ORIGINAL ARTICLE

Antihepatotoxic Activity of Gemmotherapeutically treated Neem and alcoholic extract of Neem in Albino Rats

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ABSTRACT

Gemmotherapy is a new way of treatment by herbal medicine, using extracts of embryonic tissues from fresh plants such as young buds, shoots, leaves and rootlets of neem. In this study, the hepatoprotective effect had been evaluated by observing the antihepatotoxic effect of gemmotherpeutically treated and alcoholic extract of neem in albino rats. Hepatic damage was established by the commonly used pain killer in our human population i.e., the paracetamol, confirmed by evaluating the liver function parameters i.e. AST, ALT, ALP, bilirubin, magnesium, calcium and total proteins. Studies had shown that these rejuvenating plant tissues during their active growth stage have been found to be the rich source of plant growth factors and hormones. During this study it was concluded that neem extract possesses antihepatotoxic properties and support the empirical use of plant drugs in traditional system of medicine. It had also been presumed that the output of this study can be applicable experimentally to prevent the drug induced damage of liver during the treatment of chronic malaria, tuberculosis, cancers etc.

Keywords: albino rats, paracetamol, liver damage, antihepatotoxic activity.

INTRODUCTION

Liver is the most important organ which concern to detoxify the toxic substances and synthesize use full products. Due to severe side effects of synthetic agents like pain killer drugs etc. can damage the liver. We focus our research to evaluate scientific basis for herbal medicine in protecting the liver against damages[1].The different parts of plants are used to treat different diseases; particularly parasitic,hepatic and microbial infections.Plants contain many substances like alkaloids, flavonoids, glycosides, tannins etc. All these compounds have been found to play an important role against different diseases[2].Neem which is also called AzadirachtaIndica is an ever green tree cultivated in various parts of subcontinent. It has been used in ayurveda, unaniand homeopathic medicines. This tree is still regarded as village dispensary in Pakistan[3]. Neem seeds, neem oil, neem barks and leaf extract had been used to control parasitic infestations, respiratory disorders, constipation etc. Azadirachtaindicapossess antifungal activity, antimalarial activity, antibacterial activity, antiviral activity and anticancer activity[4]. Present study was carried out in albino rats to explore the effect of gemmotherapeutically treated neem extract (GTNE) and native neem extract(NNE)on biochemical and histopathological changes associated with paracetamol induced liver damage in rats. Silymarine is used as standard hepatoprotective agent. Gemmotherapy is a new form of herbal medicine using extracts of embryonic tissues from fresh plant; such as young buds, shoots, leaves and rootlets. These rejuvenating plant tissues, during active growth stage have been found to be rich sources of plant growth factors and hormones that are not found in whole plant[5.] In present study hepetoprotective effect of gemmotherapeutically treated neem and methanolic extract of leaves of neem is evaluated.

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MATERIAL AND METHODS

Chemicals and Drugs used

1.Paracetamol Pure powder was obtained from global pharmaceutical company,Islamabad.
2.Gumaccasia was procured from a local pharmacy at Faisalabad.
3.Ethanol from Merck Germany
4.Silymarin Powder from Shani Pharmaceutical, company Faisalabad.

Diagnosis kit

1.AST (SGOT) kit catalog no BD-117000-02 manufactured by Biocon, Germany.
2.ALT (SGPT) kit Catalog No BD 118000-04 manufactured by Biocon Germany
3.ALP kit catalog no BD-162200-23 manufactured by Biocon Germany.
4.Bilirubin Merck Germany
5.Magnesium, Merck Germany
6.Calcium, Merck Germany
7.Total protein

Experimental Animals Used: Healthy albino Wistar rats (200-300g) were obtained from the NIH, Islamabad.5 animals per cage were housed in metal cages. The animals had free access to food and tap water. Animals were kept under observation for one week beforeexperimentation, under usual management conditions at 30°C (environmental temperature), in the animal room of the physiology and Pharmacology Department, University of Agriculture, Faisalabad.

Induction of Hepatic Toxicity: Hepatic injury in rats was induces separately by oral administration of paracetamol (0.64 g/kg) suspended in 2% gumaccasia solution in water. The control animals received an equal volume of the vehicle (Gumaccasia).

Administration of plant extracts to different groups of rats

Parameters	G I	G 2	G 3	G 4	G 5	G 6	G 7
Gummaccia,1ml of 2% gumaccasiadaily for 40 days	+						
GTNE 100 mg/kg b.wt. daily for 40 days		+					
Paracetamol(0.64mg/kg b.wt single dose)			+	+	+	+	+
GTNE 100 mg/kg b.wtdaily for 40 days				+			
GTNE 250 mg/kg b.wtdaily for 40 days					+		
NNE 300mg/kg b.wt. daily for 40 days						+	
Sylimarine powder 100 mg/kg b.wt. daily for 40 days							+

PREPARATION OF EXTRACTS

Gum acacia solution: 2% Gum acacia in distilled water was prepared. After 48 hours it was shacked well.

Preparation of GTNE for antihepatotoxic activity: Fresh growing shoots and leaves were collected in spring season. It was dissolved in equal amount of glycerin and alcohol. After one month, the extract was filtered and then alcohol was evaporated in hot air oven at 65°C. After the evaporation of alcohol the remaining extract was measured and dissolved in gum acacia (2% solution). Two doses were prepared one as 100 mg/kg body weight and other was 250 mg/kg body weight.

Native Neem Extract for antihepatotoxic activity: Mature neem leaves were collected, was hed and then dried in shady place. All the leaves were grinded and then dipped in Alcohol. After one month it was filtered. The alcohol was evaporated at 65°C and solution was prepared by dissolving 300 mg/kg body wt. of extract powder in 2% gum acacia solution.

Silymarin Powder: 100mg/kg body wt. of Silymarin was dissolved in 2% gum acacia solution. Itwas used to cure the liver damage.

Administration of extracts and Silymarin solution: The amount of extracts and Silymarin powder for each animal was calculated on weight basis.Measured amount was suspended in 2% gum acacia solution. Drug was administered orally to each animal by using a feeding tube connects to a 5 ml calibrated syringe. The tube was inserted in to stomach and the plunger was pressed slowly. Immediate sneezing and coughing indicated that the tube is wrongly put into lungs; it was withdrawnimmediately and another animal was taken.

Induction of HepatotoxicityofParacetamol: The amount of paracetamol powder required for each animal was calculated on body weight basis. After weighing the required amount of powder, it was suspended in 2% Gum acacia solution. The method of administration was same as that of extracts.

Collection of Sample: For getting the sample, the animals were anaesthetized with ether followed by putting it intoa desiccator. 5 ml of blood was collected by cardiac puncture using sterile disposable syringe. The used syringe was damaged and discarded according to proper precautions. Serum was separated for determination of SGPT, SGOT, Alk. P., Total Bilirubin, Ca, Mg and total protein by using kits.

RESULTS

Antihepatotoxic activity: The antihepatotoxic potential of GTNE and NNE was compared with Silymarin powder. Theresults have been presented below:-

1.Effect of gum acacia on liver enzymes, total bilirubin, calcium, magnesium and total protein.
2.Effect of GTNE on liver enzymes, total bilirubin, calcium, magnesium and total protein.
3.Effect of paracetamol on liver enzymes, total bilirubin, calcium, magnesium and total protein.

Also the recovery of paracetamol induced liver damage was managed by using the following substances.

1. GTNE
2. NNE
3. Silymarin powder

1.Effect of Gum acacia on liver enzymes, Ca, Mg and total protein: In control group, the level of liver enzymes, Ca, Mg and total protein was in normal range. It was statistically non-significanthat shows the therapeutic safety of gum acacia. These results showed that in the control group, throughout the study, the level of enzyme SGPT, SGOT, Alkaline

phosphates were ranging correspondingly 37.9-39.6, 27-28, 99-105 U/L. The values were within normal limits, which revealed that gum acacia have no harmful effect on liver. The total bilirubin, calcium, magnesium and total protein levels were also with in normal range from 0 to40 days in group 1 as shown in fig 1-7. It showed the therapeutic safety of gum acacia.

2.Effect of GTNE on liver enzymes Ca, Mg and total protein:

a. Effect of GTNE on SGPT: Results showed the effect of GTNE on liver enzyme. SGPT levels have been presented in Table 4.10. In control group, the SGPT level from 0-40 days were ranging from 37-38 U/L. In case of GTNE the values were ranging from 35-43 U/L. The values were in normal range but long term treatment with GTNE are not free from toxicity as shown in Fig. 1

b.Effect of GTNE on SGOT: Average SGOT level in control group was 27, 28, 27 U/L, whereas in GTNE(100 mg/kg body weight)treated rats was 22.8-31.6 U/L indicating an increase in SGOT level. The results in fig2showedthat GTNE when treated for longer time caused hepatotoxicity.

C.Effect of GTNE on serum Alk.P level: As shown in Fig. 3, the Alk.P level in control groups was 99-104 U/L and 99-122 UL in case of GTNE from 0-40 days. The results have shown non-significant difference from each other. A slight increase in enzyme activity level was noted by the use of GTNE after 40 days, which shows that longer term use of GTNE could be toxic to liver.

d.Effect of GTNE on total Bilirubin

The bilirubin level in control group was ranging from 4.9-5.4 mg/dl correspondingly from 0-40 days (fig4). Its level in the group treated with GTNE was ranging from 7.2-7.5 mg/dl. Difference between control group and GTNE group regarding to bilirubin was also analyzed statistically.

e.Effect of GTNE on Ca& Mg

The level of calcium and magnesium was in normal range throughout the period of 0-40 days when treated with GTNE (Fig 5,6).

f.Effect of GTNE on total protein

The levels of SGPT in control group ranged throughout the period is6.9-7.08 U/L whereas in the GTNE group values were ranging from 7.3-6.34 U/L from zero to 40 days (Fig.7).

The results have showed that the level of serum enzymes and bilirubin were slightly increased by GTNE and the level of TP was decreased. Although all the values were in normal range but data showed that long term treatment with GTNE mg/dl can be harmful for liver. These results also show that the effect of GTNE is time dependent. All the results analyzedincluded the analysis of variance.

3.Post treatment antihepatotoxic effect of plant extracts andsylimarine

i.Effect of GTNE on paracetamol induced rise of serum SGPT: The level of SGPT in group III, IV and V was in normal range. The paracetamol induced liver damage indicated the rise in SGPT in these groups that was 311.2, 285, 280 U/L.It was found that GTNE (100 mg/kg, 250 mg/kg), normalized SGPT level after 40 days (fig1).

ii.Effect of GTNE on Paracetamol induced rise of serum SGOT: The serum SGOT level in group III, IV, V was 24, 26, 24 U/L before paracetamol induction. The paracetamol induced rise in SGOT were 72, 82, 76 U/L. The GTNE extract (100 mg/kg, 250 mg/kg) lowered the SGOT level in group IV, V but more significant changes were seen in group III.

iii.Effect of GTNE on paracetamol induced rise of Alk Phosphates: Fig 3.shows that groups (III, IV, V) serum Alk P level were (98, 104, 100U/L). The Paracetamol treated groups had level upto 311, 285, 280, U/L), which show a significant increase in serum Alk.P. The post treatments with GTNEwaseffective in case of Alk. P for groups IV, V. The GTNE lower the serum AlkP. Level in groups IV, V and it was significantly different from groups III.

iv.Effect of GTNE on paracetamol induced rise of total bilirubin: The level of total bilirubin in groups III, IV, V was (90.54, 0.56, 0.42 mg/dl) in normal range at zero day. Paracetamol induced the rise in total bilirubin level in these groups. Post treatment with GTNE (100 mg/kg). 250 mg/kg) to group IV and V lower the total bilirubin level after 40 days. These values are different from group III (fig4).

v.Effect of GTNE on group III, IV and V on calcium and magnesium: The level of calcium and magnesium was in normal range after liver damage in groups III, IV and V. After GTNE treatment the levels remain in normal range as shown in fig 5,6.

vi.Effect of GTNE on paracetamolinduced decrease in total protein: The mean values total protein of group III, IV and V was in normal range at zero day. There was decreased in total protein level after hepatic injury (fig7). A increase in total protein value was noted by use of GTNE extracts in group III, IV and V.

vii.Effect of NNE extract on paracetacemol induced rise in liver enzymes and bilirubin: Fig 1-4show that group VI serum SGTP, SCOT, Alk. P and total bilirbuin was in normal range at zero day. After paracetamoltreatment the level of SGPT, SGOT, Alk. P and total bilirubin was increased above normal level indicating the hepatic injury. The treatment with NNE (300 mg/kg) was found effective in normalization of SGPT, SGOT, Alk. P and total bilirubin. After 40 days, the NNE showed significant prophylacetic effects as compared with Group III.

viii.Effect of NNE onparacetamolinduced changes in calcium andmagnesium: The level of Ca and Mg remain normal before and after hapatic injury. Fig 5,6shows that NNE also did not caused any change in levels of Ca and Mg.

ix.Effect of NNE on paracetamol induced decrease of total protein: As shown in fig 7, a decrease was seen in total protein level in paracetamol treated group VI. Treatment with NNE at 300 mg/kg dose was found successful in normalization of total protein level.

x.Effect of Silymarin on paracetamol induced rise of liver enzyme: The group VII had serum SGPT, SGOT and Alk.P levels 28, 22.6 and 97.6 U/L respectively at zero day. The paracetamol induced rise in SGPT, SGOT and Alk. P was observed in this group (Fig.1,2,3). The Silymarin 100 mg/kg successfully normalized SGPT SGOT and Alk.P levels respectively. Silymarine treatment lowered the enzyme levels significantly as compare to group III. The results showed that the most effective therapeutic efficiency of silymarin powder 100 mg/kg.

xi.Effect of Silymarin on paracetamol induced rise of total bilirubin: Total bilirubin level was in normal range at before treatment day. The fig 4 reveals the paracetamol induced elevation of total bilirubin level in group III. The treatment with silymarin at 100 mg/kg dose levels lowered / normalized the elevated bilirubin level.

xii.Effect of Silymarin on Paracetamol induced calcium and magnesiumchanges: Level of calcium and magnesium was remaining in normal range before and after Paracetamol induction. Treatment with silymarin also do not show any kind of adverse effect in group VII as shown in Fig. 5,6.

xiii.Effect of silymarin on paracetamol induced decrease of total protein: The fig 7 shows that group VII total protein level was 7.00 at zero day, the paracetamol treated group had decrease to 4.8, which shows a significant decrease in total protein level. After the treatment with silymarin power at 100 mg/kg dose for 40 days, the normal levels of protein were achieved.

Fig 1.Effect of GTNE, gum acacia and post treatment of different concentration of extracts and silymarine powder against SGPT in different days

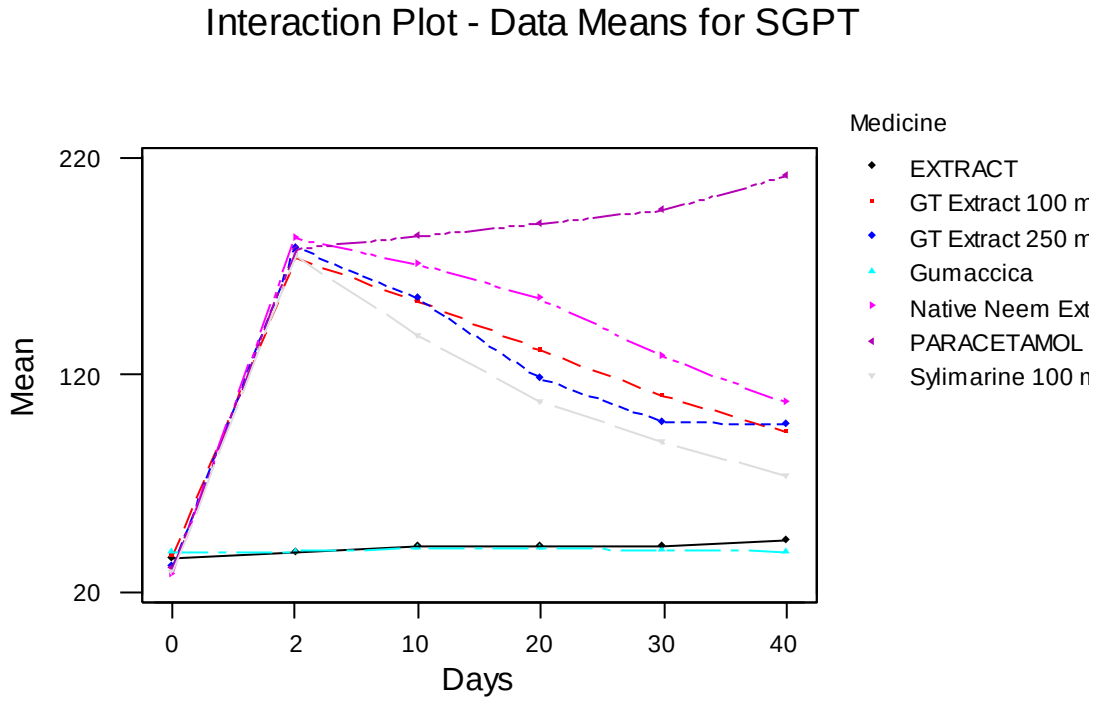


Fig. 2. Effect of GTNE, gum acacia and post treatment of different concentration of extracts and silymarine powder against SGOT in different days

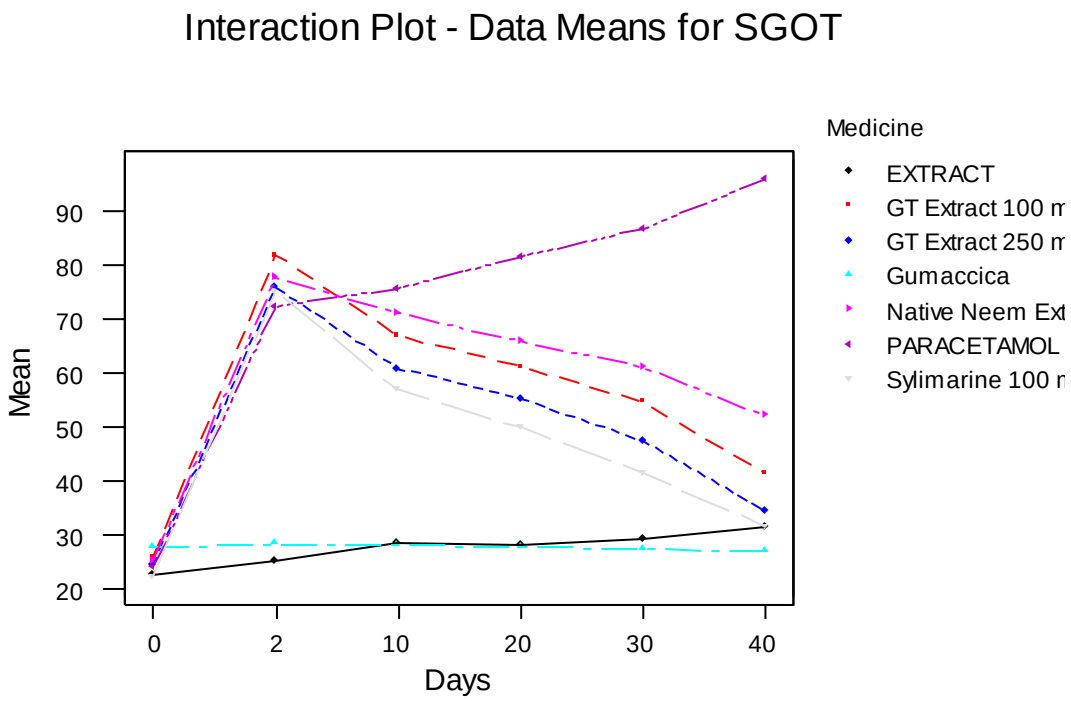


Fig 3. Effect of GTNE, gum acacia and post treatment of different concentration of extrects and silymarine powder against Alkaline phosphate in different days

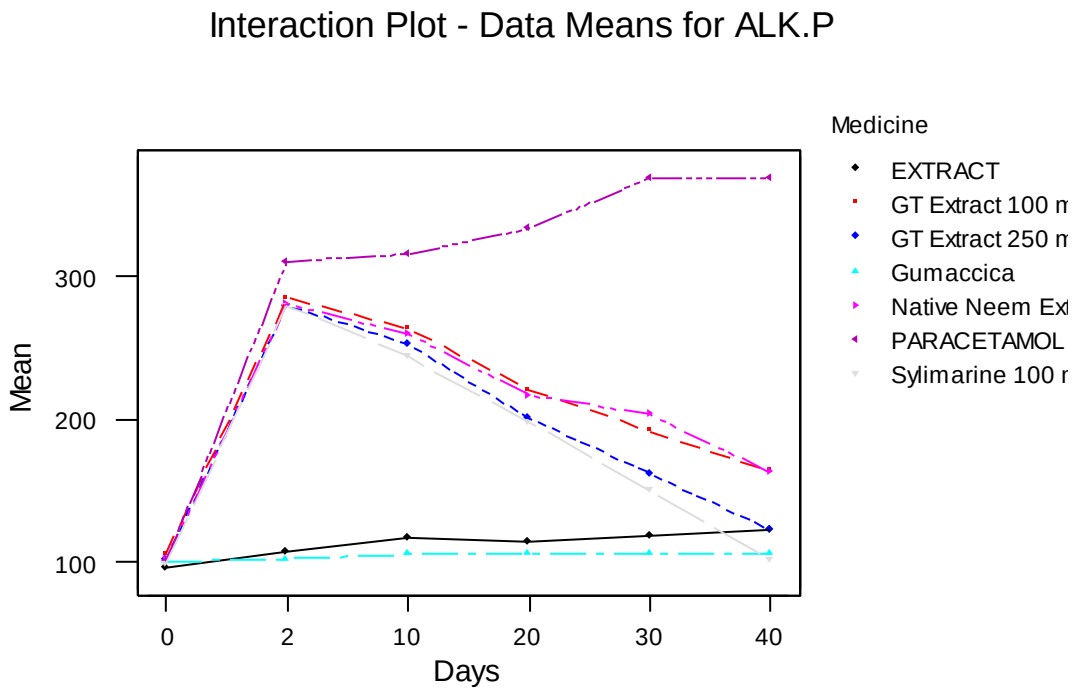


Fig. 4: Effect of GTNE, gum acacia and post treatment of different concentration of extrects and silymarine powder against total billirubin in different days

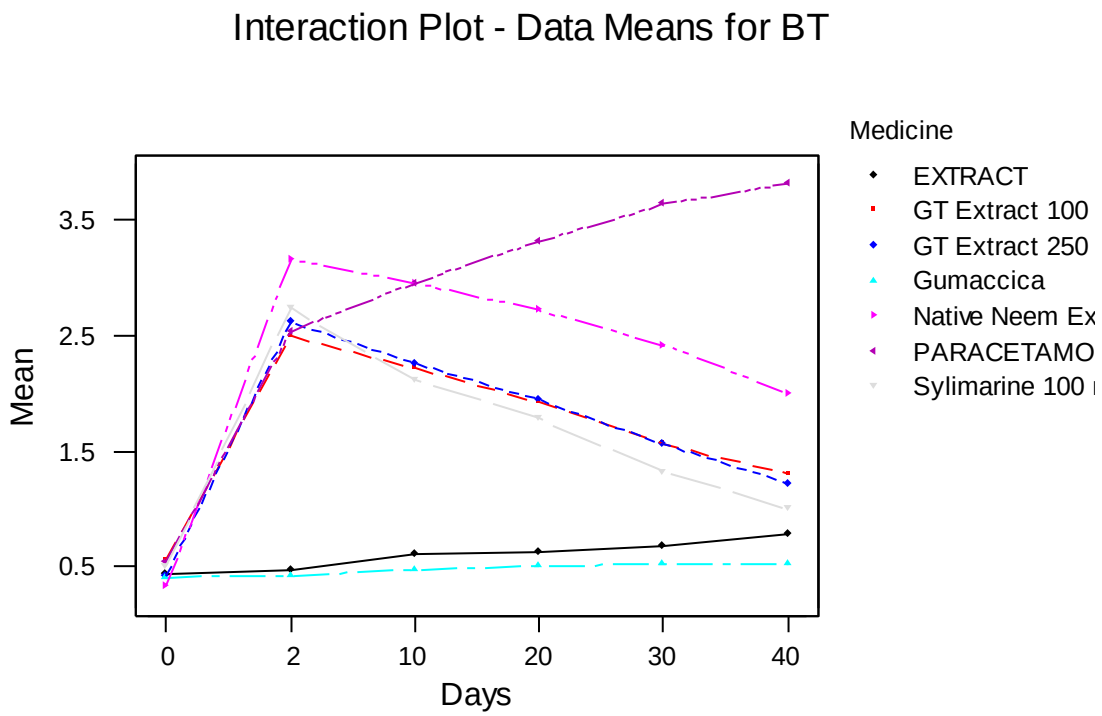


Fig. 5: Effect of GTNE, gum acacia and post treatment of different concentration of extracts and silymarine powder against calcium in different days

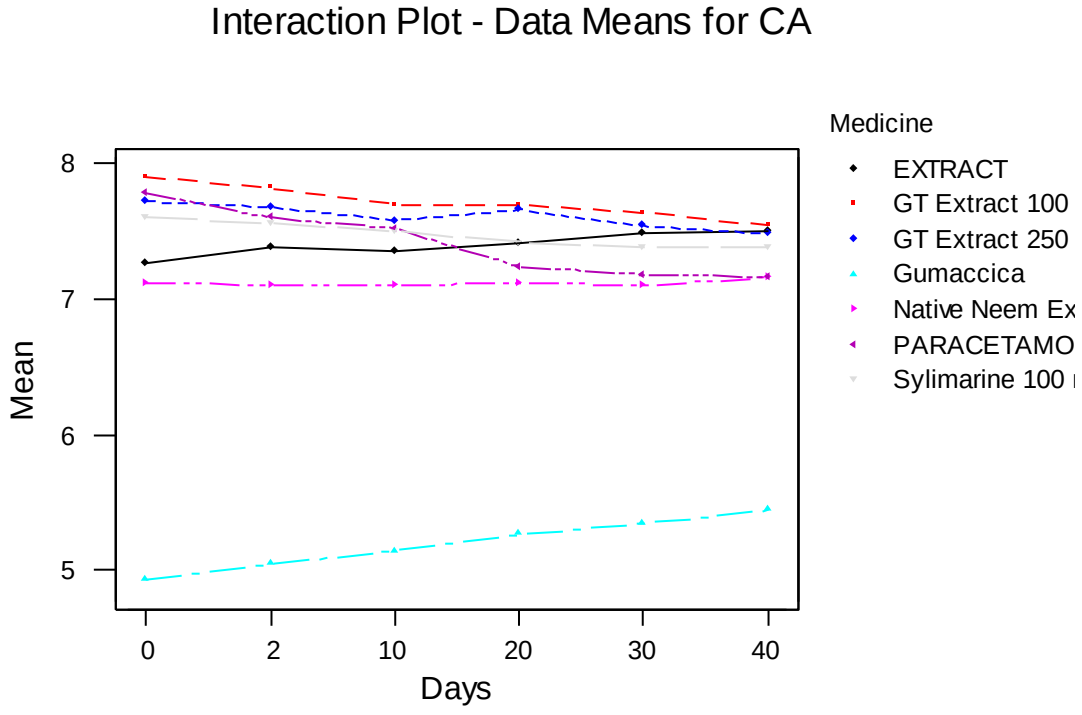


Fig. 6: Effect of GTNE, gum acacia and post treatment of different concentration of extracts and silymarine powder against magnesium in different days

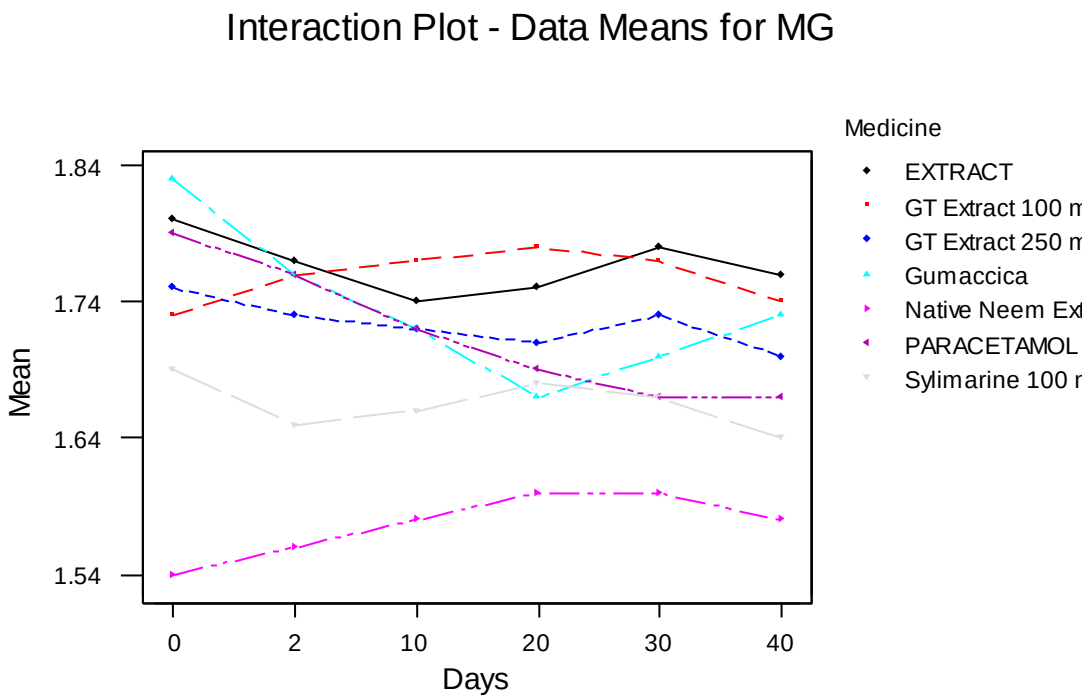
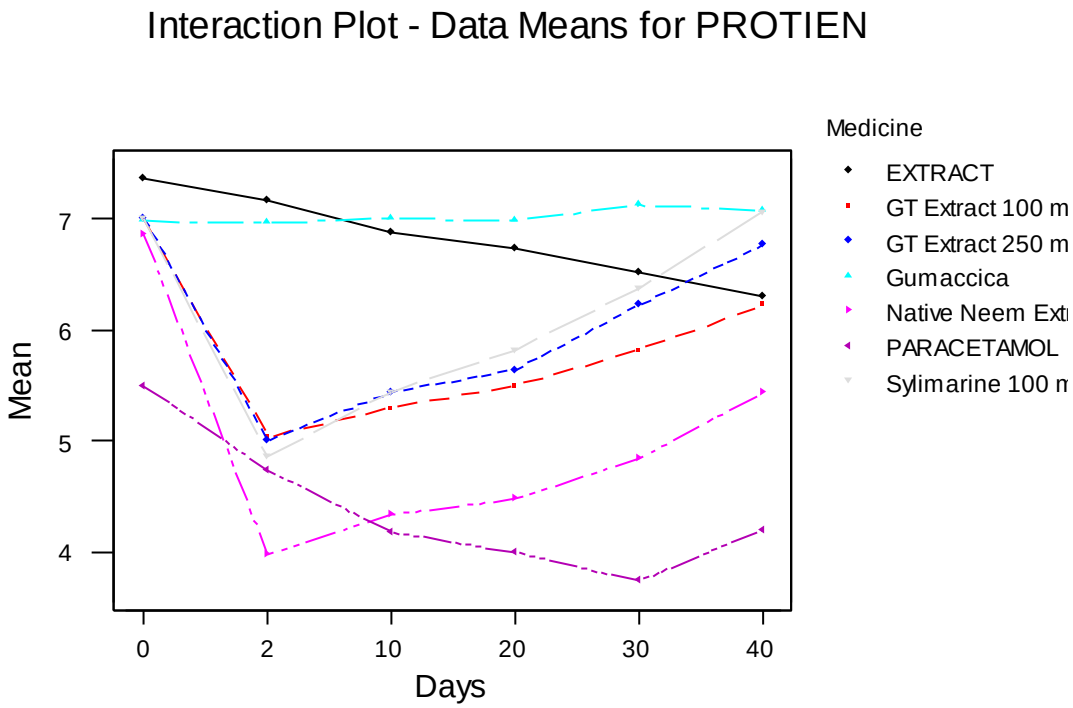


Fig. 7: Effect of GTNE, gum acacia and post treatment of different concentration of extrects and silymarine powder against total protein in different days



DISCUSSION

Paracetamol induced hepatic damage has been commonly used in experimental models for the screening of the newer hepatoprotective drugs (Slater, 1965; Plasa and Tewitt, 1982) and the extent of hepatic damage is assessed by the level of released enzymes like ALP, ALT and AST in blood circulation (Chenoweth and Hake 1962, Sallie*et al.,* 1991). Therapeutic doses of paracetamol are perfectly safe but over dosage can result in massive hepatic necrosis and may led to death from liver failure (Boyer and Fouff, 1971).

The toxicity of paracetamol is related to its metabolism. In the therapeutic doses, 60-90% is metabolized by conjugation to form paracetamol glucuronide and sulphate. A much smaller amount (5-10%) is oxidized by the mixed function oxidase enzymes to form a highly reactive compound (N - acetyl-p-benzo-genoneimine) which is then immediately conjugated with glutathione subsequently excreted as cysteine and mercapturate conjugates. Only 1-4% of the drug is excreted unchanged in urine. In over doses larger amounts of paracetamol are metabolized by oxidation because of saturation of the sulphate conjugation pathway. As a result, liver glutathione stores become depleted so that the liver is unable to deactivate the toxic metabolite. The reactive metabolite has high affinity for cell proteins and binds to liver cell macromolecules to cause hepatic necrosis (Weathena *et al* 1987) and Katzung 1998).

Paracetamol is converted to its reactive metabolite n acetual p benzoquinonemine (NAPQI) by hepatic cytochrome P-450 (Packer *et al,* 1978). The massive production of reactive species would lead to depletion of protective physiological moieties (glutathione&tocopherol, etc.) and ensuring wide spread propagation of alkylation as well as peroxidation, causing damage to the macromolecules in vital bio membranes (PeshImmam & Rechangel, 1977; Aldreigle, 1981). The rise in serum levels of AST, ALT and ALP has been attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962) because these are located in cytoplasm and are released into circulation after cellular damage (Sallie *et al.,* 1991). The inhibitors of microsomal drug metabolizing enzymes (MDME) can impair the bio-activation of paracetamol into its reactive species and, thus, provide protection, against the prevailing hepatocelluar damage (Castro*et al.,* 1974; Nelson *et al.,* 1980). The microsomal drug metabolizing enzyme (MD ME) inhibitory activity has been reported to be commonly present in medicinal plants (Shin, 1989; Gilani and Janbaz, 1995), their antihepatotoxic property was studied by dominating their effects on the serum AST, ALT and ALKP enzyme levels. The pretreatment of animals with the plant extract have resulting in the lowering of serum AST, ALT, ALP enzyme level. Therefore, it is conceivable that tested the plant extracts might contain MDME inhibitory constituents that provide hepato-protection.

The inhibitors of MDME can provide protection against hepatotoxicity when they are given before the metabolic activation of the hepatotoxic agent and fail to provide protection after generation of reactive metabolites (Gilani and Janbaz, 1995). Following ingestion, paracetmol is metabolized to its respective species within six hours (Akintonw and Essioem, 1990) and hepatotoxicity can be monitored by measuring serum transmainases at 24 hours. GTNE, NNE and a commercial preparation sylimarine powder (containing natural products) to have lowered the hepatotoxicity of the paracetamol perhaps by the inhibition of MDME.Fig 1,2,3 shows that in the post treatment the paracetamol induced rise in SGPT, SGOT, ALK.P, total bilirubin but total protein level was decreased and no effect on calcium and magnesium. These rises in liver enzyme level was decreased by GTNE, NNE and sylimarline powder. The results showed that sylimarine powder (100 mg/kg) dose exerted much more therapeutic effect.

Therapeutic trials with GTNE (100 mg/kg b.w.t) did not showed any harmful effect upto 40 days in a group of rats. Fig 1-7 showed that the levels of liver enzymes, bilirubin, total protein, calcium and magnesium which were normal before hepatic injury; were found increased along with decrease of total proteins after hepatic injury. The GTNE was effective to normalizethe liver enzyme levels and bilirubin but total protein level was elevated as compared to NNE. However, sylimierine 100 mg/kg b.wt showed good curative effect as compared to GTNE and NNE. The present results were also strongly supported by Kale *et al.* (2003) who assessed the liver damage induced by antitubercular drugs of biochemical and histopathological parameters. Moreover the aqueous extracts *of A. indica* significantly prevented the serum levels of bilirubin, total protein, SGPT,SGOT andAlk. P. He also reported that aqueous extract of *A. indica* significantly reversed the biochemical and histopathological changes. When liver was damaged with antitubercular drugs, the levels of Alk.P, SGPT, SGOT were increased. The situation with total protein was reversed, *A. indica* aqueous leaf extract administration lower down the levels of AlkP, SGPT, SGOT and total bilirbuin and increases

the level of total protein. Subapriyz in 2005 also reported that *Azadirchta indica*exhibited antioxidant property. The histopathological examinations of both prophylactic and therapeutic studies were in-line with serum enzymes level.

CONCLUSION

In conclusion the data obtained in the present study have clearly suggested that GTNE was more effective than NNE regarding hepatoprotective agent. Neemextract was found to possess antihepatotoxic properties and support the empirical use of the plant drug in the traditional system of medicine. (Slater, 1965; Plasa and Tewitt, 1982), Chenoweth and Hake, 1962 Sallie *et al.,* 1991, Boyer and Fouff, 1971, Weathena*et al* 1987) and Katzung (1998).(Packer *et al,* 1978, PeshImmam & Rechangel, 1977; Aldreigle, 1981), Chenoweth and Hake, 1962. It was also presumed that the output of this study can be applied to prevent the drug induced damage of liver during the treatment of tuberculosis, cancers etc.

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