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ASSESSMENT OF HEMATOLOGICAL MARKERS USING THYMOQUINONE AS EXPERIMENTAL AND SILYMARIN AS STANDARD DRUG AGAINST HEPATIC INSULT CAUSED BY FIRST LINE ANTI TUBERCULOSIS DRUGS

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ABSTRACT

Gram negative rod *Mycobacterium tuberculosis* is the underlying causative agent for the progress of life threatening disease tuberculosis. First line antituberculosis drugs are main choice for physician to counter this dangerous disease, by producing promising effect to counter *Mycobacterium tuberculosis*, the selected group of drugs produce hepatotoxicity. In this study Thymoquinone, main constituent of *Nigella sativa* was used as experimental drug while silymarin as standard drug. The thymoquinone found to produce hepatoprotective effects in rabbits treated anti tuberculosis drugs. Thymoquinone showed remarkable result and persisted successful in achieving level of ALT, which is 17.09 (SD±1.39) close to the usual mean value of 18.96, results were found significant (P value < 0.005). Thymoquinone in both 40mg and 20 mg dose found effective but the result by TQ 40mg were extraordinary by achieving the level to (50.27 SD±5.88) very near to the normal mean level of (48.10) in contrast with standard drug silymarin (56.96 SD± 8.36). One more intense effect was observed on day 7, when TQ cut the value down from (54.18) on same day silymarin (70.00) was unable to change the level which prove the short onset of action of thymoquinone as compare to standard drug. P value < 0.005 were found statically significant.

KEYWORDS: *Mycobacterium Tuberculosis*, Silymarin, Thymoquinone, ALT, AST.

INTRODUCTION

Tuberculosis (TB) is counted among foremost communicable diseases. Globally it is considered as one of the chief cause of morbidity and mortality.[1] It has been conversed in previous studies that around 2 billion individuals are at risk of this disease who have been exposed to this bacillus which is necessary cause of TB.[2, 3, 4] Once an individual get infection of this disease, he become carrier for many years. It is estimated that an individual with active infection of TB may infect other 10 to 15 persons on average yearly.[5] The infectious or causative agent is an airborne pathogen and transmits through air droplets.[6, 7, and 8] The best available treatment is first line antituberculosis drugs consisting of Isoniazid, Rifampicin, Pyrazinamide and Ethanbutol.[9,10] However in some studies it has been documented that prevention of infection chemotherapy is foremost effective which is also called primary chemoprophylaxis, for this isoniazid is widely used.[11, 12, 13, 14] Among the above discussed drugs Isoniazid, Rifampicin and Pyrazinamide are known to be causative agents of cytotoxicity, mainly for liver cells. This drug induced hepatic toxicity often triggers the termination of TB treatment and also the drug resistance in underlying mycobacterium population.[15] Isoniazid and Rifampicin are also considered as culprit for hepatotoxicity in the host.[16] Since years Silymarin is being used as substitutional medicine for various hepatic pathological conditions, it is well known member of Aster family. Acknowledged because of holding no detrimental impact, Silymarin could even be used at higher concentrations.[17] In studies using mice it has been observed that the cyto-protective effect of this drug in liver is actually due to inhibition of cyclooxygenase cycle and leukotrienes, these affects also reduce inflammation.[18] It is also evident from previous studies that silymarin provides protection from genomic injury , it also decrease the activeness of tumor promoters, slow down metabolism of calcium and also cause stabilization of mast cells.[19] Evidences have shown it to influence immunity by modifying anti oxidative and anti-fibrotic qualities with increasing translational and transcriptional activities.[20] Silymarin through forming a layer around hepatic cell halts the access of toxins in them.[21]

Organic remedies have long been consumed as remedy

for hepatic ailments. Nigella sativa is one such plant and its seeds have long been taken as cure for hepatic, inflammatory and also malignant malfunctions. Among the other extracted ingredients like Carvacrol and Dithymoquinone, Thymoquinone is one of the plant's main constituent. With a chemical formula $C_{10}H_{12}O_2$ and mass of 164.2g/Mol., its chemical name is 2-Isopropyl - 5Methybenzo-1, 4 Quinone. While since 2013, Thymoquinone is being used as a drug for its actions against hepatic injuries caused by anti-tuberculosis drugs. It exerts its protective effects by normalizing the different liver functions markers including Enzymes like ALT & AST, proteins like Albumin, Bilirubin and Cholesterol.[22] Owning to diverse function liver has the vital eminence in the body while hepatic diseases are themselves elementary reason of global mortality.[23] For various hepatic ailments several herbs like Silybum marianum (milk thistle) has been proven to cure them. Being the indigenous herb of Mediterranean region Silybum Marianum encloses several different Flavolignanas, including Silybin. This plant holds some active elements in their seeds consisting of four flavonolignans and these are known as silymarin.[24]

MATERIAL AND METHODS

A cross sectional comparative study was conducted at pharmacology lab of Johar Institute of Professional Studies (JIPS), Lahore to evaluate the hepatoprotective effect of thymoquinone. The study was approved by ethical committee of JIPS. Drugs and Chemicals: RIF and INH having 99% purity were obtained from Pacific Pharmaceuticals Ltd, Lahore Pakistan and Silymarin of same purity from Abbot Laboratories, Karachi, Pakistan, while TQ was purchased from Sigma-Aldrich (USA). All the chemicals and reagents used in this study are of analytical grade. Preparation of Experimental Animals and Grouping: Forty (40) Male adult albino rabbits of same breed and of 4 months of age was purchased from the animal house of University of Veterinary and Animal Sciences, Lahore, Lahore. They were future kept in animal house of Johar Institute of Professional Studies, Lahore for acclimatization before the start of experimental procedure. They were kept under the standard control temperature (25±3) and humidity. They were kept under natural light and dark cycle. All rabbits were fed on standard diet and water *ad libitum*. Rabbits were randomly divided in five groups, each group contain 8 rabbits.

S/No	Groups	Diet and Drug
1	Group 1: Normal Control	Routine Diet along with water ad-libitum for 0-28 days
2	Group 2: Treated with hepatotoxic Drugs	Routine diet + Isoniazid (50mg/kg body weight) + Rifampicin (250mg/kg body weight) P.O. for 0-28 days as hepatotoxic drugs.
3	Group 3: : Treated with hepatotoxic + standard hepatoprotective group	Routine diet + Isoniazid (50mg/kg body weight) + Rifampicin (250 mg/kg body weight) + Silymarin (100mg/kg body weight) for 0-28 days
4	Group 4: : Treated with hepatotoxic drugs + Thymoquinone	Routine diet + Isoniazid (50mg/kg body weight) + Rifampicin (250 mg/kg body weight) + Thymoquinone (20mg/kg body weight) for 0-28 days
5	Group 5: : Treated with hepatotoxic drugs + Thymoquinone	Routine diet + Isoniazid (50mg/kg body weight) + Rifampicin (250 mg/kg body weight) + Thymoquinone (40mg/kg body weight) for 0-28 days

Blood Sampling: Blood from all the rabbits were collected in heparinized tubes (for hematological analysis) and in simple glass tubes (for the collection of serum). 5 blood samples were drawn from each rabbit on day 0, 7, 14, 21, 28. Blood collected for serum extraction after half an hour put into the centrifuge machine for centrifugation at 2000-3000rpm for 2-3 minutes. After 2-3 minutes. Serum was collected in labeled Eppendorf tubes by using micropipette and stored at -80^0C for father use.

Biochemical Analysis: Liver functions and hematological parameters were assessed by calculating serum AST (aspartate aminotransferase) and ALT (alanine aminotransferase) by chemistry analyzer using commercially (randox) available biochemical kits. Erythrocyte count, packed cell volume (PCV), hemoglobin concentration (Hb), Erythrocytes sedimentation rate (ESR), leukocyte count (WBC) and platelet count were analyzed using Hematology analyzer. Statistical analysis: The data was expressed as Mean ± SD. Statistical analysis was performed using Graphed Prism software. One-way *ANOVA* applied followed by post hoc Tukey test and P value equal or less than 0.05 will be considered significant.

RESULTS

ALT

Day	Group 1	Group 2	Group 3	Group 4	Group 5
0cc	19.13	19.30	17.50	17.75	18.05
7	18.30	64.59	21.08	19.08	18.26
14	19.38	58.59	21.07	18.25	17.78
21	19.25	72.08	20.57	17.29	16.28
28	18.75	78.08	20.63	15.38	15.01
Mean	*18.96*	*58.53*	*20.17*	*17.55*	*17.08*
SD±	*0.43*	*23.14*	*1.51*	*1.38*	*1.39*
SEM	*0.19*	*10.35*	*0.67*	*0.61*	*0.62*

Table 1

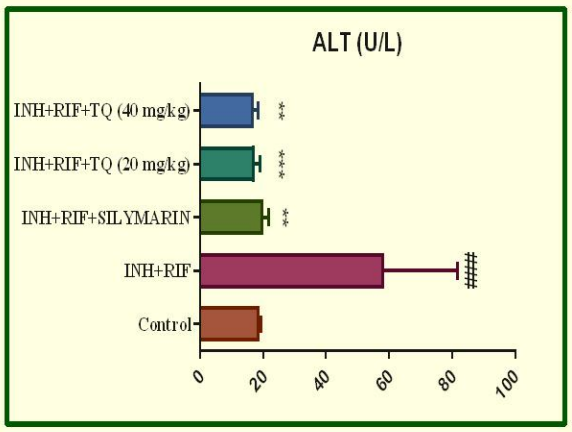

Fig 1

AST

Day	Group 1	Group 2	Group 3	Group 4	Group 5
0	43.63	49.75	48.57	46.15	44.15
7	51.63	72.67	70.00	58.18	54.18
14	48.63	77.52	59.00	59.00	57.25
21	49.51	87.85	56.13	54.28	51.50
28	47.12	91.25	51.08	47.08	44.29
Mean	*48.10*	*75.81*	*56.96*	*52.94*	*50.27*
SD±	*2.98*	*16.40*	*8.36*	*6.05*	*5.88*
SEM	*1.33*	*7.33*	*3.74*	*2.70*	*2.63*

Table 2

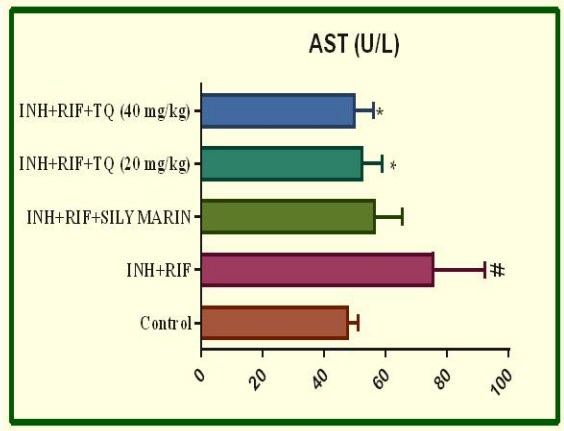

Fig 2

HEAMOGLOBIN

Day	Group 1	Group 2	Group 3	Group 4	Group 5
0	11.67	12.06	11.83	12.76	12.57
7	12.40	11.20	12.60	12.09	12.33
14	12.15	10.56	12.03	11.53	11.89
21	12.16	11.26	11.94	11.43	11.79
28	11.89	11.31	11.89	11.61	11.61
Mean	*12.06*	*11.29*	*12.06*	*11.88*	*12.02*
SD±	*0.27*	*0.53*	*0.31*	*0.55*	*0.39*
SEM	*0.12*	*0.23*	*0.13*	*0.24*	*0.17*

Table 3

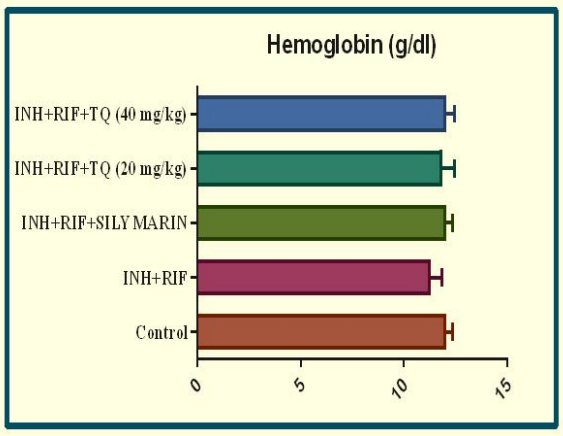

Fig 3

ESR

Day	Group 1	Group 2	Group 3	Group 4	Group 5
0	4.5	4.5	4.63	4.25	5.25
7	4.56	5.25	4.25	4.74	5.45
14	4.25	6	3.63	3.9	3
21	3.88	5.75	4	3.82	3.75
28	3.75	7	4.13	3.7	3.5
Mean	*4.188*	*5.700*	*4.128*	*4.082*	*4.190*
SD±	*0.3627*	*0.9253*	*0.3644*	*0.4210*	*1.095*
SEM	*0.1622*	*0.4138*	*0.1630*	*0.1883*	*0.4897*

Table 4

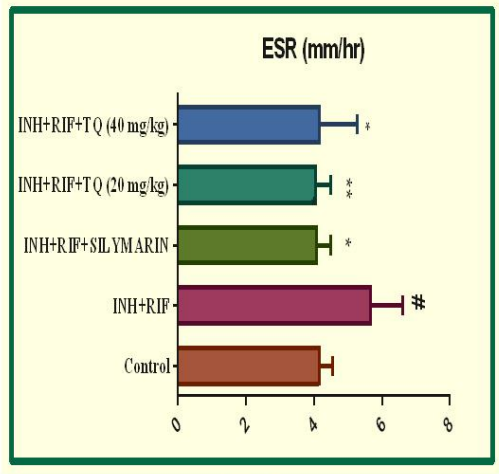

Fig 4

WBC

Day	Group 1	Group 2	Group 3	Group 4	Group 5
0	16262.5	16262	16628.57	16637.5	16637.5
7	16512.5	7866.67	13806.25	16787.5	16787.5
14	16287.5	8483.33	13937.5	16456.25	16456.25
21	15206.25	7183.33	13475	14962.5	14962.5
28	16625	7700	11650	15975	15975
Mean	*16179*	*9499*	*13899*	*16164*	*16164*
SD±	*564.6*	*3809*	*1782*	*737.8*	*737.8*
SEM	*252.5*	*1703*	*796.8*	*330.0*	*330.0*

Table 5

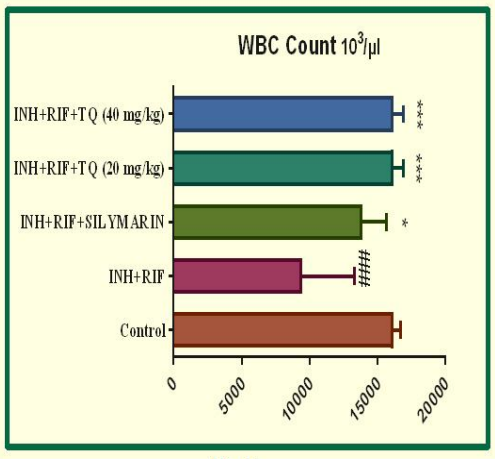

Fig 5

RBC

Day	Group 1	Group 2	Group 3	Group 4	Group 5
0	5.66	5.66	4.99	5.62	5.49
7	5.78	5.25	6.21	5.94	5.64
14	5	5.36	6.6	6.01	5.56
21	4.93	6.03	6.24	5.85	5.01
28	4.98	5.97	5.37	5.37	5.66
Mean	*5.270*	*5.654*	*5.882*	*5.758*	*5.472*
SD±	*0.4138*	*0.3503*	*0.6725*	*0.2620*	*0.2670*
SEM	*0.1850*	*0.1567*	*0.3008*	*0.1172*	*0.1194*

Table 6

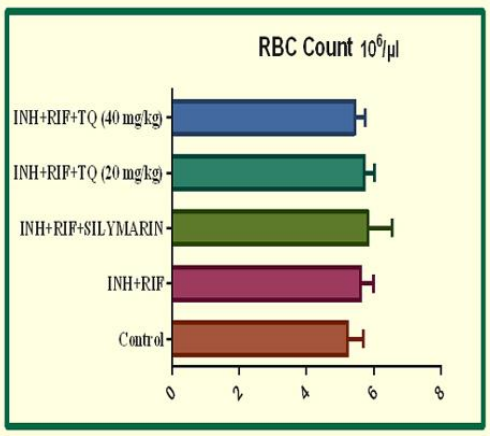

Fig 6

PLATELETS

Day	Group 1	Group 2	Group 3	Group 4	Group 5
0	254625	254625	232714.3	65500	204750
7	249125	265166.7	173500	372625	228100
14	247750	182833.3	225500	337000	385170
21	251187.5	79000	181714.3	383125	210400
28	240597.5	82083.34	200000	19200	207300
Mean	*248657*	*172742*	*202686*	*235490*	*247144*
SD±	*5197*	*89938*	*26082*	*177893*	*77699*
SEM	*2324*	*40222*	*11664*	*79556*	*34748*

Table 6

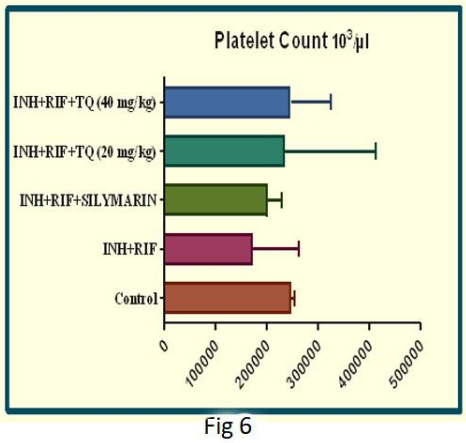

Fig 6

PACKED CELLS VOLUME (PCV)

Day	Group 1	Group 2	Group 3	Group 4	Group 5
0	37.89	37.3	38.31	37.3	37.4
7	34.98	39.78	41.6	39.06	38.06
14	38.66	41.67	41.18	40.2	38.19
21	34.65	45.8	42	39.8	38.01
28	35.49	45	39.08	37.09	36.88
Mean	*36.33*	*41.91*	*40.43*	*38.69*	*37.71*
SD±	*1.817*	*3.554*	*1.637*	*1.427*	*0.5540*
SEM	*0.8128*	*1.589*	*0.7319*	*0.6380*	*0.2478*

Table 8

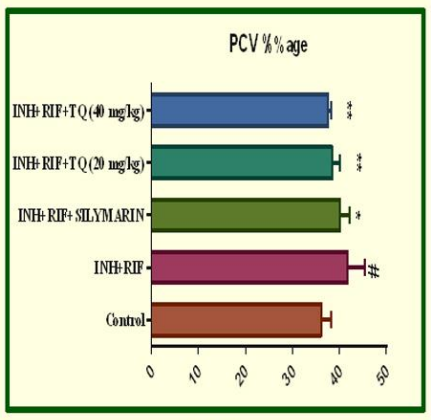

Fig 8

DISCUSSION

Group 1 of rabbits were on normal healthy rabbits so all the test results were usual. These test results were used to compare with the test results of other group to evaluate the effectiveness of drug therapy being administered for 28 days. Alanine aminotransferase (ALT), is an enzyme prepared by hepatic cells, plays a fundamental role in metabolism, the procedure that goes food into energy. ALT is generally found inside liver cells. However, when liver become impaired or inflamed, ALT can be released into bloodstream. This causes serum ALT levels to upswing. Measuring the level of ALT in a person's blood can help to evaluate liver function or determine the underlying cause of a liver problem. Raised ALT (Table-1, Fig-1) level was detected in group 2 rabbits, mean value 58.53, after treating with antituberculosis drugs. (20.17 SD± 1.51) mean value was observed after treatment with silymarin as standard drug. Thymoquinone as experimental drug, in 20mg dose, decreased the level up to (17.55 SD±1.38) while thymoquinone in 40 mg dose showed remarkable result and persisted successful in achieving level of 17.09 (SD±1.39) close to the usual mean value of 18.96, results were found significant (P value < 0.005). Aspartate aminotransferase (AST) is an enzyme found in cells throughout the body but mostly in the heart and liver and, to a lesser extent, in the kidneys and muscles. In healthy individuals, levels of AST in the blood are low. When liver or muscle cells are injured, they release AST into the blood. This makes AST a useful test for detecting or monitoring liver damage. Thymoquinone in both 40mg and 20 mg dose (Table-2, Fig-2) found effective but the result by TQ40mg were extraordinary by achieving the level to (50.27 SD±5.88) very near to the normal mean level of (48.10) in contrast with standard drug silymarin (56.96 SD± 8.36). One more dramatic effect was observed on day 7, when TQ cut the value down from (54.18) on same day silymarin (70.00) was unable to change the level which prove the short onset of action of Thymoquinone as compare to standard drug. P value were found statically significant. We report that natural origin thymoquinone in both 20mg and 40 mg dose is effective as hepatoprotective agent when given in combination with antituberculosis drug to treat infection caused by *Mycobacterium tuberculosis*. Results are in accordance as previously suggested in previous

studies **[25, 26, and 27]**. Test results shows extreme deranged values it means these rabbits are suffering from liver cell damage and the blood cells are also affected. ESR is increased, WBCs are decreased and RBCs are increased. The values of test result of group 3 shows that if silymarin is co administered with isoniazid and rifampicin there would be less hepatotoxicity and the values of blood cells are approximately normal. Thymoquinone is hepatoprotective the test results shows that the liver function is almost normal and blood cells are also approximately normal as compare to those which are treated with only isoniazid and rifampicin in group 4. We suggest that increasing the dose of thymoquinone from 20mg//kg to 40 mg /kg proved to be more effective in making the therapy more safe for the patient health. Hence, from the experimentation it is proved that Isoniazid and Rifampicin are hepatotoxic and co administration of Thymoquinon along with these hepatotoxic drugs can lead to more effective therapy for tuberculosis patients.

CONCUSION

The study was designed to rule out the protective effect of thymoquinone on hepatocytes from hepatic insult caused by first line antituberculosis drugs. Thymoquinone found with improved effects as hepatoprotective agent.

CONFLICT OF INTERESTS

We declare no conflict in interests.

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