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HEPATOPROTECTIVE ACTIVITY OF *Iphiona grantioides* AND *Pluchea arguta* FOR ACETAMINOPHEN INDUCED TOXICITY IN RABBITS

Shahida Naveed[1]*, Muhammad Ibrar and Barkatullah

ABSTRACT

The present study was aimed to explore the hepatoprotective role of Iphiona grantioides (IG) and Pluchea arguta subsp. glabra (PA) ethanolic extract (EE) in acetaminophen induced liver toxicity in rabbits. The experiment was conducted during April, 2011. Ethanolic extracts of Iphion agrantioides (IG) and Pluchea arguta subsp. glabra (PA) at three different dosages, administered orally for three days, significantly reduced the serum enzymes; Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and serum bilirubin. In this study, silymarin was used as a standard hepatoprotective drug. The results of this study showed that Iphion agrantioides (IG) and Pluchea arguta subsp. glabra (PA) ethanolic extract (EE) had hepatoprotective potential against acetaminophen induced liver toxicity in rabbits. The biochemical observations were supported with histopathological examination of rabbit liver sections. These findings exposed that the hepatoprotective activity of the ethanolic extracts of Iphiona grantioides (Bioss.) and Pluchea arguta subsp. glabra might be due to the presence of unique chemical constituents such as flavonoids, sesqueterpene, triterpenes and sterols.

Key words: Hepatoprotective, *Iphiona grantioides, Pluchea arguta*, acetaminophen, rabbit.

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INTRODUCTION

Iphiona grantioides (Boiss.) Anderb and *Pluchea arguta* (Boiss.) subsp. *glabra* Qaisar , are two medicinally important plants, growing widely in highly saline areas of district Karak, belongs to *Inuleae* and *Plucheeae* tribes of family Asteraceae, respectively (Qaiser, 2002).

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Iphiona grantioides is a perennial herb with woody base, 15-60 cm tall; the aerial parts are covered with glandular and non glandularhairs. Leaves are fleshy, lobed and flowers are yellow (Qaiser and Abid, 2003). In Sind province of Pakistan, it is used in the treatment of hepatitis, scabies and decoction of the whole plant is effective when given in snake bite (Atta-ur-Rahman, 2006). Plant paste is effective for fast healing of wounds (Qureshi, 2012) and leaves, when steeped in water, are used as a remedy for the treatment of asthma (Wazir *et al*., 2007). Chemically the plant containstriterpenes, sesqueterpenes, sterols, flavonols, flavones and flavnoides (Burdi *et al*., 1991; Ahmad *et al*., 1991b; Abid and Qaiser, 2003). *Pluchea arguta* (Boiss.) subsp. *glabra* Qaisar is a branched shrub. Other plants of this genus are traditionally used as hepatoprotective, astringent, antipyretic, anti-inflammatory, smooth muscle relaxant, nerve tonics and laxatives and as a treatment of dysentery, leucorrhoea, haemorrhoids and gangrenous ulcer (Ahmad *et al*., 1991a). Decoction of the whole plant has diuretic potential (Qureshi, 2012). Locally the plant is used as a camel fodder in district Karak. Its chemical composition is of sesquiterpenes of eudesmane type skeleton, triterpenoids and flavonoids (Ahmad *et al*., 1991a; Saba *et al*., 2011). Yogeshkumar (2012) reported that the plant has analgesic and anti-inflammatory potentials.

Liver is the largest and most important gland and second largest organ in the body; performing more than 500 functions within the body i.e., helps in digestion, manufacture and regulation of many hormones, synthesis of essential proteins and enzymes as well as the production of energy from the breakdown of food etc. It also combats infection through mobilization of the body's defense mechanisms, and removes harmful substances (drugs and toxins) not excreted by the kidneys (Ahsan *et al*., 2009), however, many drugs are known to cause hepatic injuries due to over dosage. More than 1100 drugs, world-widely, are potentially hepatotoxic (Biour *et al*., 1998) including acetaminophen (APAP),which is used as analgesic and antipyretic, however, it can cause hepatotoxicity and nephrotoxicity, when an overdose is administered (Bonkovsky *et al*., 1994).

Transferases, usually alanine aminotransferase (ALT) or aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) are enzymes normally reside inside cells (in cytoplasm) of liver, kidney, cardiac and skeletal muscles, however ALT is more specific to liver cells, than other body tissues. Elevated levels of these enzymes in serum usually represent hepatocellular damage. Similarly raised serum bilirubin is also associated with liver cell damage (Keith and Robert, 2001; Price and Stevens, 2003).

As majority of the allopathic medicines for the treatment of liver diseases have side effects (Chattopadhyay, 2003), thus it is needed to search for safer drugs to treat the liver diseases in order to replace currently used drugs of suspicious efficacy and safety. Medicinal plants are a rich source of various chemical constituents including alkaloids, flavanoids, phenols, glycosides, coumarins, monoterpenes, organic acids, lipids and xanthenes. Therefore they have been used for the treatment of liver diseases (Bhawna, 2009). It is reported that more than 160 chemical constituents from 101 plants have been isolated with hepatoprotective activity (Handa and Sharma, 1990). Similarly extracts of many plants have shown potential to cure liver disorders (Sharma *et al*., 2002). Silymarin is a plant extract, derived from the seeds of *Silybum marianum* L. It has been used for centuries as a natural medication for liver and biliary tract diseases (Saller *et al*., 2001). Silymarin protects and regenerates the liver in most liver diseases such as cirrhosis, jaundice, and hepatitis and offers good protection in various models of experimental liver disease. It acts by membrane stabilizing, immunomodulatory and liver regenerating mechanisms (Pradhan and Girish, 2006).

Several studies have been carried out to assess the hepatoprotective potential of medicinal plants e; *Inula britannica* (Song *et al*., 2000) *Inula racemosa* (Gnanasekaran *et al*., 2012), *Calendula officinalis* (Ali and Khan, 2006), *Cichorium intybus* (Butt *et al*., 2012), *Butea monosperma* (Maaz *et al*., 2010) and *Abutilon bidentatum* (Yasmin *et al*., 2012). As no hepatoprotective potential of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* has been worked out so far, so it is intended in the present work to find out hepatoprotective potential of these two plants.

MATERIALS AND METHODS

Chemicals/Reagents

Acetaminophen and Silymarin was gifted by the Chairman Department of Animal Health and Veterinary Sciences, The University of Agriculture, Peshawar- Pakistan. Standard kits of serum AST, ALT, ALP and serum Bilirubin (Bio Apex) were purchased from the market. All other reagents and solvents were of analytical grade and purchased from either Merck or Sigma.

Collection of plant sample and preparation of extract

Fresh plants of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were collected from the highly saline area of district Karak, Khyber Pakhtunkhwa, Pakistan in March, 2011. The plant samples were authenticated by Mr. GhulamJilani, Curator, Botany Department University of Peshawar and voucher specimens No. Bot. 20030 (PUP) and Bot. 20031(PUP) of *Iphiona grantioides* and *Pluchea arguta* subsp.

glabra, were deposited in the herbarium of Botany Department, University of Peshawar, Pakistan, respectively. Cleaned and shade dried plant materials were pulverized into a fine powder and 500 g powder of each plant sample was macerated in 1 liter each of 70% ethanol for 15 days with occasional shaking. The extract was then filtered and concentrated to a dark greenish semi solid residue (yield 20.5%) by a rotary evaporator at low temperature (45° C) and stored in a freezer (<4°C) until needed.

Animals

Adult healthy rabbits of local breed (*Oryctola guscuniculus*), of either six (average weight 700 - 900 g) were obtained from PCSIR Laboratories Complex, Peshawar Pakistan, for the study. Rabbits were kept at animal house of the Department of Animal Health and Veterinary sciences, Khyber Pakhtunkhwa Agriculture University Peshawar, on a 12-h light/dark cycle under controlled conditions of temperature (25 ± 1° C) and humidity (50 ± 5° C) for one week. They were fed with standard diet and water *ad libitum*. NIH (National Institute of Health, 1985) guide lines for the care and use of the laboratory animals were followed, while performing all the experiments (NIH, 1985).

Phytochemical studies

Extracts of the two plants were screened for the presence of various phytochemical constituents by performing different qualitative chemical tests (Trease and Evans, 1989; Kokate, 1994; Harnborne, 1998; Evans, 2002; Khandelwal, 2004; Kumar and Kiladi, 2009; Chitravadivu *et al*., 2009).

Preliminary acute toxicity of Plant material

Rabbits were divided into 9 groups (A- I) of 5 animals each. Group-A served as control and received saline only. Groups-B to E received ethanolic extract of IG and groups-F to I received ethanolic extract of PA through oral administration, in the doses of 0.5 g, 1.0 gm, 1.5 gm and 2.0 gm/ kg. Animals were observed after extract administration continuously for the first 4 hours to note any behavioral change and then for 48 hours to record the mortality (Jeyasekar *et al*., 1998).

Acetaminophen induced hepatotoxicity

Experimental design

Seventy two healthy rabbits of either sex were randomly divided into twelve groups (n = 6 in each group). Animals of group -1 (control) were administered with distilled water (1 ml/kg) daily for three days along with food and water. Group-2 (acetaminophen) received distilled water (1 ml/kg) once daily for three days and received acetaminophen (2 g/kg body. wt.) on day 3 and Group-3 received standard drug silymarin (100 mg/kg), orally, once daily for

three days. Animals in groups 4 , 5 and 6 were orally fed with ethanolic extract of IG in three different doses (low, medium, high) of 100, 300 and 500 mg/kg in the form of aqueous suspension along with food and water, once daily, for three days. Similarly animals in groups 7 ,8 and 9 were orally fed with ethanolic extract of PA in three doses (low, medium, high) of 100, 300 and 500 mg/kg in the form of aqueous suspension along with food and water, once daily, for three days. Rabbits of groups 10 , 11 and 12 were given mixed extract of IG and PA in the ratio of 1:1 , in three doses 100 mg /kg b. wt. (50 mg IG+ 50 mg PA),300 mg /kg b. wt. (150mg IG+ 150 mg PA) and 500 mg/kg b. wt. (250 mg IG+ 250 mg PA) for three days (Table-1). Animals in all groups (3-12) except 1 and 2 were given acetaminophen (2 g/kg) on day three, 1 hour after the oral administration of silymarin and plant extracts in either case. All the animals were anaesthetized 48 hours after the last treatment. Blood samples were collected via the vein puncture of animals ear, blood was kept in tubes (without anticoagulant) allowed to clot and serum was separated by centrifugation (2500 r.p.m. for 15 minutes) at 37° C and analyzed for various biochemical parameters (Yasmin *et al*., 2012; Prochezian and Ansari, 2005).

Table-1. Schedule of acetaminophen and plant extracts administration on a pre-treatment basis

Group 1 - Normal control (Distilled water (1 ml/kg)
Group 2 - Toxicant (Acetaminophen 2 g /kg)
Group 3- Standard drug (Silymarin 100 mg/kg) + acetaminophen
Group 4- Ethanol extract of IG (100 mg/kg) + acetaminophen
Group 5 - Ethanol extract of IG300 mg/kg) + acetaminophen
Group 6 - Ethanol extract of IG (500 mg/kg) + acetaminophen
Group 7 - Ethanol extract of PA (100 mg/kg) + acetaminophen
Group 8 - Ethanol extract of PA (300 mg/kg) + acetaminophen
Group 9 - Ethanol extract of PA (500 mg/kg) + acetaminophen
Group 10 - Ethanol extract of IG (50 mg):PA (50 mg) /kg) + acetaminophen
Group 11 - Ethanol extract of IG (150mg): PA (150mg/kg) + acetaminophen
Group 12 - Ethanol extract of IG (250mg) : PA (250mg/kg) + acetaminophen

(IG=*Iphiona grantioides*, PA=*Pluchea argutas*ubsp.*glabra*)

Liver function test

The biochemical parameters were assessed by the estimation of raised level of serum ALT, AST [Reitman, 1957), ALP (Kind and King, 1954) and serum bilirubin (Mally and Evelyn, 1937) by using the commercially available test kits.

Histopithological examination

For histopathological examination, three animals from each group were sacrificed by cervical dislocation and the abdomen was cut

open to remove the liver. A portion of the liver was cut into two to three pieces approximately of 6 mm and fixed in 10% buffered formalin. After fixation, these tissues were processed in automatic tissue processor (RX-11B, Sakura, Japan) for histopathological studies. Tissues were embedded in paraffin wax in Paraffin Dispenser (TEC, SEMT-2 Sakura, Japan) and thin section (3-5µm) were cut using rotary microtome (SRM 200, CW, Sakura, Japan). The mounted section on the glass slides were stained in automatic slide Stainer (DRS2000 J-B_2, Sakura Japan) using H&E staining as described by (Bancroft, 2007). The stained sections were made into permanent slides and examined under high resolution microscope (OLYMPUS C×41, Japan) with photographic facility and photomicrographs were taken at the resolution power of 10 × 40.

Data analysis

Data was expressed as a means ± SE. One-way analysis of variance (ANOVA) was used for the evaluation of difference between multiple groups followed by Duncan's multiple range test for individual comparisons using SAS 9.1 software (SAS Institute Inc., USA). Data was considered statistically significant when the *p* value was less than 0.05.

RESULTS AND DISCUSSION

Phytochemical screening

Qualitative phytochemical screening of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* revealed the presence of proteins, carbohydrates, tannins, alkaloids, flavonoids, saponins, glycosides, phytosterol, tri-terphenoids and volatile oil in the ethanolic extracts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra.* The result showed that both the plants are a rich source of bioactive compounds and hence is a potential source of therapeutic properties.

Acute toxicity of plant

Animals of all the groups were found alive after 24 hours and no behavioral changes were observed, that proved that the plant material is safe up to an oral dose of 3.0 g/kg.

Acetominophen induced hepatotoxicity

The present study was designed to evaluate the hepativeprotective effect of ethanol extract of *Pluchea arguta* (Boiss.) subsp. *Glabra* Qaiser and *Iphiona grantioides* (Boiss.) separately and in combination. As compared to control (Group -1), acetaminophen administration in Group - 2 significantly ($p<0.05$) increased AST, ALT, ALP and Serum Bilirubin levels. In all the test groups (Group 3-12),where ethanolic extracts of *Iphiona grantioides* (IGEE) and *Pluchea arguta* subsp. *glabra* (PAEE) were given in three different (100,300,500 mg/kg.b.wt.) doses, have significantly ($p<0.05$)

decreased these parameters towards normalization in a dose dependent manner. The results are shown in Table-2 and Fig. 1 and Fig. 2. Values of AST, ALT, ALP and S. Bilirubin in rabbits of control group were found to be 37.035± 3.581^{F} I.U / L,31.91±2.175 ^{I} I.U / L, 54.22±3.611 ^{G} I.U/Land 0.063± 0.004^{F} mg/dl (n=6), respectively; while a toxic dose of acetaminophen (1000 mg/kg) significantly raised ($p<0.05$) the respective serum values to 188.17± 7.703^{A} I.U/L, 273.83±6.231 ^{A} I.U/L,191.62±3.360 $^{\mathbf{A}}$ I.U/Land 2.190±0.164 $^{\mathbf{A}}$ mg/dl in animals of group-2. Values of AST, ALT, ALP and S. Bilirubin in animals of rest of the groups (4-12) either treated with ethanolic extracts of IG and PA or mixed extracts of IG+PA, in three different doses (100, 250 and 500 mg/kg. body. wt.) were decreased in a dose dependent manner. Extracts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* (in the doses of 100 mg/kg and 250 mg/kg) separately and in combination, slightly and moderately decreased the AST, ALT, ALP and S. Bilirubin values, while high dose (500 mg/kg) of IG extract significantly reduce the respective values up to 59.917± 2. 556^{E} I.U/L, 68.54±1.466^{F} I.U/L, 154.16±1.809^{E} I.U/L and 0.833± 0.033^{DE} mg/dl, respectively. Similarly, high dose (500 mg/kg) of PA extract reduced the values of AST (59.078± 2.174^{E} I.U / L), ALT (63.59±2.627^{FG} I.U/L), ALP (155.89±1.033^{E} I.U/L) and S. Bilirubin (0.850±0.022^{DE} mg/dl). Serum values for combined extract of IG+PA were 60.017± 2.849^{E} (AST), 60.245± 1.523^{GH} (ALT), 153.04± 1.015^{E} (ALP) and 1.007±.0201^{CD} (S. Bilirubin), that were significantly lower ($p<0.05$) than the values of toxic group and very close to the serum values of silymarin treated group which were 52.850± 6.257^{E} (AST), 54.625±3.948^{H} (ALT), 124.88±2.030^{F} (ALP) and 0.7550±0.044^{E} (S. Bilirubin).

Results of histopathology

In group-1, Normal hepatocytes showed fairly well preserved lobular texture. No cellular infiltration or circulatory disturbances were observed. Nuclei appeared normal and were centrally placed. No abnormal morphological pattern and cellular accumulations were observed in the parenchyma (Fig. 3)

In group-2 (Intoxicated acetaminophen group), sinusoidal spaces were dilated due to shrinkage of hepatocytes. A severe degree of cellular infiltration around the blood vessels was observed in some sections (Fig. 4). There was severe vacuolation, suggesting early stage cellular injury in the cytoplasm of hepatocytes.

In the groups, treated with ethanolic extract of IG the protective effect of *Iphiona grantioides* (Boiss) was evident by decrease in the changes as observed in the rabbits in group-2. A cellular infiltration of moderate degree along with mild to moderate degree of leukocytic

infiltration was observed around the blood vessels. The vaculation of lesser degree than rabbits in group B was observed (Fig. 6).

The histological picture of the liver in the group treated with ethanolic extract of PA, was almost similar to the rabbits in group treated with IG extract, showing that *Pluchea arguta* subsp. *glabra* Qaiser has similar healing effects on hepatocytes (Fig. 6). Similarly Rabbits treated with Silymarin + Acetominophen (Fig. 5) and with combined extract of IG+PA showed marked degree of regeneration. The sinusoidal spaces were normal and hepatocytes showed well preserved foamy structure. The blood vessels showed no cellular infiltrations in their surroundings (Fig. 7).

In the present study acetaminophen intoxication raised the level of AST, ALT, ALP and S. Bilirubin in the blood serum of experimental animals and causes acute hepatocellular damage and biliary obstruction. In contrast ethanolic extract of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* significantly ($P < 0.05$) decrease the raised levels of AST, ALT, ALP and Serum bilirubin (Table-2) in a dose dependent order. The present results are in line with the findings reported by Ali and Khan (2006) (*Calendula officinalis*); Butt *et al*., 2012 (*Butea monosperma*) and Maaz *et al*. (2010) (*Abutilon bidentatum*).

The efficacy of any hepatoprotective drug is essentially dependent on its ability in reducing the harmful effects or maintaining the normal liver physiology that has been disturbed by a hepatotoxin. The exact mode of hepatoprotective action of the plant extract may be speculative at this stage; however, it may be due to the presence of flavonoids and phenolic compounds, known for antioxidant and hepatoprotective activities (Di-Carlo *et al*., 1999).There are many hypothesis to explain the drug induce liver injury. The toxicity produced by acetaminophen following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by antioxidants (Harman, 1985). Calcium contents in the liver cells are increased during experimental hepatic damage (Moor *et al*., 1985) and Ca++ cannel blocking agents i.e. nifedipine, diltiazem and verapamil were found to inhibit the development of hepatic damage (London *et al*., 1986; Thibault *et al*., 1991). Some hepatoprotective drugs have been proved to have Ca^{++} chanel blocking constituents (Gilani *et al*., 1994). The other possible mechanisim is through microsomal drug metabolizing enzyme (MDME) inhibition.The inhibitors of MDME can impair the bioactivition of acetaminophen into its reactive metabolite and hence provide protection against the prevailing hepatocellular damage and since MDME inhibitory activity is reported to be common in medicinal plants (Shin, 1989) so both the plants *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* must be investigated for possessing

any such activity or not. The plant material is safe as is apparent by the lack of any symptoms of acute toxicity at an oral dose of as high as 3.0 g/kg. This study lends some support for the traditional use of *Iphiona grantioides* and plants of genus *Pluchea* in hepatobiliary diseases.

Results of histopathological studies provided supportive evidence for biochemical analysis. Histopathological examination of the liver sections confirmed that normal cellular construction was damaged with acetaminophen treatment (Fig-2). However, extract treated rabbits showed less damage of the liver structure (Fig 4-6).

CONCLUSION

The present study, although initial, has provided a strong evidence of the hepatoprotective potential of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* and it can be concluded that *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* contains some specific constituents that may be potentially effective against the liver injury induced by acetaminophen. Further studies on these two plants should be conducted for the isolation, structure determination and pharmacology of the hepatoprotective constituents. So that *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* may be used in the drug development for various hepatic diseases.

Table-2. Effect of *Iphiona grantioides* ethanol extract (IGEE), *Pluchea arguta* ethanol extract (PAEE) on serum enzymes and Bilirubin contents of rabbits.

S. #	Groups	AST I.U / L	ALT I.U / L	ALP I.U / L	S. Bilirubin mg/dl
1	Control	37.035± 3.581^{F}	31.91±2.175 ^{I}	54.22±3.611 ^{G}	0.53± 0.004 ^{F}
2	Silymarin+ acetaminophen	52.850± 6.257 ^{E}	54.625±3.948 ^{H}	124.88±2.030 ^{F}	0.7550±0.044 ^{E}
3	Acetominophen 2 g / kg	188.17± 7.703^{A}	273.83±6.231 ^{A}	191.62±3.360 ^{A}	2.190±0.164 ^{A}
4	IGEE 100 mg + acetaminophen	122.50± 473^{B}	132.06±2.213 ^{C}	180.89±2.295 ^{B}	2.048±0.039 ^{A}
5	IGEE 300 mg+ acetominophen	85.215± 2.576 ^{D}	84.28±1.375^{DE}	168.23± 4.047^{CD}	1.0417±0.009 ^{CD}
6	IGEE 500 mg+ acetominophen	59.917± 2.556^{E}	68.54±1.466 ^{F}	154.16± 1.809 ^{E}	0.833±0.033 ^{DE}
7	PAEE 100 mg+ acetominophen	129.83± 2.614^{B}	151.43± 2.661^{B}	172.99± 1.780 ^{C}	1.703±0.206 ^{B}
8	PAEE 300 mg+ acetominophen	102.98± 2.573^{C}	90.43±1.117^{D}	164.36± 1.157 ^{D}	1.025±0.004 ^{CD}
9	PAEE 500 mg+ acetominophen	59.078± 2.174 ^{E}	63.59±2.627 ^{FG}	155.89±1.033 ^{E}	0.850±0.022^{DE}
10	IGEE+PAEE100m+ acetominophen	108.75± 3.024^{C}	130.89±2.199 ^{C}	184.88± 1.724 ^{B}	1.183±0.046^{C}
11	IGEE+PAEE300mg+ acetominophen	77.12±5.785 ^{D}	77.348±2.294 ^{E}	164.43±1.480 ^{D}	1.035±0.008^{CD}
12	IGEE+PAEE 500mg +acetominophen	60.017± 2.849 ^{E}	60.245±1.523^{GH}	153.04± 1.015 ^{E}	1.007±.0201^{CD}

IGEE = *Iphiona grantioides* ethanolic extract, PAEE= *Pluchea argut*ae thanolic extract
Means with different superscript letters are significantly different (P<0.05)

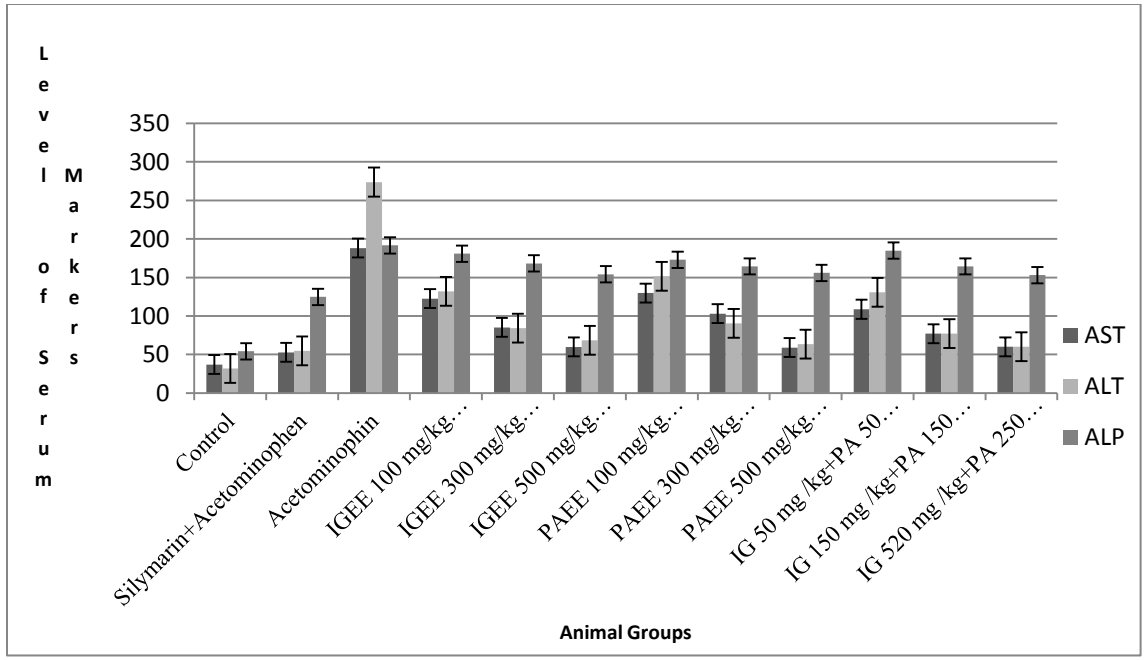

Figure 1. Comparison of different concentration of serum markers AST, ALT and ALP in rabbits after administering different doses of plant extracts with control, silymarin and acetominophen

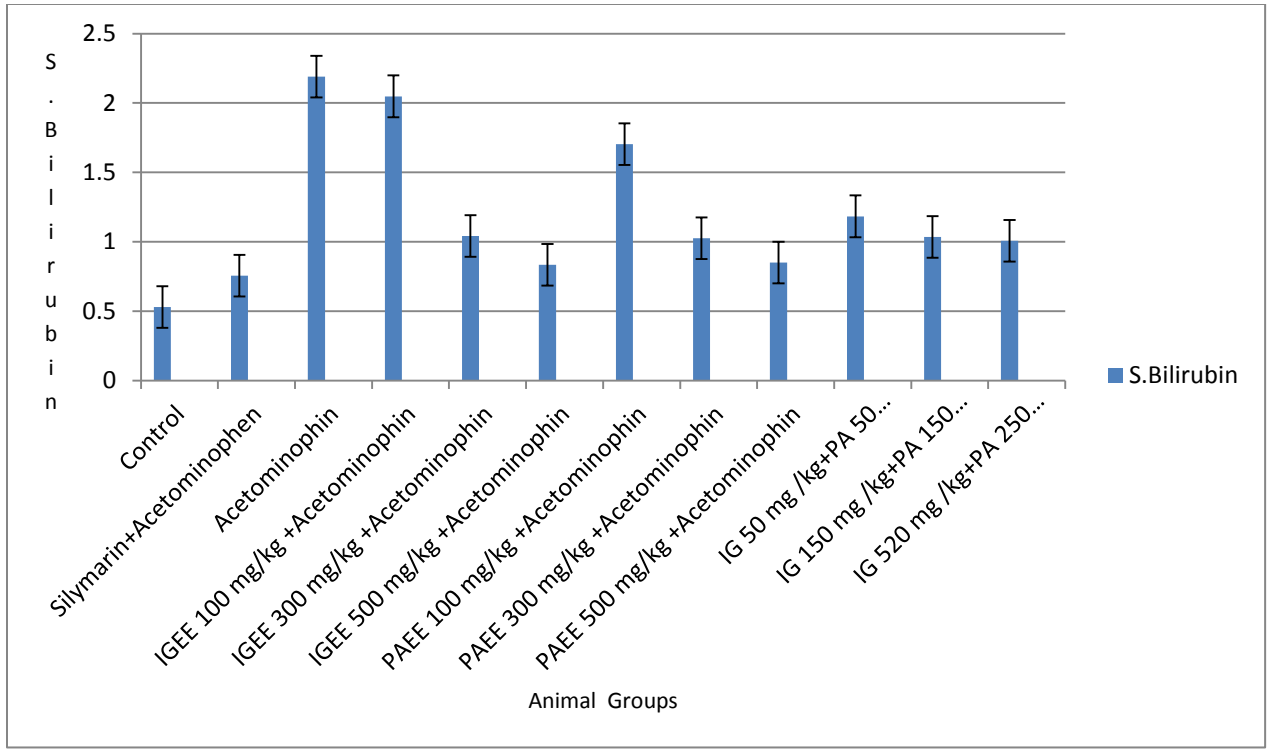

Figure 2. Comparison of different concentration of serum Bilirubin in rabbits after administering different doses of plant extracts with control, silymarin and acetominophen

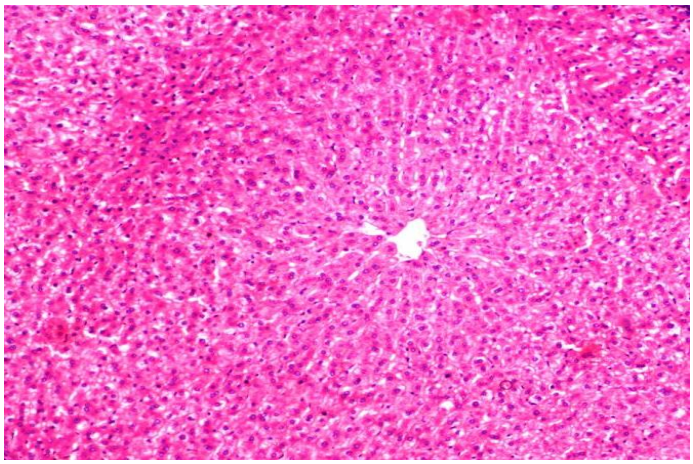

Figure 3. Liver of rabbit treated with Normal diet

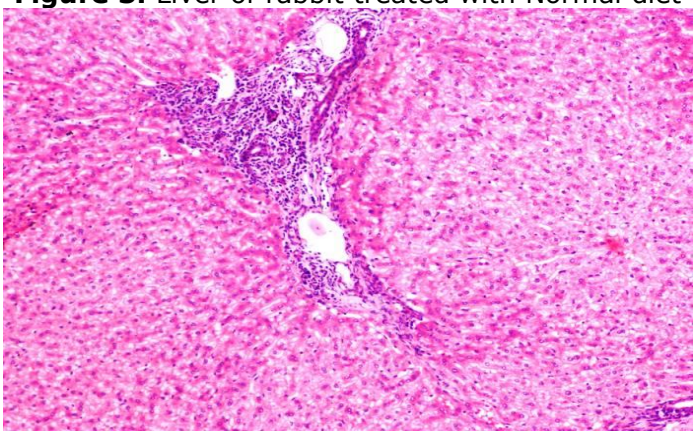

Figure 4. Liver of rabbit treated with Acetominophen

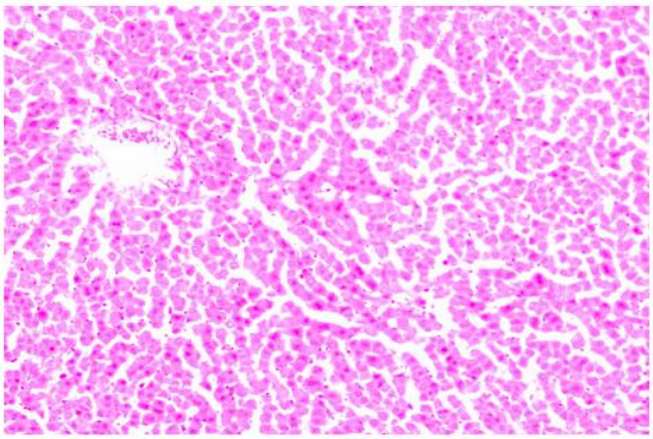

Figure 5. Liver of rabbit treated with silymarin+ acetominophen

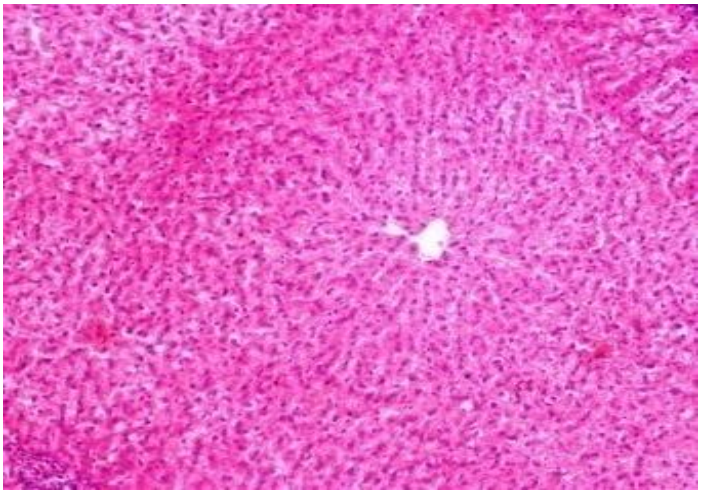

Figure 6. Liver of rabbit treated with ethanolic extract of IG

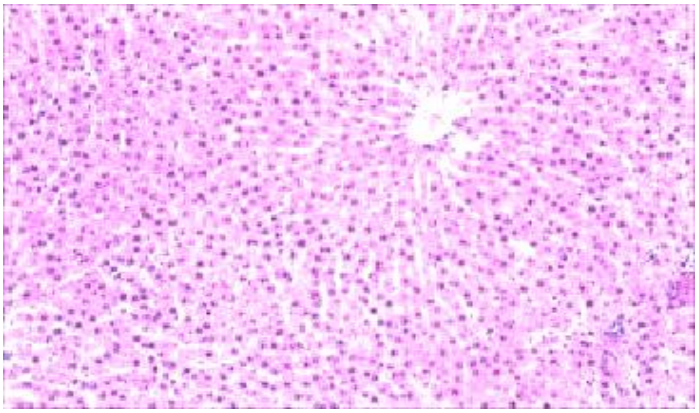

Figure 7. Liver of rabbit treated with ethanolic extract of PA

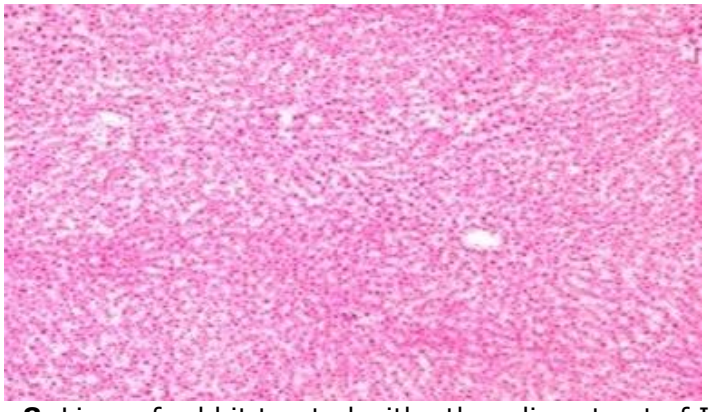

Figure 8. Liver of rabbit treated with ethanolic extract of IG+PA
(IG= *Iphiona grantioides*, PA= *Pluchea arguta* subsp *glabra*)

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