

Research Article

**Effect of *Fagonia arabica* dry leaves against
hepatotoxicity of Acetaminophen**

**Bhagyashree R. Patil, Hussein M. Ageely¹,
Manal Abdel Latif and Yasmin O. El-Amir²**

Department of Medical Biology, ¹Department of Internal Medicine,
Faculty of Medicine, Jazan University, Jazan, Gizan, KSA
² Department of Pathology and Clinical Pathology,
Faculty of Veterinary Medicine, Assiut University, Egypt
bhagyashreerpatil@gmail.com

ABSTRACT

Plants and plant parts are being utilized for drug discovery and development in recent years. Out of the flora from Arabic peninsula, *Fagonia arabica* is claimed to have many medicinal properties. Current project was undertaken to assess the anti-hepatotoxic efficacy associated with *Fagonia arabica* dry leaves powder against acetaminophen induced hepatotoxicity. Single hepatotoxic dose of acetaminophen at a concentration of 1000mg/kg BW was given to induce the hepatotoxicity. The same time, two different concentrations of 250 mg/Kg BW, and 500 mg/kg BW of *Fagonia arabica* dried leaf powder were used to assess possible hepatocyte protection. Evaluation was carried with help of liver and kidney function tests and liver histopathology.

Key words- *Fagonia Arabica*, liver, Acetaminophen, hepatoprotection

INTRODUCTION

Fagonia species has gained interests by the researchers recently, for its medicinal properties. [1-8] In lieu of this present work was carried out to assess hepatoprotective efficacy claimed to be associated with *Fagonia arabica* leaves using acetaminophen as a hepatotoxin. At therapeutic doses, acetaminophen is safe, however it acts as an hepatotoxin above the recommended doses.[9] Hence hepatotoxicity is the most remarkable feature of APAP overdose. Acute overdoses of APAP can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same. APAP toxicity is foremost cause of acute

liver failure. [10] So in this work hepatoprotective efficacy of the *Fagonia arabica* dry leaves powder was examined against hepatotoxic dose of acetaminophen. The assessment parameters used were the liver and kidney function tests.

MATERIAL AND METHODS

Animals: Healthy male albino rats originally derived from Sprague-dawley strain were used throughout this project. The animals were maintained, reared and bred at the registered animal house at College of Medicine and Medical research Center, Jazan Univeristy, Jazan,

Kingdom of Saudi Arabia. The animals were availed from the animal house with appropriate permission and ethics committee approval of the institute. The animals were maintained in standard condition of day-light, temperatures as well as animals were fed with standard chow feed and water *ad libitum*. Animals were acclimatized in laboratory conditions prior to beginning of experiment. Animals were observed for proper normal behaviors. Animals were weighed, numbered and grouped in cages throughout the experimental period.

Fagonia arabica Collection: The plants were collected, in the month of December, near Wadi Eidabi, (Jazan province), in KSA. As the genus *Fagonia* is confined to warm and arid areas, it was collected in similar environment. The plant was collected in wild, grown in vicinity of a fresh-flowing water body well away from urban and industrial influence. Being grown in wild, it was supposed to be free from most often occurrences of chemical pesticides and fertilizers. The plant had a woody base and height varied from 30 to 55 cm. The plant identity was confirmed systematically [11] and sample is preserved and deposited in College of Medicine.

Preparation of whole leaf powder: The plant was collected from site described above and the fresh plants were quickly transferred to the laboratory. Leaves were removed carefully with the forceps and washed thoroughly using sterilized water to remove any dust or soil particles from the plant surfaces. The leaves were blotted briefly prior to the drying. The leaves were air dried in shade till a constant weight was obtained continuously for three days. The dried leaves were crushed mechanically in aseptic condition to obtain fine powder. The dried whole leaf powder was collected in sterilized and aseptic conditions and was stored in refrigerator till further use and completion of experiment.

Chemicals: Highly pure and analytical grade chemicals were used throughout the experiment.

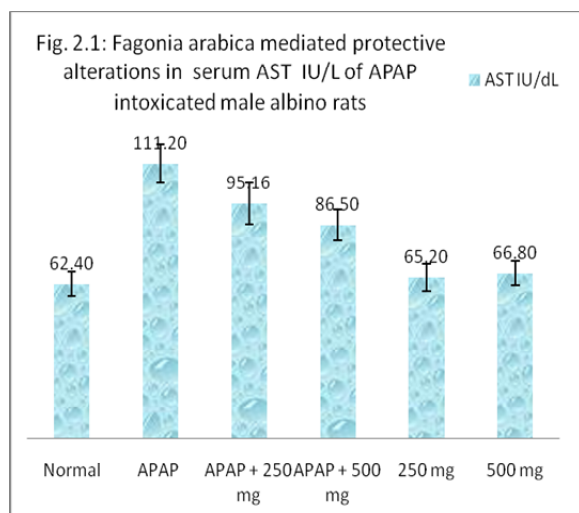
Experimental Design: The animals were divided into six groups comprising 5 animals in each

group. The Group I was not given any treatment, and served as the normal for comparison. Other groups receiving acetaminophen and/or dry leaf powder received it orally, dissolved/suspended in sterilized water. Group II to group IV were given 1000mg/kg BW of acetaminophen to induce hepatotoxicity. Group III and IV however additionally received 250 and 500 mg/kg BW of *Fagonia arabica* whole leaf powder. The group V and VI received only 250 and 500 mg/kg BW of *Fagonia arabica* whole leaf powder respectively. All the treatments were given to overnight fasted animals between 8.00-9.00 am and the animals were sacrificed the next day morning between the same time.

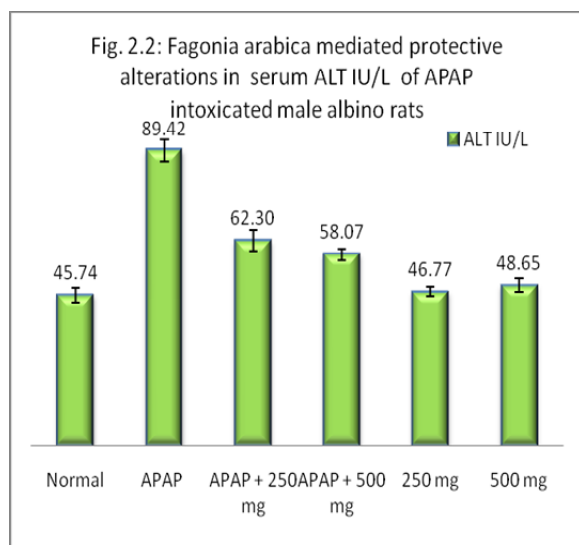
Post-treatment procedure: Animals were sacrificed giving deep anaesthesia. The blood was drained and serum was prepared. Liver and kidney function tests, viz AST, ALT, ALP, Total serum proteins, bilirubin, urea and creatinine tests were carried by using standard kits with autoanalyzer. The liver was perfused immediately after dissection and fixed in 10% neutral buffer formalin to study histopathology. The results are presented with tables and figures in the next section. The statistical analysis of the biochemical marker data was carried and the values are expressed as \pm SE of 5 sets.

RESULTS AND DISCUSSIONS

The AST enzyme activity in serum of normal rat group was found to be 62.42 ± 5.23 units per dl. This activity showed highly significant increase by 1000mg/kg BW oral dose of APAP and reached at 111.20 ± 7.96 units/dl of AST. This increased activity by APAP treatment was decreased in groups with APAP+250 (95.16 ± 8.30) and APAP+500 mg/kg BW (86.50 ± 6.26) co-treatment of FaLP, however still remained higher than the normal levels noted. Toxicity study of 250 and 500 mg/kg BW FaLP didn't result any significant alteration in the AST levels and remained 65.20 ± 5.57 and 66.80 ± 4.87 IU/dl respectively.

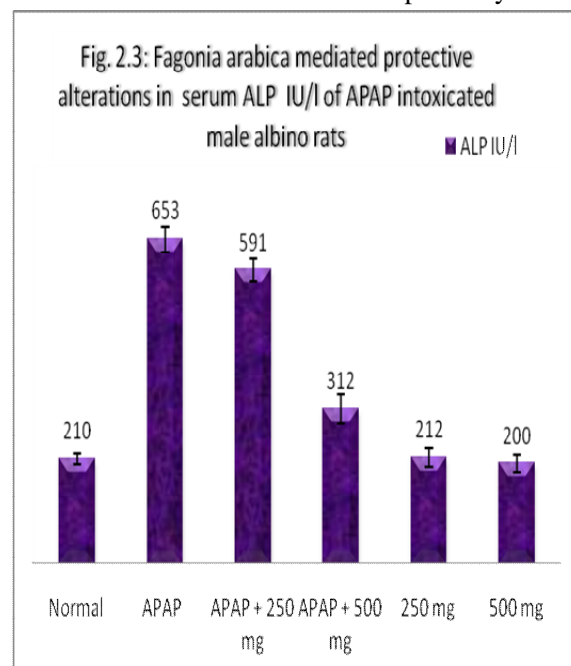


The untreated group of experimental rats showed 45.74 ± 2.33 units of alanine transaminase activity in per dl of serum. A highly significant increase wherein $p < 0.001$ vs normal was noted by APAP treatment of 1000 mg/kg BW. This group was recorded to have 89.42 ± 3.45 u/dl of ALT activity in serum. FaLP treatment of 250 mg/kg and 500 mg/kg BW showed a concentration dependent reducing trend (62.30 ± 3.10 and 58.07 ± 1.78 respectively) however the levels were still remaining marginally significant. The FaLP only treatment of 250 and 500 mg/kg BW could maintain the normal levels without any notable alterations corresponding to 46.77 ± 1.37 and 48.65 ± 2.06 U/dl.



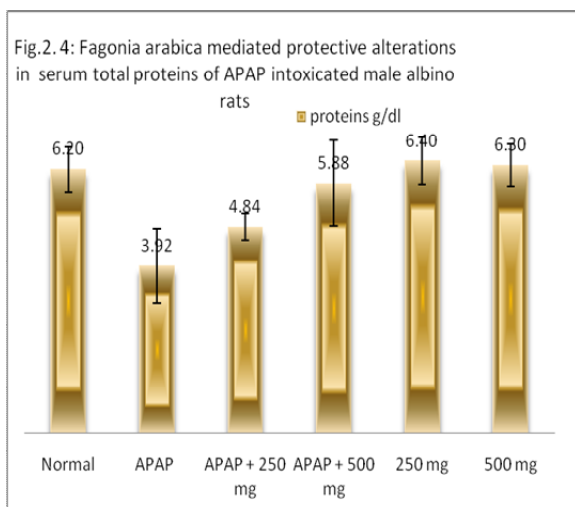
The serum activity of alkaline phosphatase enzyme in the untreated group was noted

210 ± 11.11 IU/dl of serum. The APAP treatment increased the alkaline phosphatase by highly significantly and the activity was recorded as 653 ± 25.61 u/dl. Both the 250 (at 591 ± 22.13 U/dl) and 500 mg /kg BW (at 312 ± 28.74 U/dl) treatment showed a concentration dependent tendency to reduce the elevated levels of ALP however failed to retain them at or near normal levels. The 250 and 500 mg/kg BW FaLP only maintained the ALP levels near normal, that is 212 ± 18.19 and 200 ± 16.68 u/dl respectively

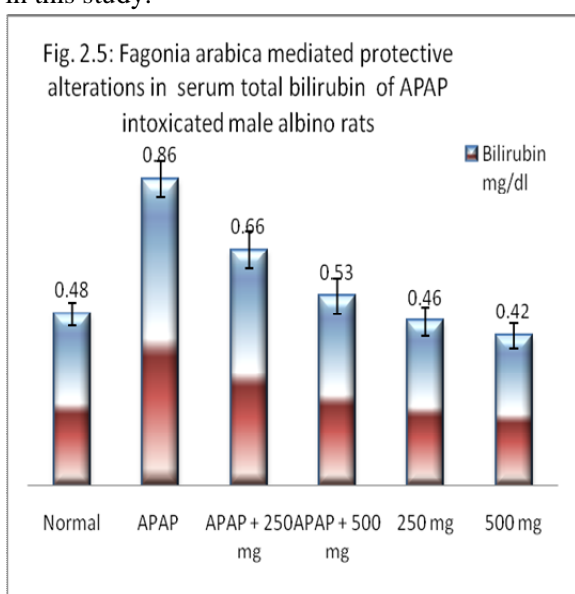


Group of experimental animals which did not received any treatment showed protein levels at 6.20 ± 0.52 g/dl. APAP oral dose of 1000 mg/kg BW reduced the protein levels significantly and the proteins were noted to be 3.92 ± 0.87 g/dl of serum.

Although APAP+250 mg/kg BW FaLP could not maintain the protein levels near normal and the protein levels were observed at 4.84 ± 0.31 g/dl, the 500 mg/kg BW dose of FaLP along with the APAP toxicity dose of 1000mg/kg BW normalized the protein levels and were recorded at 5.88 ± 1.01 g/dl. The 250 and 500 mg/kg BW FaLP maintained the protein levels without any significant alterations respectively at 6.40 ± 0.56 and 6.30 ± 0.49 g/dl of serum.

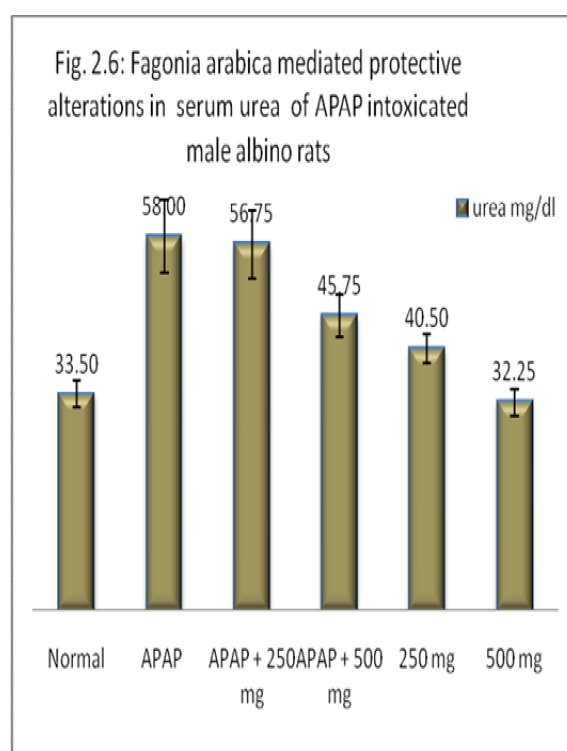


In normal group bilirubin levels were recorded as 0.48 ± 0.031 mg/dl in the normal group of experimental rats. After the treatment of 1000mg/kg BW the bilirubin levels showed an increase which was statistically significant and reached a peak of 0.86 ± 0.051 mg/dl. The serum bilirubin levels of FaLP treated group were reduced, but still were higher than normal (normal- 0.48 ± 0.031 mg/dl and 0.66 ± 0.053 mg/dl with 250 mg/kg BW and 0.46 ± 0.039 mg/dl with 500 mg/kg BW FaLP) with treatment of 250 mg/kg BW of FaLP, a similar trend was observed with 500 mg/kg BW FaLP, but the levels were not normalized. The FaLP treatment of 250 and 500 mg/kg BW did not showed any notable alterations in this study.



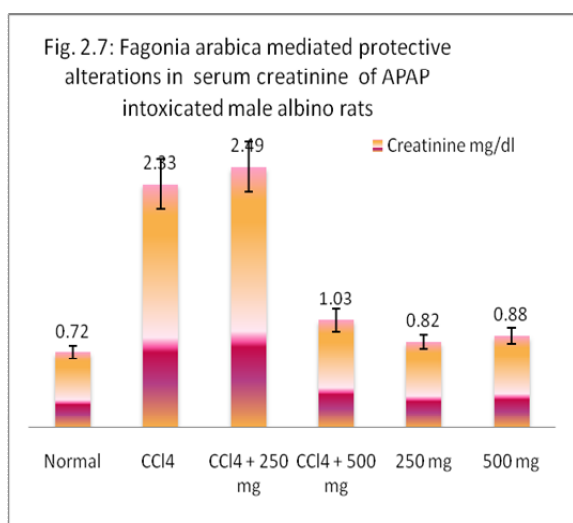
The urea levels in serum were noted as 33.50 ± 2.06 in normal group. The APAP treated group elevated the urea levels significantly and the levels were noted as 58.00 ± 5.74 .

The APAP + 250 mg/kg BW FaLP and 500 mg/kg BW FaLP treatment could not reduce the elevated urea levels (56.75 ± 5.21 mg/dl and 45.75 ± 3.33 mg/dl) and the levels were noted a higher range. With the Toxicity doses of 250 and 500 mg/kg BW FaLP urea levels were in normal (respectively 40.50 ± 2.20 and 32.25 ± 2.15 mg/dl) range.



Normal experimental group showed 0.72 ± 0.06 mg/dl of creatinine activity in the serum. The toxicant APAP elevated the levels highly significantly ($p < 0.001$ vs normal) at levels of 2.33 ± 0.24 mg/dl.

The APAP and 250 mg/kg BW FaLP treatment reduced the levels but still remained highly significant when compared to normal. While APAP and 500 mg/kg BW FaLP treatment reduced the levels even further, still failed to normalize. The 250 and 500 mg/kg BW also kept the levels of creatinine elevated than the normal



The elevated levels of aspartate transaminase, alanine transaminase and alkaline transaminase with the treatment of hepatotoxic dose of acetaminophen indicate the hepatocyte injury. The increased levels of urea, creatinine and bilirubin as well as decreased levels of total serum proteins in the above experiment as a result of acetaminophen treatment are also indicative of the hepatocyte injury.

Histopathology: The histopathological observations (Table 2) showed normal histology in control liver (Fig. 2A). Administration of paracetamol for one day induced mild congestion and mild necrotic changes in liver represented by foamy cytoplasm, pyknosis of nucleus and even loss of nuclei (Fig. 2B). Administration of plant at dose of 250 ppm FaLP didn't induced any improvement (Fig. 2C). Administration of the plant at a dose of 500 ppm FaLP induced great improvement in liver cells (Fig. 2D). No changes can be observed in liver of rats administered 250 ppm FaLP and 500 ppm FaLP alone (Fig. 2E-F).

As a result of large single dose ingestions of acetaminophen the massive centrilobular necrosis is observed that could be fatal in both humans and experimental animals. [9]. During this experiment no mortality with the 1000mg/kg BW single treatment of acetaminophen was observed in contrast that the same dose in mice (1g/kg BW acetaminophen given orally) induces 100% mortality [12]

Major of the acetaminophen dose within therapeutic limits (>90%) of APAP is glucuronidated or sulfated and then excreted. However a small percentage is metabolized by cytochrome P450 enzymes (CYP) to the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI), which is readily detoxified by conjugation with glutathione (GSH). Studies with rodents indicate that much the higher doses saturate the glucuronidation and sulfation pathways, resulting in formation of excess N-acetyl-p-benzoquinone imine (NAPQI). The additional reactive metabolite depletes liver GSH and binds to proteins [13-15] The hepatocyte function markers studied in this project indicate that there is a good potency of hepatocyte protection associated with the *Fagonia arabica* leaf powder as observed by reduced leakage of hepatocyte specific enzymes in serum indicating stabilization of hepatocyte membrane.. The present work observes that the lower dose of 250 mg/kg BW of *Fagonia arabica* dry leaves powder considerably failed to protect the hepatocytes against toxic dose of acetaminophen. However 500 mg/kg BW dose of *Fagonia arabica* dry leaves powder offered considerable protection to the hepatocytes. Still this study is limited to conclude firmly and establish the mechanism. Hence further studies are required for full understanding of synergic action of the leaves as well as for the study of active ingredients. The reduced leakages of hepatocyte and renal biomarker enzymes viz aspartate transaminase, alanine transaminase, alkaline phosphatase, blood urea, creatinine indicate possible protection offered by the leaves. Similarly the depleted protein levels by the toxicant are restored to a greater extent indicate that the leaves possess the capacity to regularize the mechanisms altered due to toxicity induction.

Polyphenols from various plant sources are known to function as antioxidants [16] so also flavonoids [17]. *Fagonia arabica* studies in the past have indicated that there is remarkable anti-oxidant activity associated with *Fagonia Arabica* due to

its considerable amount of total polyphenols.[18]. In this experimental work *Fagonia arabica* dry leaves powder with its antioxidant potential may be reducing oxidative stress generated by Acetaminophen, which later leads to mitochondrial dysfunction and necrosis. A lot of macroelements and micro elements constitute leaves of *Fagonia arabica* plant [19]. Among the constituents, Zn is estimated to be present at levels of 21.99 µg/ g of leaves. With the selected dose in this study, a considerable zinc should have been reached the metabolic pathways of the intoxicated animals. It has been established long ago that Zn acts as an anti-oxidant in animals [20] as it plays an important role of controlling levels of oxygen radicals. Apart from zinc's role as an essential metalloenzyme, it also plays an important role in the maintenance of membrane structure and function [21]. So possibly this elevated intake of zinc may also contributing towards the membrane integrity of the cells as well as mitochondria leading the reduced leakages and ultimately giving protection. One of the study conducted, *in vitro* using a close species of *Fagonia arabica*, *F. cretica* [22] on rats protected neurons possibly by enhancing GSH levels, resulting in reducing oxidant levels via direct scavenging. *Fagonia arabica* may have acted similar to its proximate species exerting some protective effect on hepatocytes against paracetamol. Other constituents may have had an added effect and the final result can be a synergic action by all the constituents. However this study is limited in understanding the underlying molecular mechanism of protection as well as in elucidating the synergic effect of some active antagonist constituents which must be present simultaneously along with the active hepatoprotective ingredients. This is a high

possibility considering the number of constituents present at high concentrations in leaves and owing to this study being primary in this area has utilized the whole leaf powder. I

CONCLUSION: This study was undertaken to assess the protective efficacy of *fagonia arabica* dry leaves powder against the hepatic injury induced by acetaminophen toxicity. The 250 mg/kg BW dose of *Fagonia arabica* dry leaves powder failed to protect the hepatocyte at this concentration of the toxicant (1000mg/kg BW of acetaminophen) acetaminophen however the higher dose of 500 mg/kg BW protected the hepatocytes from injury as indicated by the reduced leakage of the enzymes studied in this project. Further investigation is important to understand the possible mechanism of action.

FINANCIAL DISCLOSURE

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Note:²Author is currently working in Faculty of Applied Medicine, Jazan University, Jazan.

Table:1. - *Fagonia arabica* mediated protective alterations in histopathology of acetaminophen intoxicated male albino rats (One day treatment study Fig. 2.8)

	Control	APAP	APAP+250 FaLP	APAP+500 FaLP	250 FaLP	500 FaLP
Congestion	-	++	++	-	-	-
Necrobiotic changes	-	++	++	-	-	-

++ moderate, - no lesion

Fig.2 *Fagonia arabica* mediated protective alterations in histopathology of acetaminophen intoxicated male albino rats

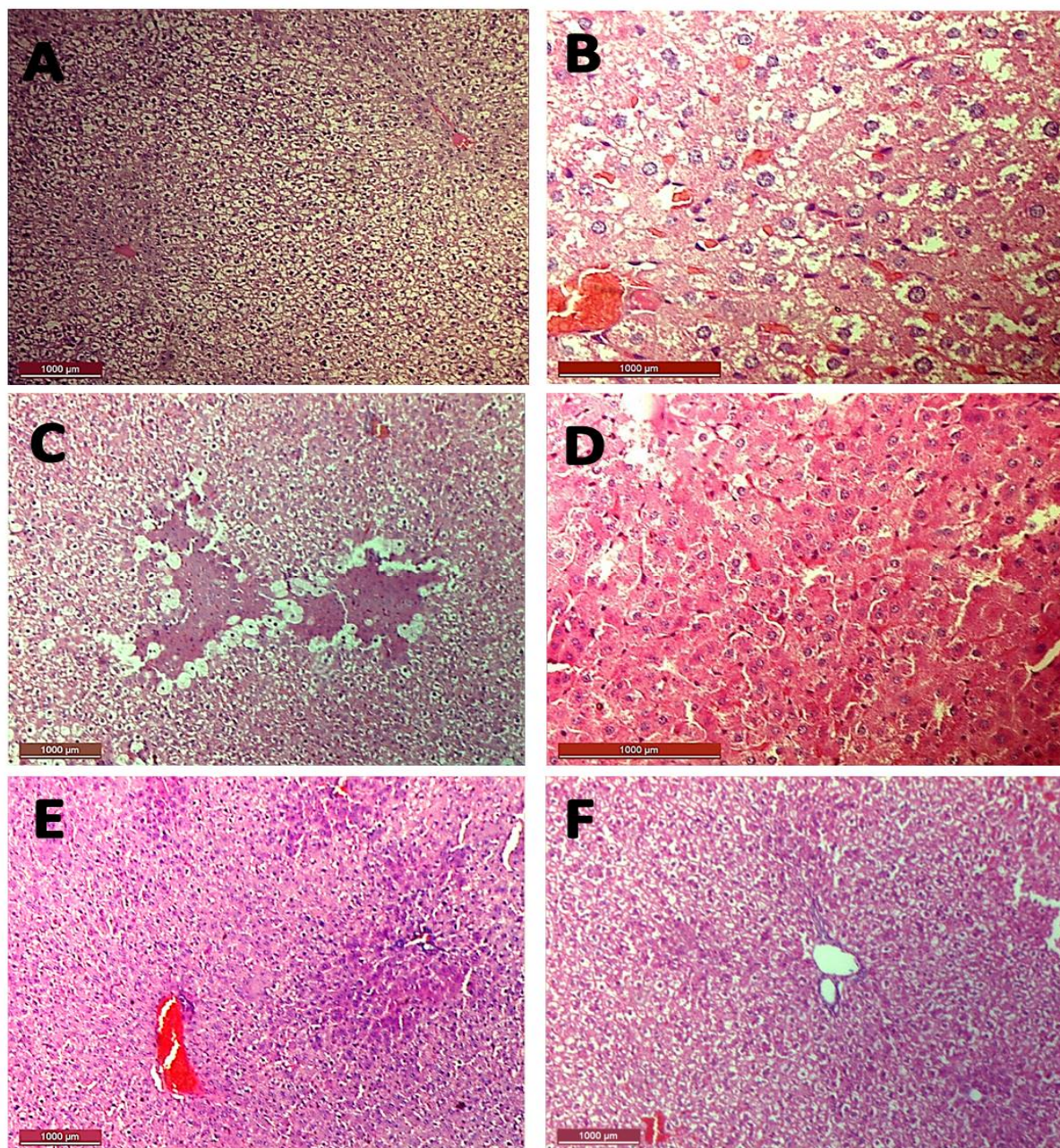


Figure-2 Legend and Table caption A-Normal/Untreated, B-APAP, C- APAP+ 250 mg/kg BW FaLP, D- APAP+ 500 mg/kg BW FaLP, E- 250 mg/kg BW FaLP, F- 500 mg/kg BW FaLP

REFERENCES:

1. Qureshi H., Asif S., Ahmed H., Al-Kahtani HA, Hayat K., 'Chemical composition and medicinal significance of *Fagonia cretica*: a review' *Nat. Prod Res.* 2016;30(6) pp625-39
2. Ageely HM., Gupta SK, Manal Abdel Latif, Patil BR., 'Review of Ethnomedicinal plant

- Fagonia*', *Int. J of Pharmaceutical Applications* Vol.5, Issue 2, 2014 pp 22-28
3. Kasture V.S., Gosavi SA, Kolpe JB, Deshapande SG., 'Phytochemical and Biological Evaluation of *Fagonia* species: A Review' *World Journal of Pharmacy and*

- Pharmaceutical Sciences, Vol 3, Issue 5, 1206-1217,
4. Hammiche Victoria, MaizaKhadra, (2006) 'Traditional medicine in Central Sahara: Pharmacopoeia of TassiliN'ajjer, Journal of Ethnopharmacology, Vol 105, Issue 3, pg 358-367
 5. V. Prashanth Kumar, ChauhanNeelam S., Padh Harish, M. Rajani, (2006) 'Search for antibacterial and antifungal agents from selected Indian medicinal plants' Journal of Ethnopharmacology Volume 107, Issue 2, pg 182-188 doi:10.1016/j.jep.2006.03.013
 6. Prasad Sweta, KashyapRajpal Singh, DeopujariJayant Y, PurohitHemant J, Girdhar M Taori and DaginawalaHatim F, (2007) Effect of *Fagonia Arabica* (Dhamasa) on in vitro thrombolysis, BMC Complementary & Alternative Medicine, Vol-7 Issue-36 doi:10.1186/1472-6882-7-36
 7. Khanzadi Fatima Khattak, (2012) 'Microbiological quality assessment of commercially available medicinal plants in Peshawar city, Pakistan, Pak. J. Bot, Vol-44 Issue-4 pg 1203-1208
 8. Ahsan Hussain, Zia Muhammad, MirzaBushra, (2007) 'Cytotoxic and Anti-tumor Potential of *Fagoniacretica* L.', Turkish J. Biol. 21 19-24
 9. Shayiq RM., Roberts DW., Rothstein K., Snawder JE., Benson W., Ma Xiang, Black M., 'Repeat Exposure to Incremental Doses of Acetaminophen Provides Protection Against Acetaminophen-Induced Lethality in mice: An Explanation for High Acetaminophen dosage in Humans without Hepatic Injury', *Hepatology* February (1999)pp451-463
 10. Payasi et al., 2010 Payasi A., Chaudhary M, Singh BM, Gupta A., Sehgal R., (2010) "Sub_Acute Toxicity Studies of Paracetamol Infusion in Albino Wistar Rats", Int J. Pharmaceutical Sciences and Drug Research 2(2), 142-145
 11. Alam Eman, A. (2011) 'Morphological, phytochemical and biological screening on three Egyptian species of *Fagonia*' Academia Arena, Vol 3, Issue 1, pg 18-27
 12. Janbaz K.H., Saeed S.A., Gilani A.H.,(2004) 'Studies on the protective effects of caffeic acid and quercetin on chemical-induced hepatotoxicity in rodents' *Phytomedicine* 11(5) 00424-430
 13. JMitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *J Pharmacol Exp Ther.* 1973;187(1):185-194.
 14. Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. *J Pharmacol Exp Ther.* 1973;187(1):195-202.
 15. Potter WZ, Davis DC, Mitchell JR, Jollow DJ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. 3. Cytochrome P-450-mediated covalent binding in vitro. *J Pharmacol Exp Ther.* 1973;187(1):203-210
 16. Higdon JV., Frei Balz, 'Tea catechins and polyphenols:Health Effects, Metabolism and antioxidant functions' *J Critical Reviews in Food Sci and Nutrition* Vol 43, 2003, issue 1 <http://dx.doi.org/10.1080/10408690390826464>
 17. Catherine A., Rice-evans, Nicholas J. Miller, Paul G. Bolwell, Peter M. Bramley and John B. Pridham' The relative antioxidant activities of plant derived polyphenolic flavonoids' *Free radical research* vol 22, 1995 issue 4
 18. SatputeRavindra M., Kashyap, Rajpal S., DeopujariJayant Y., Purohit, Hemant J. TaoriGirdhar M., DaginawalaHatim F. (2009) Protection of PC12 cells from chemical ischemia induced oxidative stress by *Fagoniaarabica*, Food and Chemical Toxicology Vol 47, Issue 11, pg 2689-2695
 19. Shad AA, Shah H., Khattak F.K. Dar N>G., Bakht J., 'Proximate and Mineral constituents of Medicinal Herb *Fagonia arabica*' , Asian J

- of Plant Sciences vol 1, Number 6, 710-711, 2002
20. Bray and Betterger 1990 from 19
 21. Bettger WJ., O'Dell BL., 'A critical physiological role of zinc in the structure and function of biomembranes' *Life Sciences* Vol 28, issue 13, March 1981, pp 1425-1438
 22. Rawal AK, Muddeshwar MG, Biswas SK., 'Rubia cordifolia, Fagonia cretica linn and Tinospora cordifolia exert neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation' *BMC Complementary and Alternative Medicine* 2004 **4**:11
DOI: 10.1186/1472-6882-4-11