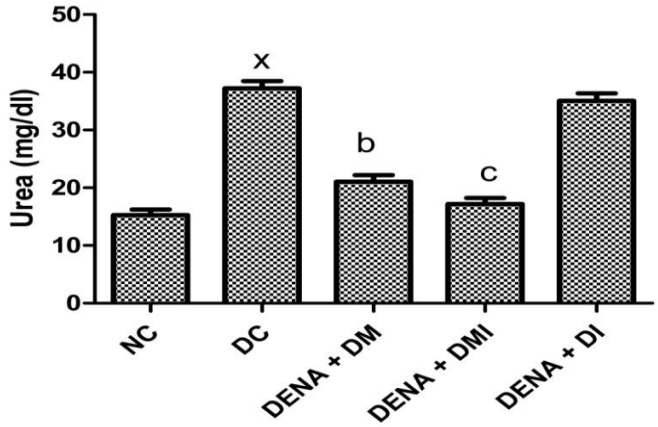
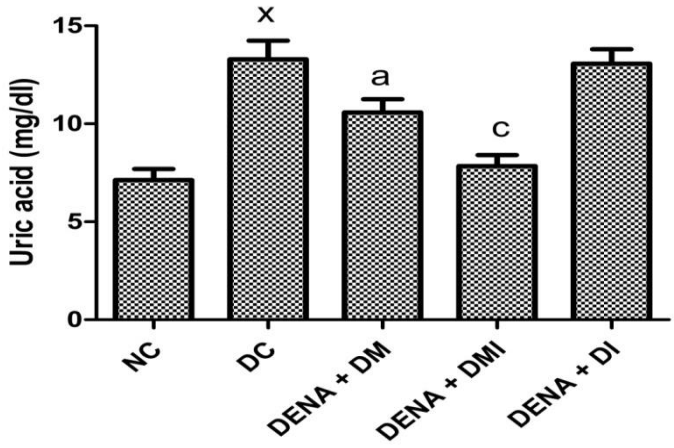
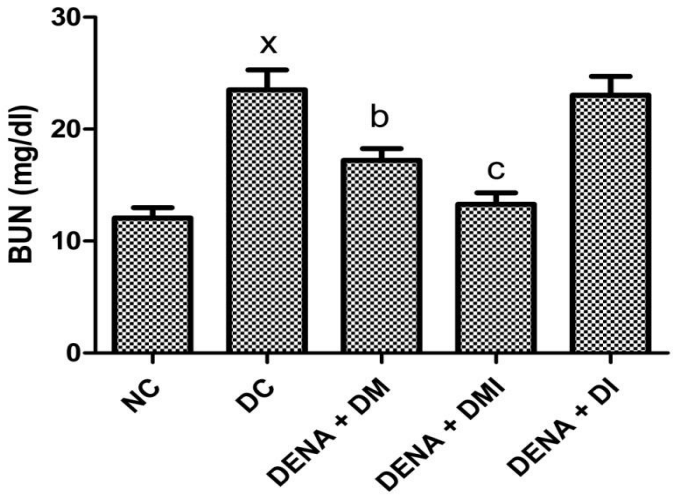


Table: 5 Effect of ibuprofen and methotrexate combination on kidney function parameters in DENA induced hepatocellular carcinoma

Groups	Urea (mg/dl)	Uric acid (mg/dl)	BUN (mg/dl)
NC	15.27 ± 0.94	7.13 ± 0.56	12.05 ± 0.94
DC	37.22 ± 1.26[x]	13.29 ± 0.95 [x]	23.51 ± 1.78 [x]
DENA + DM	21.08 ± 1.09 [b]	10.58 ± 0.67 [a]	17.22 ± 1.04 [b]
DENA + DMI	17.20 ± 1.02 [c]	7.84 ± 0.56 [c]	13.29 ± 1.03 [c]
DENA + DI	35.05 ± 1.32	13.07 ± 0.74	23.05 ± 1.67







Discussion

The results of present research showed a synergistic effect of ibuprofen with methotrexate against DENA induced hepatocellular carcinoma (HCC) in rats. A significant ($P<0.001$ for ibuprofen with methotrexate) levels of Specific marker bioenzymes like LPO, SOD, CAT, GPX, SGOT, SGPT, ALP, T.P., T.B., AFT, in DENA induced hepatocellular carcinoma rat model indicates synergistic effects of ibuprofen with methotrexate.

The research analysis showed that LPO level remained significantly elevated in DENA induced group-II as compared to control-group-I. The LPO intensity was significantly increased in Group- IV (Ibuprofen with methotrexate combination).

The research showed that SOD, CAT, and GPX levels remained significantly diminished in DENA induced group-II as compared to control group-I. The superoxide, catalase and GPX levels were significantly increased in Group-IV.

The results of the haematological parameters like Haemoglobin, number of Erythrocytes, Leukocytes, Neutrophils, Lymphocytes, and Monocytes indicates synergistic effect of ibuprofen with methotrexate on the haemopoietic system.

A significant ($P<0.001$ for ibuprofen with methotrexate) levels of Cholestrol, Triglycerides and High density lipoproteins in DENA induced hepatocellular carcinoma rat model indicates synergistic effect of ibuprofen with methotrexate.

A significant ($P<0.001$ for ibuprofen with methotrexate) levels of Urea, Uric Acid, BUN, in DENA induced hepatocellular carcinoma rat model indicates synergistic effect of ibuprofen with methotrexate.

The assessment of biochemical parameters like serum total bilirubin (T.B), SGOT, SGPT, ALP, T.P. and alpha-fetoprotein (AFP) parameters tested which showed a significant ($P<0.001$ for ibuprofen with methotrexate) effect of ibuprofen with methotrexate combination. The analysis showed that serum total bilirubin (T.B.), SGOT, SGPT, and ALP remained significantly elevated in DENA induced group-II as compared to control group-I. The T.B. SGOT, SGPT, and ALP levels were reduced significantly in Group-IV.

The results showed that T.P. remained significantly decreased in DENA induced group-II and This total protein level was increased extensively in Group-IV that indicates combination of ibuprofen and methotrexate was very effective to treat hepatocellular carcinoma.

The alpha-fetoprotein level was mainly common interpreter for diagnosis of liver cancer. The research analysis showed that ibuprofen and methotrexate combination decreases the serum alpha-fetoprotein level that was increased significantly in the DENA-induced group-II. The results of serum SGOT, SGPT, T.B. and ALP were extensively increased in the DENA-induced group-II as compared with the control group-I but these results were decreased significantly by ibuprofen with methotrexate combination. In addition, DENA induced the significant lowering the level of GPX, CAT, T.P. and SOD activities of liver enzymes and increased significantly in Group-IV that indicates combination of ibuprofen and methotrexate was very effective to treat liver cancer.

Summary and Conclusion

The liver has an essential role in life via its metabolic and detoxification capability. The present research was carried out to generate synergistic data against in-vivo hepatocellular carcinoma of ibuprofen with methotrexate combination. DENA (Diethylnitrosamine), a potent hepatotoxic agent that induces HCC. In present research the ibuprofen with methotrexate combination was used for evaluating the synergistic effect of Ibuprofen with methotrexate. The synergistic activity of Ibuprofen with methotrexate was confirmed by significant enhancement in SOD, CAT, GPX and decrease in LPO, ALP, SGOT, SGPT, AFP, T.B. Levels and also decrease WBC count in DENA induced rats.

Acknowledgements

This was PhD work of Mr. Tarique Hussain under supervision of Dr. Abdul Hafeez. Authors are thankful for Glocal University, Saharanpur, Uttar Pradesh, India for providing research facilities.

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