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HEPATOPROTECTIVE EFFECTS OF GLYCYRRHIZ GLABRA AND CICORIUM INTYBUS IN PARACETAMOL INDUCED TOXICITY IN RATTUS NORVEGICUS

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Abstract:

Aims and Objectives: The present study was aimed to determine hepatoprotective role of two plants *Glycyrrhiza glabra* and Cicorium intybus against paracetamol (acetaminophen) exposure hepatotoxicity in albino rats (Rattus norvegicus). It has been demonstrated that *G. glabra* and *C.intybus* extract has biological capabilities of anti-oxidation, detoxification and anti-infection.

Methodology: So, to evaluate the effects of *G. glabra* and *C.intyubs* administration on the liver's histology and some biochemical parameters were investigated. Rattus norvegicus male rats (n= 48) were randomly distributed into four groups. Experimental groups were given acetaminophen with 2.5mg/kg BW /day for 5 days respectively to induce hepatotoxicity, then aqueous extract of *G. glabra* (Roots) and *C.intybus* (Seeds) at 200 mg/kg/day was given for a period of 10, 20 and 30 days. After the completion of experiment, tissues were taken and blood was collected in order to evaluate histopathological changes, and for studies of biochemical parameters separately. The obtained results showed hepatic necrosis and centrilobular hepatic congestion with a decrease in hepatocytes diameter, and increase in sinusoidal spaces was also observed in the liver of treated rats.

Results: Liver enzymes ALP, AST and ALT and other biochemical factors Albumin, Bilirubin showed a significant increase in 200 mg/kg extract received rats in comparison with control ($P<0.05$) and ($P<0.01$). *G.glabra* and *C.intybus* have shown a remarkable and effective impact on RLW which was statistically significant ($P<0.05$), percentage increase and decrease showed that both plants have good effective on the recovery of body weight, affected by the paracetamol (acetaminophen) toxicity.Some harmful effects of *C.intybus* were seen on integrity of liver in 30 days consumption of this plant.

Keywords:
Hepatoprotective effects, Glycyrrhiza glabra, Cichorium intybus, paracetamol-induced toxicity, Rattus norvegicus, liver dysfunction, medicinal plants, antioxidative properties, detoxification, anti-inflammatory, liver tonic.

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INTRODUCTION

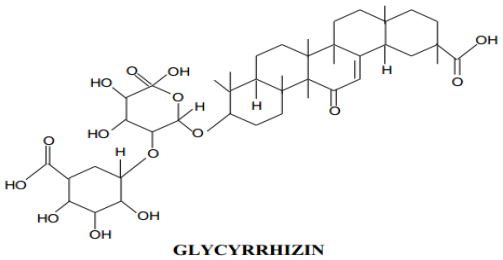
In human's body, liver is the largest organ with a weight of approximately 1500 g, at upper right corner of abdomen, the liver performs more than 500 crucial metabolic functions (Naruse *et al.*, 2007*)*. Hepatotoxicity is the dysfunction of liver or the damage of liver due to profusion of xenobiotic or drugs (Navarro *et al.*, 2006). The chemicals which are responsible for liver dysfunction are known as hepatotoxic ants or hepatotoxins. These are the exogenous compounds including medicinal drugs, industrial waste and chemicals, herbal remedies, dietary supplements,and natural chemicals such as microcystins.(Willett *et al*., 2004, Papay *et al.*, 2009). Acetaminophen, paracetamol, or N-acetyl-p-aminophenol (APAP) is a frequently used antipyretic and analgesic drug. (James *et al.*, 2003). An overdose in case of therapeutic context and in suicidal case can induced extreme hepatotoxicity (Bunchorantavakul *et al.*, 2013). Nearly about 50% cases of acute liver failure are due to toxicity of APAP and carry about 30% mortality (Ostapowicz *et al., 2002*). Davidson and Eastham 1996, were the first to report that acetaminophen was hepatotoxic in overdose. Acetaminophen occurs as white crystalline powder with odorless smell, and have bitter taste when used orally. It has a melting point of 169-172°C and is non-flammable with a specific gravity of 1.293. The compound is prepared at a pH of about 5.5-6.5, it's miscible with water, methanol and ethanol (Foye *et al*.,2014).

Metabolic action of acetaminophen. Immense studies has been conducted on the metabolism of action of acetaminophen in the body and with well-known pathway.(Gelotte *et al.*, 2007).In the metabolism of acetaminophen, liver is a key player. In metabolism of acetaminophen, three pathways are involved, which are significant for about 55-60% of the metabolism. A conjugation reaction "Sulfation" accounts for 20-30% acetaminophen metabolism, dehydration, less than 15% reactions of conjugation of glutathione and N-hydroxylation reactions account for it. The major enzyme involved in metabolism is referred as the hepatic cytochrome "P450" that is involved in the formation of "N- acetyl-p-benzoquinoneimine (NAPQI)" which are alkylating metabolites which has also been referred as N-Acetyl imidoquinone. CYP2E1 and CYP3A are two specific isoenzymes of P450 involved in the reactions (Gelotte *et al.*, 2007).

Cicorium Intybus. It is an perennial herbal plant which belongs to Asteraceae (Mohamed *et al*., 2017). Plant contains number of medicinally important compounds such as sesquiterpene lactones, inulin, coumarins, vitamins and flavonoids it used as anti-hepatotoxic, anti-inflammatory, liver tonic, cholagogue, depurative, diuretic, emmenagogue, alexeteric and also as tonic, anticancer and other medicinal uses.(Ranjitha and Nandagopal 2007).

Hepatoprotective Plants. In current study we have used two plants "*Glycyrrhiza glabra*" and " *Cicorium intybus".*

Glycyrrhiza glabra. *Glycyrrhiza Glabra L.* belongs to family, Fabaceae, Leguminosae which is known as popular medicinal plant (Shibata *et al*., 2003).. Ali Louei Monfared (2013) reported that *Glycyrrhiza glabra* extract has biological capabilities which included anti-infection, anti-oxidation and detoxification properties, and there is little data available about its probable side effects on the liver integrity.



GLYCYRRHIZIN

Body condition and body weight Following treatment, the rats were observed over a 12-hour period and no mortality was recorded over that period. Rats administered with acetaminophen at doses of 2.5mg/ kg body weight/day after 2-3 days were sick and less active as compared to normal rats group,they also had a rough coat and poor appetite. While the control rats, and those treated with C.*intybus* and *G.glabra* were alert and active, had glossy coat, and were showing good appetite.

Animals. Forty-eight albino rats (*Rattus norvegicus*) of 200-300 g body weight were used in the current study. The rats were given free access to water and food. They were allowed to acclimatize for one week. They were maintained at 12 hours light, and 12 hours dark cycle during experiment. The animals were collected from the animal house of University of Lahore

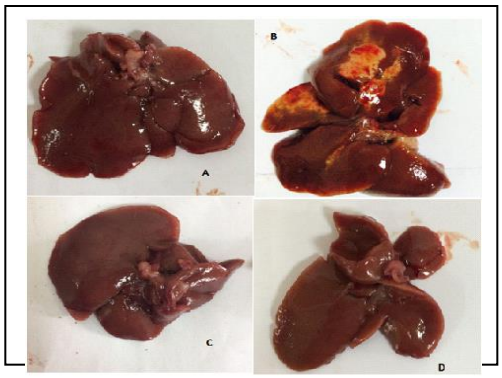


Figure 2: Morphological characteristics of rat livers: (A) Control group (Alert and glossy) (B) Acetaminophine 2.5mg/kg body weight (Less active and sick) (C) *Cicorium intybus* 200mg/kg treated (Active) (D) *Glycyrrhiza glabra* 200mg/kg treated (Active).

Group-1 (Control): This group was used as control rats and were given free access to food and water. Number of rats (n=4) were used in control group.

Group-2 (Paracetamol): It contain four animals (n=4) which were given 2.5mg to induced hepatotoxicity in animals. Rats received it for a period of 5 days.

Group-3 (Paracetamol+*G.glabra*): 200mg of *G.glabra* given orally to the four animals (n=4) that was dissolved in the water. According to the experimental design the treatment was given accordingly 10, 20 and 30 days.

Group-4 (Paracetamol+*C.intybus*): An dosage of 200mg was used for four (n=4) that was dissolved in water. The plants were administrated after the five days application of acetaminophen. The hepatoprotective effects were checked after respectively 10,20 and then 30 days.

Biochemical analysis:

The samples were processed and analyzed for the estimation of aspartate aminotransferase (AST), alkaline phosphate (ALP), alanine aminotransferase (ALT), bilirubin total (BILT), total protein (TP). For the quantitative determination of above enzymes in plasma or serum Cobas c 111 systems has been used.

Table-1: Groups treatement for 5,10,15,20,25,and 30 days.

Group/ Treatment	5 Days	10 Days	15 Days	20 Days	25 Days	30 Days
Control	3.7	8.6	21.7	37.0	57.5	76.3
P+ *C.intybus*	-7.9	8.1	14.0	23.6	31.6	42.6
P+ *G. glabra*	-11.4	12.0	24.0	40.4	66.7	76.0

%increase(+) or decrease (-) in Body weight gain following *G.glabra and C.intybus* to acetaminophen exposed rats.

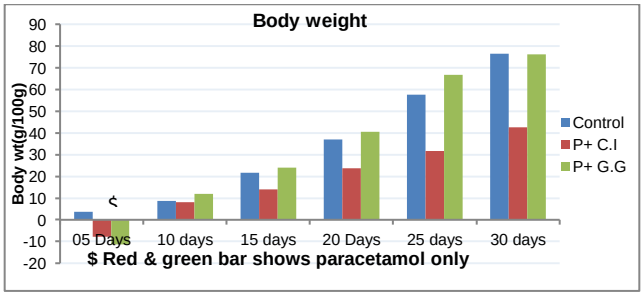


Fig-2:Administration of *G.glabra* and *C.intybus* to paracetamol exposed rats:Percent increase(+) or decresae (-) in body weight gain following administration of *G.glabra* and *C.intybus* to paracetamol exposed rats.

20 days showed that, *G.glabra* treated group and Control group are has almost near values i.e 40.4±14.1 and 37.0±3.8, indicating the clear effective role of *G.glabra* against the paracetmol induced decrease in body weight of rats, and was statistically significant p<0.05. 25 days experimental work showed that, *G.glabra* has an small elevation in value as compared to the control group, which was 66.7± 12 while in the case of control group it was 57.5±9.7, But at 30 days treatment the *G.glabra* treated group and Control group had the same value that was 76±14.3 and 76.3±3.3 which gave absolutely good result of *G.glabra* as good body weight gainer and it showed an excellent recovery in body weight of rats. *G.glabra* value within 25, 30 days was observed as statistically significant P<0.05.

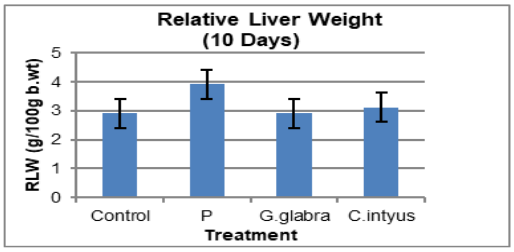
Relative Liver Weight (RLW). It was observed that, the relative liver weight (RLW) in the case of paracetamol induced toxicity rats was increased 3.9±0.16 in 10 days as compared to control group that was observed as 2.9±0.03, *G.glabra* has shown a value of 2.9±0.03 thaw was almost equal to the control group suggesting potential role of it in the organ weight. While *C.intybus* has shown an value of 3.1±0.12 which wasn't near to control group. Statistically significant value p<0.05 of RLW was observed in the 10 days. In 20 days experiment, an increased value of 3.7±0.16

as compared to control group *i.e* 2.8±0.02, declare abnormality in liver of rats. After administration of *G.glabra* (3±0.06) and *C.intybus* (2.9±0.08) remarkable recovery in liver size was observed, which showed their role in the improvement of liver size. Statistically significant value of RLW *i.e* P<0.05 was found in 20 days. In the case of result of 30 days experiments, paracetamol treated group give a raising value of 4±0.18 as compared to control group 2.7±0.15, this elevation is due to aberration of liver. This abnormal size of liver is potentially recovered back to control group with values of 2.6±0.02 in the case of *G.glabra* and 2.7±0.20 in the case of *C.intybus* . Within the 30 days statistically significant value p<0.05 was observed.

Table-2:Treatement of Groups in 10,20,and 30days

Group/ Treatment	10 Days	20 Days	30 Days
Control	2.9±0.12	2.8±0.02	2.7±0.15
Paracetamol	3.9±0.16*	3.7±0.16**	4±0.18**
G. glabra	2.9±0.03*	3±0.06**	2.6±0.20**
C. intybus	3.1±0.12*	2.9±0.08**	2.7±0.20**

Fig-3:Relative liver Weight of treatement in 10days



Relative Liver Weight (RLW) of 10 days treatment of G.glabra and C.intybus following 5 days acetaminophen expose rats

Fig-4: Relative Liver Weight ofTreatement in 20 days

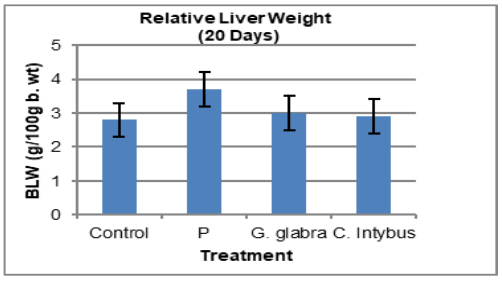


Fig-5:Relative liver Weight of treatement in 30days

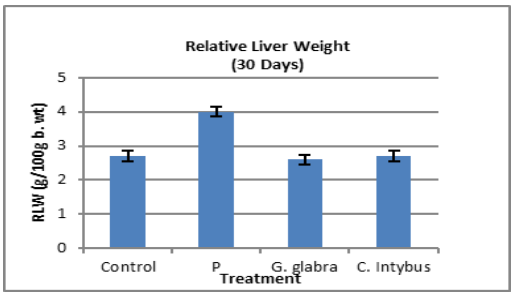
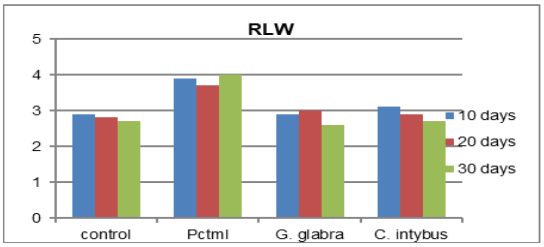


Fig-6:administration of G.glabra and C.intybus exposed rats in 10, 20 and 30 days .



RLW following administration of G.glabra and C.intybus exposed rats in 10, 20 and 30 days .

In 10 days experimental work, graphically it can be clearly evaluated that, paracetamol treated group has shown an increase in the relative liver weight, and its increase from the control group bar shows an abnormality. Administration of *G.glabra* and *C.intybus* has shown an notable recovery of relative liver weight (RLW) back to the control group bar as shown above. It is clearly evident from graph bar of Fig 9 that, RLW of paracetamol treated group is crossing the control group bar indicating unusual result, while treated groups bars *G. glabra* and *C intybus* are showing a marked improvement in the RLW. 30 days treatment group shows that RLW of paracetamol was increased from the normal range (control group) showing clearly the adverse effect of acetaminophen on organ weight, while graph bar of both administrated plants shows that their values of 2.6±0.20 and 2.7±0.20.

BIOCHEMICAL ANALYSIS OF 10 DAYS EXPERIMENT

Results in the above Table 5 showed the hepato-protective effect of *Glycyrrhiza glabra* and *Cicorium intybus* administration following hepatic damage induced by Acetaminophen (paracetamol),

Alanine Aminotransferase (ALT)

The liver function enzyme, ALT activity in the 10 days control group was (53.2± 4.8) IU/L, which has been increased in the case of paracetamol (84.0± 17.8) which shows marked increase

from the normal group. Percentage comparison difference between Control and Paracetamol was 58%.The marked alleviation in ALT level was also observed in *C.intybus* (66.6± and G.*glabra* administrated groups, showing hepato-protective role. Statistically significant value ($P<0.05$) was observed in *C.intybus* and *G.glabra* groups, respectively.

Aspartate Transaminase (AST)

AST in the table showed that, in normal group (73.5±6.25) in the case of paracetamol it was found (74.6± 1.20) with slight elevation, *Glycyrrhiza* administration in 10 days rats showed the marked recovery (72.3±2.60) while *Cicorium* showed an value of (80.0 ± 12.1). The AST in the 10 days was statistically moderately significant ($P<0.01$). During % comparison a 2% decreased was observed which showed paracetamol hepatotoxicity.

Alkaline Phosphatase (ALP)

ALP has shown a value of (211±37.1), while the paracetamol showed a decreased value of (94.3±19.5) which indicate the injury in the liver, and % comparison difference between both of these was 55%. *Cicorium intybus* showed a hepato-protective effect with an value of (222±36.8)

Bilirubin (BIL)

Bilirubin content of the of blood remained almost same from Control 0.057±0.14 to Paracetamol and *G.glabra* (0.053±0.080 and 0.040±0.01) except *C.intybus* (0.086±0.01) Paracetamol showed percentage decrease of 7% when compared with the control group. Comparison of Control with *C.intybus* and *G.glabra* showed a percentage of 2% and 6%

Total Protein (TP)

In the case of Total Protein, statistically significant activity was observed after the administration of hepato-protective plants, Glycyrrhiza and Cicorium $P\leq0.05$ (5.60±0.23 and 5.16±0.14), while control showed a value of (5.50±0.1). Percentage % comparison between normal and paracetamol group showed a difference of 26% which indicate the hepato injury by paracetamol in rats.

Albumin

Albumin activity was observed statistically significant $P\leq0.05$ among the 10 days groups, normal group has shown an value of (4.07±0.13) while paracetamol group's value decreased (2.43±0.06), with a percentage difference of -40.2% which signify liver dysfunction. Hepato-protective role of Cichorium and Glycyrrhiza was clearly found (4.26±0.11 and 5.16±0.14)

BIOCHEMICAL ANALYSIS OF 20 DAYS EXPERIMENT

Alanine Aminotransferase (ALT)

ALT activity in 20 days table has shown a value of 53.2±4.8 in control group, Paracetamol group has shown an exalted value of 67.7±14.6 which declared deliberately the damage by liver tissues, values alleviate back towards the normal after the administration of both hepato-protective plants, 46.0±9.0 and 44.2±5.1, respectively. Values showed that they are statistically moderately significant $P<0.01$.Percentage comparison exhibited 27% in the case of Control and paracetamol, *C.intybus* and *G.glabra* had showed 13% and 16% comparison with control group. .

Aspartate Transaminase (AST)

AST content observed was 73.5±12.5, while no noticeable results were found for *C.intybus* and *G.glabra*.

Alkaline Phosphatase (ALP)

ALP value in Paracetamol 278.2±26.92 had increased from normal group 211.0±74.3 that showed liver tissues injury, a percentage difference of 31% was observed.

while in the case of *G.glabra* its value was 224.2±36.1 and *C.intybus* didn't showered any marked recovery.

Bilirubin

Bilirubin content in *G.glabra* and *C.intybus* had not shown prominent recovery after the paracetamol hepatotoxicity which was extended as 0.097±0.71 from normal group 0.057±0.028 with a percentage % of 70%. In the case of *G.glabra* it was just 8%.

Total Protein

Total protein content was found insignificant throughout the days, Control has shown a value of 5.50±0.1 while its value slightly increased due to toxicity induced by paracetamol, *C.intyus* and *G.glabar* had showed the same value of 5.07±0.36. If the % comparison was observed Control and paracetamol 9% , both plants showed a same % which was 7.81%.

Albumin

Albumin content of the 20 days experiment showed statistically significant values through groups and days (P≤0.05) as shown in the table. Its extended in the paracetamol 4.45±0.08, *G.glabra* didn't showed marked recovery 4.60±0.2 as compared to the *C.intybus* which has a value of 4.10±0.38.

BIOCHEMICAL ANALYSIS OF 30 DAYS EXPERIMENT:

Alanine Aminotransferase (ALT)

ALT was observed as statistically moderately significant P<0.01 in 30 days, Control goup has shown a value of 44.8±4.11, when paracetamol value was compared with Control it was clearly reported the abnormality in the liver, *C.Intybus* and *G.glabra* (43.6±5.78 and 43.2±4.32) had showed a good recovery and values reaches back towards the normal group. Control and paracetamol group had showed a % difference of 27%, while C and G had given 13.5 and 16.9% difference.

Aspartate Transaminase (AST)

AST activity in 30 days was also found as statistically moderately significant P<0.01, paracetamol (107±20.6) didn't showed marked elevation from normal group so hepatotoxicity wasn't observed in the case of acetaminophen. G.glabra (170±22.7) showed notable heaptoprotective effect as compared to the C.intybus (131±12.7).A prominent percentage difference of 67% was perceived between normal and paracetamol group. While C.intybus has given a 92% and G.glabra 77%.

Alkaline Phosphatase (ALP)

30 days data of ALP showed a control value of 181±23.4, paracetamol value was lower as compared to the normal group 153±13.7, in the case of *G.glabra* the obtained value was 213±12.8 which was higher than control value indicating the abnormality may be due to long period administration of *G.glabra* and *C.intybus* had shown a value of 168±31.0 and no marked recovery was observe in the case of both plants. ALP was found statistically significant P<0.05 in 30 days, while insignificant in the case of groups.

Bilirubin (BIL)

Bilirubin was observed as statistically significant P<0.05, normal group has shown a value of 0.66±0.35,while value of this parameter has been decreased prominently in the case of paracetamol 0.16±0.06,which indicate the abnormality of liver function regarding to this parameter. A notable change of 0.75±0.01 was seen in Bilirubin content after *G.glabra* application but no noteworthy hepatoprotectve effect of *C. Intybus* was observed with an lesser value of 0.36±0.01 as compared to control value.

Total protein (TP)

Total protein content was found insignificant throughout the days, control has shown a value of 5.50±0.1 while its value slightly increased due to toxicity induced by paracetamol, *C.intyus* and *G.glabra* had showed the same value of 5.07±0.36. If the % comparison was observed Control and paracetamol 9% , both plants showed a same % which was 7.81%.

HISTOPATHOLOGYControl

Tissues of liver of normal rats preserved by Hematoxylin and Eosin (H&E).Control group showed sinusoidal spaces and normal hepatic architecture with discrete liver cells (Fig 23,A).The hepatocytes were in proper arrangement of cuboidal hepatocytes separated from each other by blood sinusoids lined. The hepatocytes appeared polyhedral in shape with large rounded vesicular nuclei. It was observed that, hepatocytes were in proper arrangement and of separated from each other by blood sinusoidal lines. There was no malignancy, atypia or pathological changes. (Figure A)

Table-3: Comparative Analysis of Hepatic Function Biomarkers Across Different Treatment Groups Over Time: Unveiling the Impact of Pctml, C. Intybus, and G. Glabra on Liver Health

Group/ Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Total Protein (mg/dL)	Albumin (g/dL)
Control						
10 days	53.2 ± 4.8	73.5±6.25	211±74.3	0.057±0.028	5.50±0.14	4.07±0.13
20 days	53.2±9.6	73.5±12.5	211±74.3	0.057±0.028	5.50±0.29	4.07±0.26
30 days	44.8±4.11	172±12.2	181±23.4	0.66±0.35	5.36±0.27	3.90±0.26
Pctml						
10 days	84.0±17.8^{a**}	74.6±1.20**	94.3±19*	0.053±0.08^{a*}	4.03±0.11^{a}	2.43±0.06^{a*}
20 days	67.7±14.6^{a**}	123±27.2**	278.2±26.92*	0.097±0.71^{a*}	5.97±0.095^{a}	4.45±0.115^{a*}
30 days	50.6±5.89^{a**}	107±20.6**	153±13.7*	0.16±0.06^{a*}	4.53±0.37^{a}	4.23±0.02^{a*}
C. Intybus						
10 days	66.6±7.97^{b**}	80.0±12.10**	222±36.8*	0.086±0.01^{b*}	5.60±0.23^{b}	4.26±0.11^{b*}
20 days	46.0±9.0^{b**}	141±37.5**	164.0±79.3*	0.147±0.041^{b*}	5.07±0.72^{b}	4.10±0.77^{b*}
30 days	43.6±5.78^{b**}	131±12.7**	168±31.0*	0.36±0.01^{b*}	5.60±0.31^{b}	3.73±0.28^{b*}
G. glabra						
10 days	64.3±6.35^{c**}	72.3±2.60**	65.0±5.8*	0.040±0.01^{c*}	5.16±0.14^{c}	5.16±0.14^{c*}
20 days	44.2±5.1^{c**}	130±18.0**	224.2±36.1*	0.160±0.43^{c*}	5.07±0.72^{c}	4.60±0.40c*
30 days	43.2±4.32^{c**}	170±22.7**	213±12.8*	0.75±0.01^{c*}	5.47±0.15^{c}	4.10±0.21^{c*}

= P<0.05 **= P<0.01, ± show SEM (Standard Error),a= significant difference between para and ctrl, b= significant difference between para and *C. Intybus,* c= significant difference between para and *G.glabra

Paracetamol:
Acetaminophen treated rat revealed markedly disturbed parenchymal architecture of the hepatocytes in the form of cytoplasmic vacuolation, degeneration, and acute necrosis of hepatocytes. Microabscess was observed that involve few necrotic debris and inflammatory cells. Other histopathological changes such as extensive accumulation of connective tissue that results in the formation of the continuous fibrosis septa. Steatosis was also observed that leads to the liver cirrhosis and finally to the liver fibrosis, and accumulation of hepatic tissue fibrosis septa around cental vein with areas of hemorrhages in blood vessels and steatosis.

***Glycyrrhiza glabra*:**
Liver histopathology of *Glycyrrhiza glabra* treated rats was observed at different magnification of microsope to elucidate any marked change by this hepato-protective plan. Portal triade (PT), sinusoids are also visible clearly(X200). There was small congestion and dilation of sinusoidal spaces at some places of liver tissue.

Cicorium intybus:
Different magnification power of microscope was used to evaluate the any hepatoprotective histopathological changes caused after the administration of *Cicorium intybus*

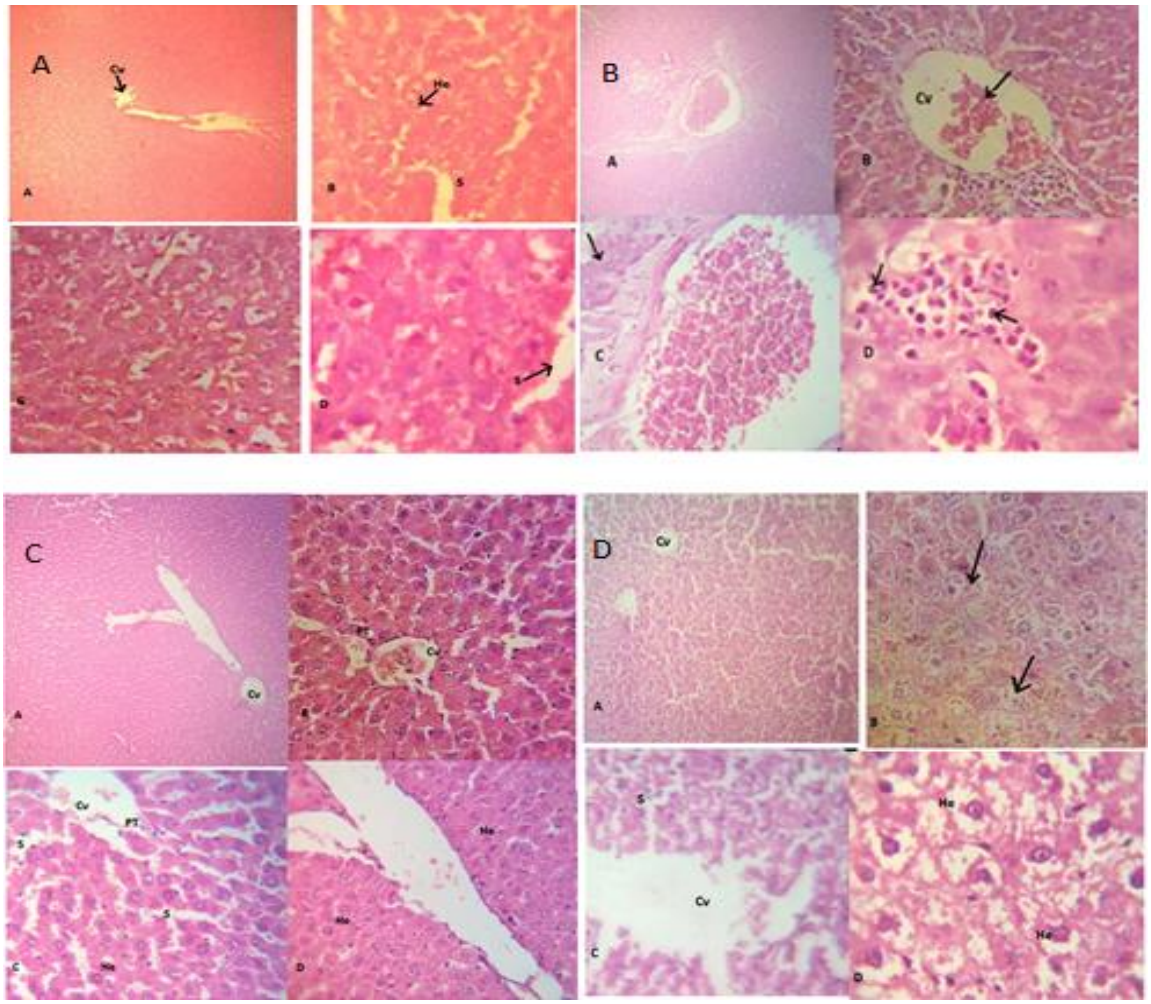


Fig-7: A.Histopathology of rat liver (control group). B.Histopathology of acetaminophen treated rat *C.* Histopathology of *G. glabra* treated. D. Histopathology of *C.intybus* rat.

DISCUSSION

Different studies showed recently that acetaminophen overuse in mice and rats can cause extensive and severe necrosis cells in the centrilobular area of liver, and increased serum ALT and AST levels in rats (Khorsandi. *et al.*, 2010). Acetaminophen toxicity within the centers of liver lobules causes hepatocytes necrosis, extending throughout them. In different species some differences are seen in sensitivity to paracetamol, so that in most rat strains acetaminophen is primarily hepatotoxic, but acetaminophen also shows nephrotoxic effects in others such as Fischer 344 strain (McGee *et al.*, 1992, Kumar *et al.*, 2005). Cell death mechanisms due to APAP consumption in humans and mice are initiated by the formation of the reactive metabolite N-acetylpbenzoquinone imine (NAPQI) which is generated by the cytochrome P450 enzymes Cyp2E1 and Cyp1A2. NAPQI is usually detoxified by conjugation with GSH but the availability of GSH is limited in case of overuse (Jaeschke *et al.*, 2010). Investigations also showed that in humans and mouse models, APAP-induced liver injury involves oxidative stress, mitochondrial damage, c-jun N-terminal kinase (JNK) activation and nuclear DNA fragmentation. However, the mechanisms of injury and cell death are different in rats and happen almost always due to apoptosis (Heard *et al*., 2014).

In current study Alanine amino transferase (ALT) was significantly elevated following administration of acetaminophen overdose when compared to the control ($p< 0.01$). The Leakage of ALT into the serum indicate damage to the endoplasmic membranes of hepatocytes (Thapa and Walia,2007). ALT is a specific parameter for liver damage and it also defines the integrity of the liver cells. It has also been reported that cell membrane

injury and necrosis of the cells is a result of free radical attack caused by acetaminophen (trichloro-) molecules (Konrad *et al.,* 2000).

Aspartate amino transferase (AST) activity was also significantly elevated ($p < 0.01$) in rats treated with acetaminophen at a dose of 2.5mg/kg. The elevation of AST level is a further indication of acetaminophen-induced hepatotoxicity. Alanine aminotransferase (AST) is produced within the cells of the liver and elevated serum levels of this enzyme suggest that there was damage to the integrity of the liver hepatocyte membranes. Previous studies showed that a acetaminophen at a dose of 400 mg/kg body weight was able to significantly increase the activity of AST at 4 and 6 hours after administration (Gujral *et al.,* 2002; Ganey *et al.,* 2007; Kanter et *al.,* 2010). However, lower doses of acetaminophen (16-66 mg/kg) showed an insignificant elevation of AST (Payasi *et al.,* 2010).

In present study the alkaline phosphatase (ALP) was elevated significantly ($p < 0.05$). Elevation in serum ALP has been associated with cholestatic liver disease (David, 1999) and liver damage as a result of pernicious anemia, zinc deficiency and hypophosphates (Thapa and Walia, 2007).

Total bilirubin values were within the normal range in all treatment groups except acetaminophen treated group. It was significantly elevated ($p< 0.05$) indicating hepatotoxicity. Liver damage promotes increase in both conjugated and unconjugated bilirubin.This is promoted by drugs that cause damage to the liver, chemical toxins, drinking excessive alcohol, brucellosis, hepatitis, and typhoid. Similar findings at a dose of 325 mg/kg of acetaminophen showed hyperbilirubinaemia, which was suggested to be as a result of the damage to the RBCs by acetaminophen administered orally (Harvey *et al.,* 1986).

In normal cases, albumin activity is elevated in states of acute, severe dehydration and excessive synthesis in the liver (Banaee *et al.,* 2008). It is also suggested that liver dysfunction may lead to increase in protein synthesis in the liver as a mechanism of sustaining the liver protein balance (Sagar and Vidyasagar, 2010). Similarly, production of corticosteroids and thyroid hormones is also known to increase the formation of albumin in the hepatocytes in the liver (Thapa and Walia, 2007). A positive presence of coumestans polypeptides, polyacetylenes, triterpenes, flavonoids and steroids could be responsible for the elevation of the albumin levels of treated groups of mice as a result of the regeneration of the liver.

The present study was performed to determine the hepatoprotective activity of two plants *G.glabra* and *C.intybus* against paracetamol (acetaminophen) induced hepatotoxicity in albino rats. It has been shown that most damage in acute acetaminophen toxicity, including, occurs within 24 hours of drug administration.

Caspase-1 and Akt-1 are top targets among array of all other potential compounds. High expression level of Akt-1 can be related to tumorigenesis, while it can also prevent apoptosis (Darr *et al.,* 2014). Caspase-1 is involved in apoptosis which is mediated by death receptor. Methyl 4-hydroxyphenylacetate and 4-hydroxylphenylacetic acid showed hepato-protective activity by two aspects, firstly, these compounds inhibit the expression of caspase-1 and that's why reduces the degree of liver damage via preventing apoptosis, and secondly, the prevention of the tumors formation because they can also down regulate abnormally high expression of Akt-1.(Edinger *et al.,* 2004, Wang *et al.,* 2001)

It is supported by significant decrease in the length, width and weight of kidney as gross anatomical assay. In *G. glabra* adminstrated rats, liver histological findings indicate a clear increase in sinusoidal spaces, with prominent decrease in hepatocytes diameters, with, centri-lobular hepatic congestion, and focal necrosis. Alteration in hepatocyte tissues could be caused by metabolism of plant extract in the liver (Louei *et al*.,2012). Increased metabolic activity can be detected by any changes in shape and size of of hepatocyte´s nucleus. Animal's excessive activity in order to get rid of the toxicants from body results in the focal necrosis of the liver tissues during the process of detoxification, which can be visualized in the experimental group, and liver's incapability to regenerate new cells can lead to necrosis or damage (Patel *et al.,* 2011).When orally administrated, intestinal bacteria containing β-D-glucuronidase will metabolizes "glycyrrhizin" to "glycyrrhetinic acid" (Hattori *et al.,* 1985). When glycyrrhizin intravenously administered, it is metabolized in liver by the activity of "lysosomal β-D-glucuronidase" to "3- monoglucuronide glycyrrhetinic acid". This metabolite is then excreted into the intestine along with bile, where it can be then be re-absorbed.(Akao *et al.,* 1991). Gentamicin-induced acute renal failure, in the rat model "Glycyrrhizin" could ameliorate renal defects.(Sohn *et al.,* 2003). In the mouse, glomerular disease model, "Glabridin" showed an anti-nephritis effect (Fukai *et al.,* 2003).

CONCLUSION

Evaluation of *G.glabra* and *C.intybus* for the prevention of paracetamol-induced hepatotoxicity was successfully performed in rats. Both plants hasshown the ability to protect the liver against hepatic injury when a toxicdose of paracetamol was administered to rats. Hepatotoxicity always remained the prime concern for scientists, doctors and drug developmental agencies. On the basis of body

weight it was observed that the *G.glabra* has shown an prominent recovery as compared to *C. intybus*. Relative body weight (RLW) of paracetamol treated rat was increased as compared to control rat, the administration of both plants has played crucial recovery of RLW. Futhermore, biochemical and histopathological study, it is evaluated that, both *G.glabra* and *C. intybus* have good efficacy in the prevention of hepatic injury caused by paracetamol (acetaminophen) at a dosage of 200mg/kg. It is concluded that after treatment with *G,glabra* and *C.intybus* hepatic enzymes and body weight has recovered, and level of biochemical parameters ALT, AST, ALP, Bilirubin, Albumin, Total protein were improved towards normal, which was disturbed by toxification and metabolic activation of acetaminophen can be altered by using different medicinal plants or herbs. For future prospect additional plant and herbs extracts can screened and the isolation of different hepatoprotective phytochemicals from these plant extract can decrease the possibility of hepatotoxicity and liver necrosis.

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