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EVALUATION OF HEPATOPROTECTIVE EFFECTS OF SILYBUM MARIANUM AND CICHORIUM INTYBUS EXTRACTS

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Abstract:

Plants have been used as medicinal agents from centuries of years. Cichorium intybus and Silybum marianum are wildly grow in Pakistan. Cichorium intybus and Silybum marianum had been used for liver injuries since ancient times.

Aims and Objectives: *To evaluate the liver profile in damage induced liver of rabbits and effect of Cichorium intybus and silybum marianum plants extracts*

Methodology: *Ethanol extracts are prepared of Cichorium intybus and Silybum marianum separately. Liver of rabbits are damaged with CCl4. The elevated values of liver enzymes (ALT, AST, ALP, Total protein, Albumin and Bilirubin) are measured with Randox kit. Then these extracts are used in the treatment of damaged liver for 60 days.*

Results: *Cichorium intybus and Silybum marianum decreased the elevated levels of damaged liver enzymes. Silybum marianum has more potential than Cichorium intybus. The combine effect of both plants is more than individual plants.*

Conclusion: *Cichorium intybus and Silybum marianum play heaptoprotective role in damaged liver.*

Key words: *Cichorium intybus, Silybum marianum, Carbon tetrachloride (CCl4), Ethanol (99.9%).*

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INTRODUCTION:

Research on plants have been increased all over the world. There are number of evidences showed the great potential of medicinal plants have been used traditionally for years. The components obtained from plants are called phytochemical components. During the different reactions in body various Reactive oxygen species (ROS) are produced. These Free radicals can start degenerative process that damage biomolecules. The compounds which scavenge these ROS are called Antioxidants. Antioxidants have ability to eliminate free radicals. There are number of different plants that have abundant of antioxidant compounds. The medicinal plants have no side effects so they may be used safely [1]

Kasani (Cichorium intybus):

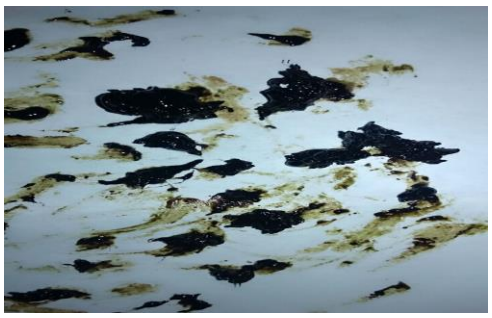
Cichorium intybus commonly known as kasani.The major component of cichorium intybus in fresh plant is inulin. The mixture of Cichorium intybus and Cinnamomum zeylanicum decrease the elevated values of liver enzymes in non alcohlic fatty liver diseases. The water and methanolic extract of kasani have hepatoprotective role. Liver injury induced by acetaminophen and carbon tetrachloride (CCl4) in rats was reduced by treatment of cihorium intybus extract due to its antioxidant potential. Oxytetracyclin induced fatty liver diseases in albino rats. Then extract was given to rats which significantly decrease the fat of liver. [2,3,4]

Milk thistle (Silybum marianum):

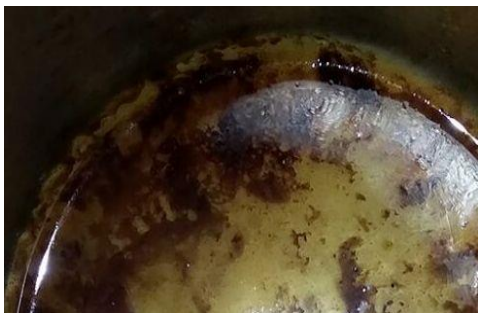
Silybum marianum commonly known as Milk thistle and In Europe Silybum marianum is used in liver diseases such as jaundice and some other biliary diseases. Silymarin is a major ingredient of the extract of milk thistle. [5].Silymarin protect hepatic tissues against many liver toxins in humans and animals. Desplace and its coworkers practically investigated that 20 milligrams per kilogram of silymarin produced benefial effect on Amanita poisoning in patients. Vogel induced liver injury by ethanol, acetaminophen and carbon tetrachloride. Madani and its coworkers experimentally performed that values of liver enzymes such as ALT and AST were significantly decreased by the administration of silymarin. [6]. Silymarin decreased the values of liver enzymes and oxidative stress. [7]

Methodology:

Plants ethanol extract was obtained by socking method with some modifications described by Imranrt al., 2012. After the process of extraction, filtration, The filtrtae kept in beaker for 15 days under shade. The ethanol evaporates from filtrate. The left over ethanol evaporate through incbator. At 60 degree temperature filtrate kept in incubator for 3 days. All ethanol evaporate from extract and crystals remain in the beaker. These crystals are polar compounds of plants. These compounds are saved for used as medicine. [8]



Extract of Silybum marianum



Extract of Cichorium intybus

Animals and treatment protocols:

The protocol for experiments of animal was approved by ethics committee of the Faculty of biochemistry (advance board of study) Minhaj University Lahore. Male rabbits weighing 1-1.5 kg are used. All animals are divided into 5 groups (G1, G2, G3, G4, and G5). Each group consists of 5 rabbits. Group 1 of rabbits fed with normal diet. This group remains without any treatment for control group for whole study. The blood withdraws from the ear of animals and following tests (ALT, AST, ALP, Serum Albumin, Total Proteins and Bilirubin) are performed. The 2nd group of rabbits administered with carbon tetra chloride (CCl4) 3ml/kg twice a week and standard diet. After the 12 hours of treatment with CCL4, the blood with draw from the ear of animals and above mentioned tests are performed for further process. The third group of rabbits administered with CCL4, milk thistle extracts (50 mg) and finally divided form of milk thistle (100mg). The powder form of the plant mix with few drops of water and made tablet. Each tablet of 100 mg is given to each rabbit orally daily. The extract (50 mg) is also given to rabbits daily for 60 days. The 4th group of rabbits received

CCl4, kasani extract and finally divided form (powder) of kasani. The CCL4 is given 3ml/kg to rabbits twice a week. Each rabbit fed orally 100 mg powder form of kasani and 50 mg extract of this plant for 60 days. All animals are maintained in well airy rooms and give proper tap water. The 5th group of rabbits administered CCl4 (3 ml/kg) twice a week. This group is treated with both of plants milk thistle and C. intybus daily. The milk thistle extract (50mg) and C. intybus (50 mg) given orally to each rabbit daily. These rabbits also treated 100 mg powder of these plants daily with tap water for 60 days. The blood from control group collected in the beginning of work. The control group samples are parameters for further experiments. After the 60 days of treatment with drugs and plants extracts the blood samples are taken. The blood vein is dilated with the xylene. The blood samples have taken from the ear of rabbits. Serum alkaline phosphates (ALP), serum albumin, total proteins; bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) measured by using commercially available kits (Randox) using standard methods.

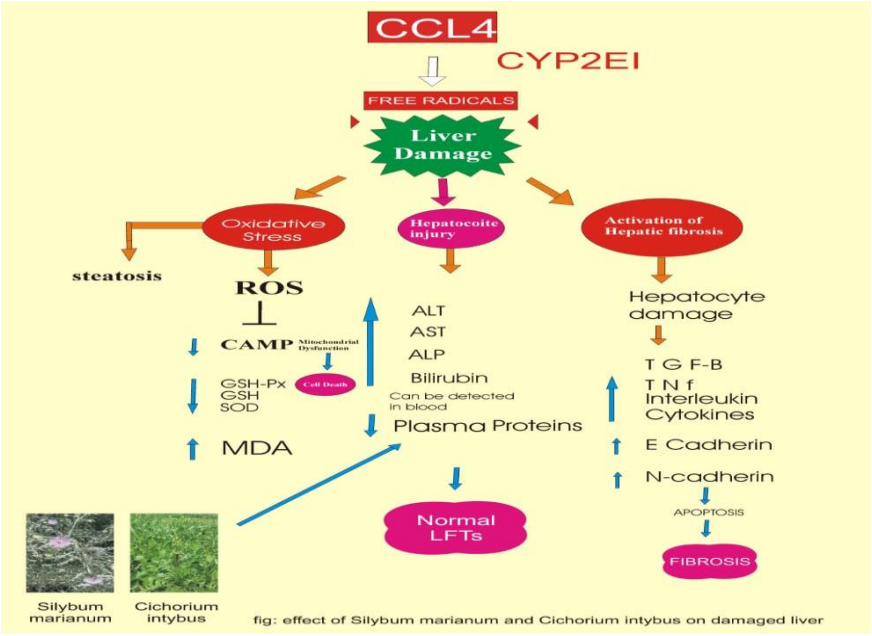


fig: effect of Silybum marianum and Cichorium intybus on damaged liver

RESULTS:

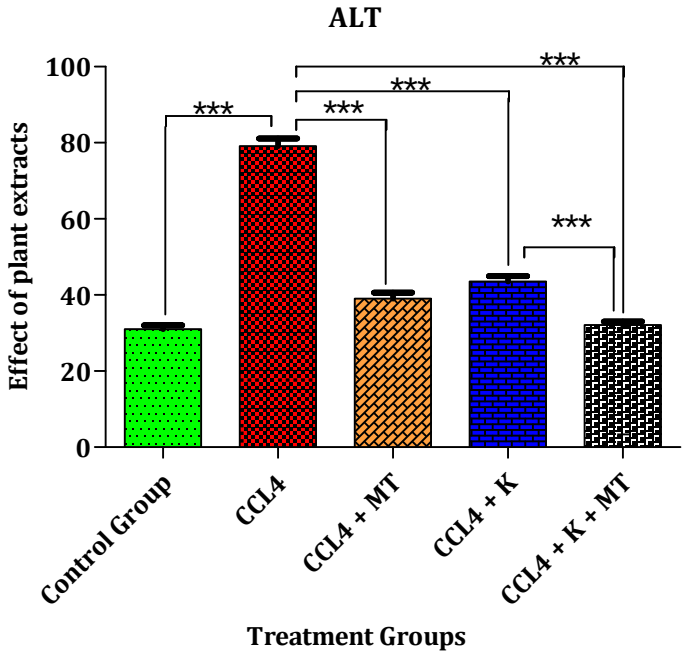
The data obtained from experiments are used to conclude results. The one way ANOVA is applied on data to analysis. The results are following

Effect of plants extract on ALT level

Table 1 The values of ALT obtained from experiments

Rabbits	Control (G1)	G2 (CCl4)	G3 (CCl4+MT)	G4 (CCl4+Kasani)	G5 (CCl4+kasani+MT)
1	28.4	80.2	38.4	40.2	29.2
2	30.2	77.9	39.9	42.4	31.6
3	32.3	81.3	44.3	45.5	32.9
4	34.2	72.5	33.6	41.7	35.1
5	30.2	83.9	33.2	47.9	31.7

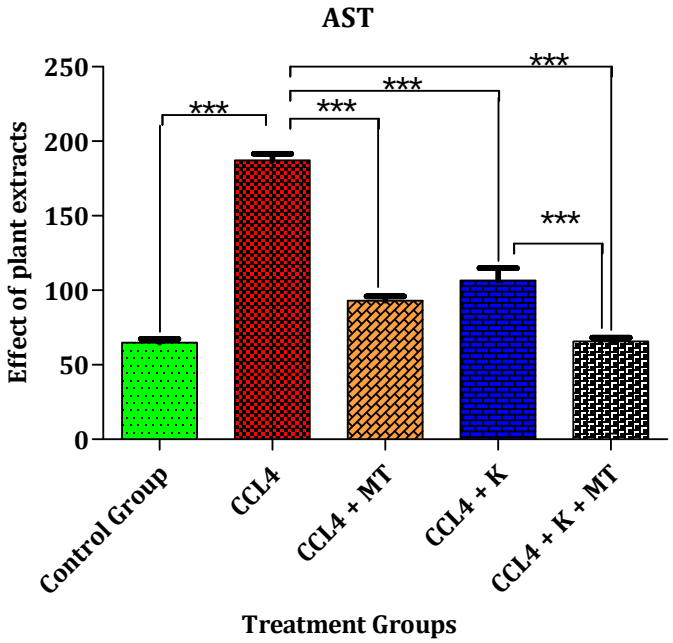
The graph A for the comparison of MSD values of ALT of five groups



Effect of plants extract on AST level
Table 2 The values of AST obtained from experiments

Rabbits	Control (G1)	G2 (CCl4)	G3 (CCl4+MT)	G4 (CCl4+Kasani)	G5 (CCl4+kasani+MT)
1	61.3	192.3	89.1	90.0	62.1
2	59.4	177.6	92.4	84.7	71.0
3	63.9	199.8	84.8	119.6	71.9
4	68.2	188.7	98.1	128.7	65.2
5	72.1	177.2	101.0	109.6	58.3

The graph B for the comparison of MSD values of AST of five groups

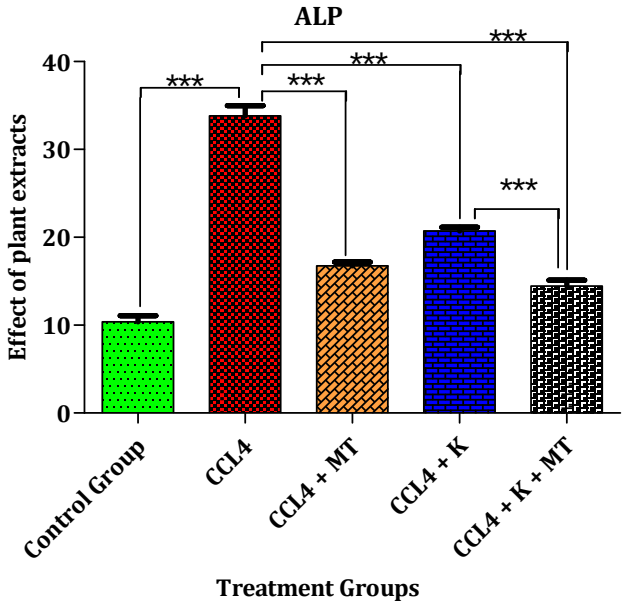


Effect of plants extract on ALP level

Table 3 The values of ALP obtained from experiments

Rabbits	Control (G1)	G2 (CCl4)	G3 (CCl4+MT)	G4 (CCl4+Kasani)	G5 (CCl4+kasani+MT)
1	10.3	37.2	16.3	20.9	14.2
2	9.4	31.9	16.1	19.7	13.9
3	12.8	35.6	18.2	21.1	16.1
4	8.6	30.8	15.9	22.1	12.4
5	10.8	33.6	17.2	19.7	15.7

The graph C for the comparison of MSD values of ALP of five groups

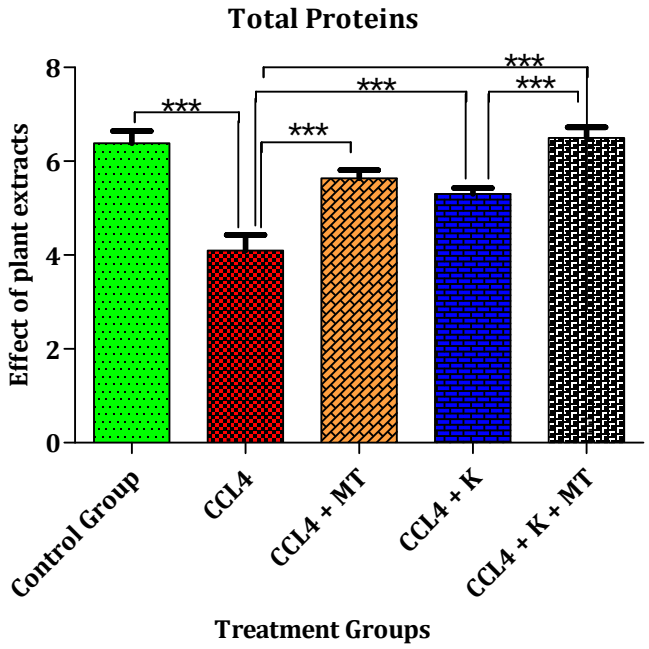


Effect of plants extract on Total Proteins

Table 4 The values of Total proteins obtained from experiments

Rabbits	Control (G1)	G2 (CCl4)	G3 (CCl4+MT)	G4 (CCl4+Kasani)	G5 (CCl4+kasani+MT)
1	5.8	3.2	5.9	5.1	6.2
2	6.2	3.6	5.5	4.9	6.4
3	6.9	4.2	5.6	5.4	6.9
4	5.9	4.4	6.1	5.6	7.1
5	7.1	5.1	5.1	5.5	5.9

The graph D for the comparison of MSD values of Total proteins of five groups

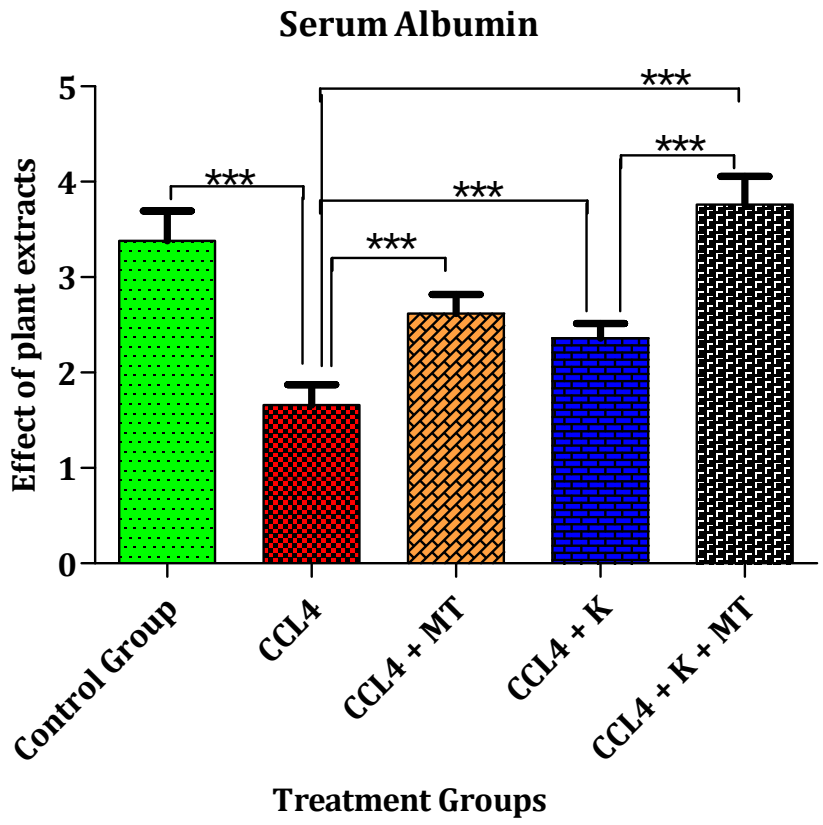


Effect of plants extract on Serum Albumin

Table 5The values of serum albumin obtained from experiments

Rabbits	Control	CCL4	G3(CCl4+MT)	G4(CCl4+Kasani)	CCl4+kasani+MT
1	2.6	1.9	2.2	2.5	3.7
2	3.2	2.0	2.8	2.6	4.2
3	2.9	1.1	2.1	2.1	3.4
4	4.3	2.1	3.1	2.7	2.9
5	3.9	1.2	2.9	1.9	4.6

The graph E for the comparison of MSD values of Serum albumin of five groups

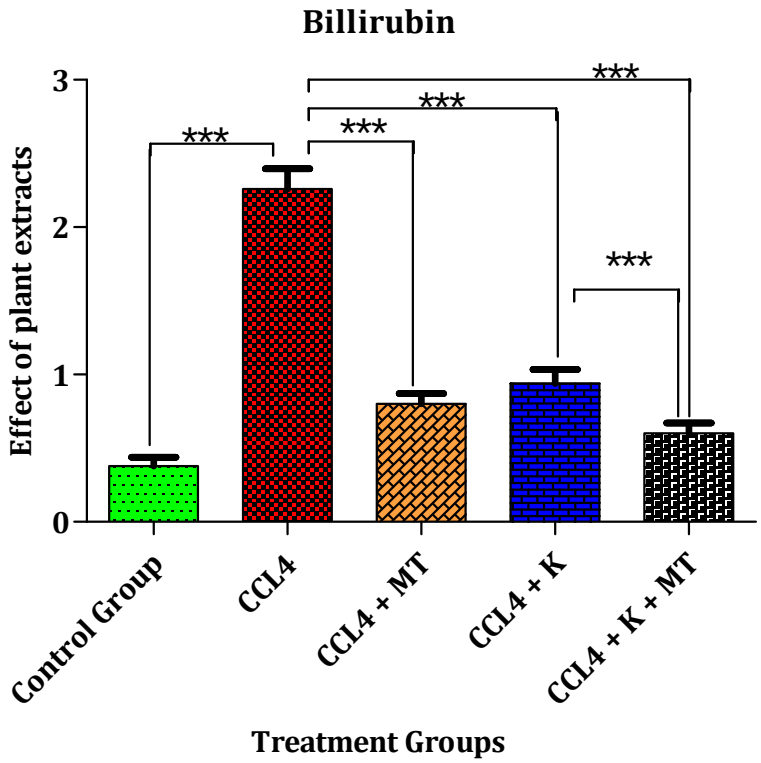


Effect of plants extract on Bilirubin

Table 6 The values of Bilirubin obtained from experiments

Rabbits	Control (G1)	G2 (CCl4)	G3 (CCl4+MT)	G4 (CCl4+Kasani)	G5 (CCl4+kasani+MT)
1	0.2	3.2	0.7	0.9	0.5
2	0.3	3.1	0.9	1.1	0.4
3	0.5	2.9	0.8	1.2	0.7
4	0.4	2.8	1.0	0.8	0.8
5	0.5	3.6	0.6	0.7	0.6

The graph F for the comparison of MSD values of Bilirubin of five groups



The overall table of MSD of Liver profile (Enzymes)

Liver enzymes	G1 MSD	G2 MSD	G3 MSD	G4 MSD	G5 MSD
	Control (N)	CCl4	MT+ CCl4	K+CCl4	MT+K+CCl4
ALT	31.1 ± 2.2	79.2 ± 4.3	37.9 ± 4.6	43.5 ± 3.1	32.1 ± 2.1
AST control 10-120 u/l	64.98±5.2	187.1±9.7	93.08±6.6	106.5±18.8	65.7±5.8
ALP control 4-20 u/l	10.38±1.6	33.82±2.6	16.74±0.9	20.7±1.0	14.46±1.5
T. PROTEIN control 5.4-7.3 g/dl	6.38±0.6	4.1±0.7	5.64±0.4	5.3±0.3	6.5±0.5
S. Albumin control(2.4-4.5 g/dl)	3.38±0.7	1.66±0.5	2.62±0.4	2.36±0.3	3.76±0.7
Bilirubin control 0-1.0 mg/dl	0.38±0.1	2.26±0.3	0.8±0.2	0.94±0.2	0.6±0.2

The ALT normal value of rabbits is 10-45 U/L . The MSD of G1 (control group) is 31.1 ± 2.2. The MSD of G2 group is 79.2 ± 4.3. It is highly significant (***) to control group $p<0.05$. The treatment with C. intybus and S. marianum significantly decrease the value of ALT. The MSD of G3 and G4 are respectively 37.9 ± 4.6 and 43.5 ± 3.1. The MSD of G5 is 32.1 ± 2.1. It is highly significant (***) to G2 group because these plants decrease the value of ALT close to control group. The AST normal value of rabbits is 10-120U/L. The MSD of G1 group is 64.98±5.2. The MSD of G2 group is 187.1±9.7. It is highly significant (***) to control group $p<0.05$. The MSD of G3 and G4 is 93.08±6.6 and 106.5±18.8 respectively which is significant to G2 group. The MSD of G5 is 65.7±5.8. It is highly significant (***) to G2 group. The ALP normal value of rabbits is 4-20U/L. The MSD of G1 group is 10.38±1.6. The MSD of G2 group is 33.82±2.6. It is highly significant (***) to control group $p<0.05$. The MSD of G3 and G4 is 16.74±0.9 and 20.7±1.0 respectively which is highly significant (***) to G2 group. The MSD of G5 is 14.46±1.5. It is highly significant (***) to G2 group. The T. Proteins normal value of rabbits is 5.4-7.3 g/dl. The MSD of G1 group is 6.38±0.6. The MSD of G2 group is 4.1±0.7. It is highly significant (***) to control group $p<0.05$. The MSD of G3 and G4 is 5.64±0.4 and 5.3±0.3 respectively which is highly significant (***) to CCl4 group. The MSD of G5 is 6.5±0.5. It is highly significant (***) to G2 group. The Serum Albumin normal value of rabbits is 2.4-4.5 g/dl. The MSD of G1 group is 3.38±0.7. The MSD of G2 group is 1.66±0.5. It is highly significant (***) to control group $p<0.05$. The MSD of G3 and G4 is 2.62±0.4 and 2.36±0.3 respectively which is highly significant (***) to CCl4 group. The MSD of G5 is 3.76±0.7. It is highly significant (***) to G2 group. The Bilirubin normal value of rabbits is control 0-1.0 mg/dl. The MSD of G1 group is 0.38±0.1. The MSD of G2 group is 2.26±0.3. It is highly significant (***) to control group $p<0.05$. The MSD of G3 and G4 is 0.8±0.2 and 0.94±0.2 which is highly significant (***) to CCl4 group. The MSD of G5 is 0.6±0.2. It is highly significant (***) to G2 group. The P value is < 0.000 which is highly significant in all groups.

DISCUSSIONS:

The study on plants has been increased throughout the world. There are many evidences shows the great potential of medicinal plants has been used traditionally for centuries. Phytochemical components are those components which obtained from plants. These phytochemical components extract from plants by different methods. Our body's metabolism bears a number of beneficial and harmful chemicals daily. During the different reactions in body various Reactive oxygen species (ROS) are produced. Plants had been used as medicinal agents from centuries of years. Free oxidative radicals incessantly produced and removed in biological systems. But the oxidizing compounds present in our biological systems are not enough to eliminate these free oxidative radicals so an external source will be required to fight this problem. Different Plants provide such types of sources of antioxidants. In this study different compounds have been found to have antioxidant activities extracted from Cichorium intybus and Silybum marianum. These antioxidant compounds were extracted from plants so can be used in biological systems and for food preservations as well. Now these days' liver and pancreatic diseases are increasing due to our life styles, diet and sewage systems. Many drugs are available in market to combat these disease but they are at risk due to their side effects so we have to adopt such methods which have no side effects. So I have used herbal medicines in my experiments to overcome these diseases. Herbal medicines have no side effects. Cichorium intybus and Silybum marianum are wildly grow in Pakistan. C.intybus has different important chemicals such as alkaloids, inulin, sesquiterpene lactones, coumarins, Vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins and tannins. The major component of C. intybus in fresh plant is inulin. Among phytochemical of kasani inulin comprises 68 %. The other components of kasani are 14% sucrose, 5% cellulose, 6% protein, 4% ash, and 3% other compounds. In dried plant inluin comprises 98%. Kasani plant can grow to 5 feet. In Europe and other countries Silybum marianum had used in liver diseases such as jaundice and some other biliary diseases. Silymarin is used as in food poisoning. Boiled root of milk thistle is eaten. The entire plant is used for treatment of fevers and uterine problems. The leaves are used as salad. Seeds are used in alleviate of jaundice, gall-bladder and hemorrhages. Seeds play helpful role in digestion. Seeds are also used in coffee. Silymarin is a major constituent of the extract of this plant. It is a complex mixture of flavonoids. Silymarin is about 80% of the extract. Silymarin consists of a variety of flavolignans such as silybin or silybinin, isosilybin, silydianin, silychristin, taxifolin, quercetin, betaine and silybonol. The present study is performed on rabbits. All the rabbits are divided into 5 major groups. Each group consists of 5 rabbits. In this study liver damage is done by CCl4. Carbon tetra chloride (CCl4) orally given (3mg/kg) to rabbits twice a week. The CCl4 is a powerful chemical which can destroy the hepatic tissues. After 48 hours of CCl4 administration blood is drawn from the ear of rabbits

for the measurement of liver enzymes. The elevated values of different enzymes (ALT, AST, ALP, Total Proteins, Serum Albumin and Bilirubin) are measured with randox kits available in the market. The ethanolic extract is prepared with the help of analytical grade of ethanol (99.9%) of these plants separately. The extracts are prepared almost in 30 days. These plants are collected from Khiali a town of Gujranwala. The collected plants washed with tap water to remove dust. Then these plants are dried under shade. The dried plants grind into fine powder. The finally divided form mix in the analytical grade of ethanol (99.9%) and place air tight in vessel for 9 days. After 9 days the opened the vessel and filter the extract to remove residues. Then incubate the filtrate and to gain crystals form of extract. The extracts and powder form of plants administered to rabbits orally for 60 days. The rabbits kept under standard condition. The C. intybus extract decreased the elevated values of liver enzymes and blood sugar approximately to control (normal) values. The protein level decreased after 10 days of CCl4 treated. According to my best knowledge based on my experiments Silybum marianum has more potential against damaged liver than Cichorium intybus. I checked the effect of plants extracts combine and individually. It is interesting to know that the combine effect of these plants is more than individually plant. The elevated liver profile (ALT, AST, ALP, Bilirubin, serum albumin and total protein) due to CCl4 return to almost control values. The rabbits which feed with both plants' extracts grow healthy. The damaged liver enzymes significantly return to their almost normal values. There were no mortality found in my whole experiments.

CONCLUSIONS:

Cichorium intybus and Silybum marianum have antioxidant poteinal which reduce the free radicals. They play protective role in damage liver. According to my best knowledge based on my experiments Cichorium intybus has potential to recover damaged liver profile but Silybum marianum has more potential against damage liver than Cichorium intybus. The combine effects of Cichorium intybus and Silybum marianum are more than individual plants. There are no adverse effects and mortality seen in this study.

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