

ROLE OF *CASSIA OCCIDENTALIS* IN THALASSEMIC PATIENTS

N. Farooq, Azizullah, M. F. Qamar and *S. Nadeem

Department of Zoology, Government College University, Lahore.

*Department of Chemistry, Government College University, Lahore,

Corresponding Authors: dr.fiazqamar@gmail.com

ABSTRACT: Effect of the leaves suspension of *Cassia occidentalis* on the elimination of excess of iron from the artificially iron overloaded rabbits was studied. Leaves suspension of *C. occidentalis* (250mg/kg) were used for the elimination of iron from iron overloaded rabbits. Rabbits were divided into three groups; overloaded for five weeks and then treated with selected herb for four weeks. Iron concentrations in serum and body organs like heart, liver and kidneys were assayed in all groups of rabbits after iron overloading with jectosol plus injections as per dose of 0.3 ml (15 mg iron) per kg body weight intramuscularly daily for 5 weeks. There was a significant increase in iron concentration in the sera and organs studied. In iron overloaded animals changes, like loss of weight, loss of hair, loss of activity and increased heart rate were observed. After four weeks of herbal treatment (250mg/kg), there was a significant decrease in iron concentration in the serum and organs as compared with iron overloaded untreated with herb group. The decrease was more significant in kidneys and liver as compared to the heart. It was concluded that *C. occidentalis* leaves suspension was significantly effective in reducing the iron level from serum and organs studied.

Key words: *Cassia Occidentali*, Thalassemia, Iron, Rabbits.

(Received 30-8-13

Accepted 22-2-15)

INTRODUCTION

Thalassemia is a form of hemolytic anemia and is produced due to inherited abnormality of globin chain production (Pignatti and Galanello, 2009). Thalassemia is thought to be the most frequent genetic disease worldwide. Thalassemia is highly prevalent in the regions of the North and West Africa, the Middle East, the Indian subcontinent and the Southern East and Southern Asia (Abolghasemi *et al.*, 1997).

The most common cause of iron overload is haemochromatosis which damages body organs (Pietrangelo *et al.*, 2010). When the ferritin level exceeds 1000ng/ml then the body is considered to be iron overloaded and if the ferritin level is less than 1500ng/ml then the most complications can be avoided (Telfer *et al.*, 2000).

Iron overload can be transfusional or non transfusional. In transfusional overload humans, there is not any physiological mechanism to expel extra iron from the body (Porter *et al.*, 2008). However, in non transfusional iron overloading, iron accumulation is due to hereditary reasons that lead to high absorption of dietary iron in the intestines. The liver, heart and endocrine glands are the major iron storage organs and harmful effects are commonly observed in these organs (Porter, 2001; Kwiatkowski and Cohen, 2004; Abetz *et al.*, 2006; and Cappellini and Taher, 2009). The thalassemic patients because of obligatory blood

transfusions accumulate large amount of iron in their bodies which proves to be toxic and fatal.

Managemental and chelation treatments are two ways to treat the transfusion related iron overloading in which our goal is to achieve a safe and sound tissue iron level (Kushner *et al.*, 2001; Porter, 2001 and Vichinsky, 2001;). Deferoxamine, Deferasirox, Deferiprone, diethylenetriaminepenta-acetic acid, Deferasirox (ICL670A) and Pyridoxalisonicotinoylhydrazone (PIH) are potent chelating drugs actively used in thalassemic patients (Hoffbrand, 1995; Pignatti and Galanello, 2009; Waldmeier *et al.*, 2010).

It is reported in some recent studies that a naturally present flavolignan and Silybin extracted from the fruits of silibiummarianum, possess some antioxidant and radical scavenging abilities that works as an iron chelator (Borsari *et al.*, 2001). ROS i.e. free radicals and reactive oxygen species are produced continuously by means of many common physiological processes. Free radicals induce the oxidative stress that causes harm to the cells and tissues and is protected by the antioxidants (Ozsoy *et al.*, 2008). Plant phenolic compounds have been considered to be the most important sources of antioxidants activity. The antioxidant activity of phenolics is because of their redox properties, which permits them to work as reducing agent, redox singlet oxygen quenchers and hydrogen donor. Flavonoids and other plant phenolics, like phenolic acids, lignin and tannins are particularly frequent in leaves, woody parts of plants like stem, bark and roots (Amarowicz *et al.*, 2004;

Shaheen *et al.*, 2013 and Zafaret *et al.*, 2011;). Many synthetic antioxidants are very valuable but they have toxic properties for human health. That is why; they seek for natural antioxidants of plant origin which have achieved momentum in the past few years (Mathew and Abraham, 2006).

Many plants and their products have been used by human beings to treat various diseases/ailments (Abida and Azizullah, 2009). In the present study the role of *Cassia occidentalis* particularly its leaves in the elimination of excessive iron from the bodies of artificially iron overloaded rabbits has been studied. *Cassia occidentalis* (Leguminosae) is present in several tropical regions and is extensively used as a medicinal plant in many countries. The objective of the present study was to find out any drug of plant origin which could be orally used in thalassemic patients and to eliminate the excessive iron from the body. Therefore, the rabbits were artificially overload with iron and then treated with *Cassia occidentalis* (leaves) to evaluate its potential efficiency in iron elimination.

MATERIALS AND METHODS

15 rabbits (*Oryctolagus cuniculus*) were purchased from Tollintinmarket and were kept in clean, spacious and well aerated rooms for about 9 weeks in animal House, Department of Zoology, Government College (GC) University, Lahore. They were all males with the body weights of 1-1.5 kg and their ages were between 5-5.5 months. They were provided with fresh food consisting of fodder (Trifolium), grains, fruits, vegetables and water. The food and water were supplied ad libitum. The rooms were cleaned daily.

These rabbits were divided into 3 groups of 5 rabbits each and were marked with numbers on the internal side of both ears with permanent marker.

Group I was the normal control group and was not treated with iron overloading and the drug *Cassia occidentalis*. They were fed only normal diet i.e. fodder, grains, vegetables and water.

Group II: the treated group; where each rabbit was injected with jectosol injection as per dose of 0.3 ml (15 mg iron) per kg body weight intramuscularly daily for 5 weeks (Brugnara, 1999). At the completion of 5 weeks, these rabbits were kept for 4 more weeks without any further iron injection.

Group III: The rabbits were treated as in Group II; but the difference was of administration of *Cassia occidentalis* suspension which was given orally for 4 weeks at the dose of 250mg/kg body weight after 5 weeks of iron load as described by (Sharma *et al.*, 2000).

The *Cassia occidentalis* leaves were procured from the herbal market and their identification was

confirmed by the Botanist of Botany Department, GC University, Lahore. The fresh leaves of *Cassia* were removed from the stalks. After cleaning they were allowed to dry for few days. The leaves were crushed and ground to obtain powder. The suspension was made in water and the solution was administered orally with the help of syringe to the rabbits.

Two to three millilitre (2-3 ml) blood was collected from ear vein by puncture for serum preparation. Hemolysis was avoided by using clean apparatus, pouring the blood gently into the receiver and avoided frothing during the withdrawal of the blood (Gomella and Haist, 2007)

Blood samples collected in Eppendorf's were allowed to clot for about 2-3 hours at room temperature of 37°C and were then stored in refrigerator overnight at 4°C so that clot retraction may become complete (Lewis, *et al.*, 2006). The serum was removed by a pasture pipette and transferred to another tube, which was then rapidly centrifuged at 4000 rpm for about 15 minutes to get rid of suspended RBCs. Blood serum was assayed for iron overload.

Rabbits were dissected for iron determination in tissues after 9 weeks of the experiment. The tissues of heart, kidney and liver were removed for ash formation. The iron in the tissues was detected by the procedure described by (Nilsson, *et al.*, 2002).

Statistical analysis: For statistical analysis, the analysis of variance (ANOVA) and student t- test were used. Student t-test was applied on the means of iron serum concentrations in normal and iron overloaded rabbits after iron overloading for 5 weeks. ANOVA was applied on the means of serum concentrations in normal, iron control and iron overloaded plus drug treated groups after four weeks of herbal treatment (McDonald, 2008).

RESULTS AND DISCUSSION

Iron concentrations in blood sera of Rabbits: Parenteral iron overloading through intramuscular injections of iron preparation (Jectosol injections @ 0.3 ml/kg containing 15 mg elemental iron per kg body weight) led to an increase in the blood sera of rabbits Table-1. The increase was highly significant ($P < 0.01$) after 5 weeks of daily iron injections. An increase of 265.2% was observed in their blood sera. The iron concentration in the blood sera of rabbits decreased highly significantly ($P < 0.01$) on treatment with leaf suspension of *C. occidentalis*. After four weeks of herb's treatment, the iron concentration (ppm) decreased from 118.7 ± 2.08 to 49.98 ± 1.40 (57.9%). In the iron overloaded rabbits left untreated with the herb (iron control), the iron concentrations decreased by only 28.2% which was considerably less decrease as compared to the decrease in herb treated iron overloaded rabbits (Table 2; Fig 1).

Iron concentrations in Kidney, Liver and Heart: Iron overloading (parenteral) in addition to increase the iron concentration in the blood sera also led to the accumulation of iron in various organs like heart, liver and kidney etc. In kidneys, the iron concentration increased by 4.70 fold i.e., 370%. In liver, the iron concentration increased by 4.78 fold i.e., 378% whereas in heart the iron concentration increased by 3.84 fold i.e., 284.1% (Table 3). Iron concentrations in kidney, liver and heart in iron overloaded rabbits also decreased on treatment with the herb. After four weeks of treatment with leaf suspension of *C. occidentalis* i.e., 250mg/kg daily, the iron concentration in kidneys decreased by 64.3%. This decrease was statistically highly significant (P<0.01)

The iron concentration in the liver of iron overloaded rabbits on treatment with the herb for four weeks also decreased significantly (P<0.01) (51.6%). In the heart of iron overloaded rabbits on treatment with the herb for four weeks, the iron concentration decreased by 39.5% (Table4).

The present study revealed that the administration of iron injections jectosol plus for 5 weeks resulted in iron overloading which led to 265.2% increase of iron in their blood sera. The iron concentration increased significantly (P<0.01) to 24.5% in the first week, 65.8% in second, 99.04% in third, 182.7% in

fourth and 265.2% in fifth week as compared to normal rabbits. The artificial iron overloading has already been reported in rats, dogs rabbits, turbot, baboons and in pigs by (Adams *et al.*, 1997). After the four weeks of herb treatment the leaves suspension of *C. occidentalis* had significantly reduced (57.9%) iron concentration in the blood sera of iron overloaded plus herb treated rabbits. While the bodies of iron control group (left untreated with herb) had reduced 28.2% iron concentration in blood sera of rabbits. The decrease of iron in the control group was statistically significantly less as compared to iron overloaded plus herb treated group. This indicated that herbal treatment gave highly significant effect (P<0.01) in reducing iron as compared to the self-elimination of body. In addition to blood serum, iron was also deposited in all the organs studied. The damage of iron overload on some organs, like skin was small, while hemosiderotic damage was seen in liver, which could be lethal (Bassett *et al.*, 1986). Liver was the main organ for storage of iron which could store up to 70% or more body iron. The iron in excess quantity resulted in fibrosis and cirrhosis of liver (Porter, 2001). In the present study, parenteral administration of large amount of iron also affected the liver, kidneys and heart. In the liver cells the iron concentration increased by 378% as compared to the amount in normal rabbits.

Table-1: Showing Mean iron concentrations (ppm) in blood sera of Normal and Iron Overloaded rabbits during 1st to 5th week of iron overloading.

Sr. No.	WEEKS	GROUPS	
		NORMAL M±SEM	IRON OVERLOADED M±SEM
1	0	30.6±1.63 ^{NS}	28.9±1.90 ^{NS}
2	1	30.2±1.84 ^a	37.6±2.23 ^b
3	2	29.6±2.35 ^a	49.1±2.87 ^b
4	3	31.4±2.40 ^a	62.5±2.55 ^b
5	4	34.2±1.80 ^a	96.7±2.07 ^b
6	5	32.5±2.01 ^a	118.7±2.08 ^b

P <0.01 is statistically highly significant among normal and experimental groups
M ±SEM Indicates Mean ± Standard Error Mean

Table-2: Showing Mean Iron concentrations (ppm) in blood sera of Normal, Iron control and Iron overloaded plus Herb treated Rabbits (250mg/kg) after four weeks of herb treatment.

Sr. No.	Groups	Weeks				
		0 M±SEM	1 M±SEM	2 M±SEM	3 M±SEM	4 M±SEM
1	Normal	32.5±2.01 ^{NS}	35.7±2.49 ^{NS}	40.1±2.10 ^{NS}	39.3±2.06 ^{NS}	43.2±2.60 ^{NS}
2	Iron control	118.7±2.08 ^{NS}	105.5±1.36 ^{NS}	100.2±2.13 ^{NS}	95.6±1.92 ^{NS}	85.2±2.64 ^{NS}
3	Iron overloaded and herb treated	118.7±2.08 ^a	95.3±1.51 ^a	78.4±2.08 ^a	61.8±1.65 ^a	49.98±1.40 ^a

^a P <0.01 is statistically highly significant among three experimental groups
M ±SEM Indicates Mean ± Standard Error Mean

Table-3: Showing Mean Iron concentrations (mg/100gm) in the kidneys of Normal, Iron control and Iron overloaded plus herb treated (250mg/kg) Rabbits before and after treatment.

Sr. No.	Groups	IRON CONCENTRATIONS IN KIDNYES (mg/100gm)		IRON CONCENTRATIONS IN Liver (mg/100gm)		IRON CONCENTRATIONS IN Heart (mg/100gm)	
		Before Treatment (M±SEM)	Before Treatment (M±SEM)	After Treatment (M±SEM)	After Treatment (M±SEM)	After Treatment (M±SEM)	After Treatment (M±SEM)
		1	Normal	1.521 ± 0.63 _{NS}	1.521 ± 0.63 _{NS}	3.870 ± 0.018 _{NS}	3.953 ± 0.084 _{NS}
2	Iron control	1.521 ± 0.63 _{NS}	1.521 ± 0.63 _{NS}	3.870 ± 0.018 _{NS}	18.50 ± 0.874 _{NS}	0.599 ± 0.019 _{NS}	2.301 ± 0.414 _{NS}
3	Iron overloaded and herb treated	7.156 ± 0.941 _{NS}	7.156 ± 0.941 _{NS}	18.50 ± 0.874 _{NS}	8.953 ± 0.682 ^a	2.301 ± 0.414 _{NS}	1.393 ± 0.0819 _{NS}

^aP<0.01 value is statistically highly significant among experimental groups; M ±SEM Indicates Mean ± Standard Error Mean

Table-4: Showing Mean Iron concentrations (mg/100gm) in the kidney, heart muscle, and liver of Normal, Iron control and Iron overloaded plus herb treated (250mg/kg) Rabbits.

Sr. No.	Organs	NORMAL (M±SEM)	IRON CONTROL (M±SEM)	IRON OVERLOADED PLUS HERB TREATED (M±SEM)	% Iron Decreased with Herbal Treatment
1	KIDNEY	1.521 ± 0.63 ^a	7.156 ± 0.941 ^a	2.552 ± 0.216 ^b	64.3%
2	LIVER	3.870 ± 0.018 ^a	18.5 ± 0.874 ^a	8.95 ± 0.682 ^b	51.6%
3	HEART	0.599 ± 0.019 ^a	2.301 ± 0.414 ^a	1.393 ± 0.0819 ^a	39.5%

Different letters a and b P<0.01 value is statistically highly significant among experimental groups; M ±SEM Indicates Mean ± Standard Error Mean

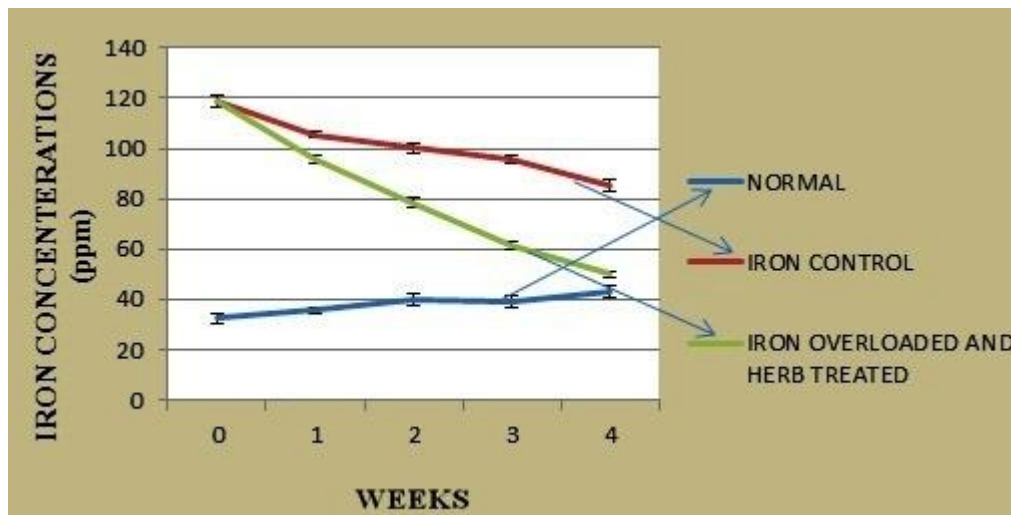


Fig-1: Showing Iron concentrations (ppm) in sera of Normal and Iron control and iron overloaded plus herb treated (250mg/kg) group of rabbits after treatment with Herb during 1st to 4th week.

Our results were contrasting to the findings of (Brown *et al.* 1957) who reported that no damage to liver was observed on the administration of iron to the experimental animals. But this was in agreement to (Bonkovsky, 1991) who reported that liver was the

conspicuous victim of excess iron deposition. The herb *C. occidentalis* significantly (P<0.01) decreased i.e. 51.6% iron from the liver of iron overloaded plus herb treated rabbits. Other organs such as heart and kidneys were also affected by large amount of iron administration. In

kidneys, the iron concentration increased by 370% when compared with group treated with herb which was helpful in decreasing significantly ($P < 0.01$) 64.33% iron from kidneys. In heart, the iron concentration increased to 284.1% in iron overloaded rabbits as compared to the amount in control rabbits while the herb decreased by 39.5% from heart of iron overloaded plus herb treated rabbits. Iron deposition in heart muscles also lead to cardiac failure and life-threatening arrhythmias (Kwiatkowski and Cohen, 2004). Cardiac failure could occur even due to very little amount of iron deposition because the total amount of iron was less important than the unbound or toxic iron subset. The level of unbound iron in tissues could not be calculated because it was present in very small quantity. This 'toxic' iron was the constituent bound and deactivated by chelators. So, cardiac injury in patients with transfusional iron overload was best prohibited by maintaining the small level of chelator in the blood circulation (Link *et al.*, 1985).

Oral administration of *C. occidentalis* (250mg/kg) brought considerable ($P < 0.01$) decrease in the concentration of iron in organs studied. This decrease was more prominent in kidneys as compared to liver and heart. *C. occidentalis* decreased by 64.33%, 51.6% and 39.5% iron from kidneys, liver and heart respectively in iron overloaded rabbits. It could be concluded that *C. occidentalis* may eliminate iron from the kidneys of iron overloaded rabbits via urine.

All the difficulties in the administration of standard iron chelators called for attempts to find out new orally active iron chelators. Medicinal plants and their derivatives were used to substitute allopathic medicines in many parts of the world. In the present study, the suspension of leaves of *C. occidentalis* (250mg/kg) was used to eliminate iron from the body. It was reported by (Yen *et al.* 1998) that the antioxidant activity of extract of *C. occidentalis*. Toxicological studies further revealed the strong toxic effects of *C. occidentalis* seeds in animals (Haraguchi *et al.*, 2003) but other parts of this plant may not be toxic. Symptoms of *C. occidentalis* seeds poisoning in general, included muscles weakness, ataxia and loss of body weight, ultimately leading to death. The lethal dose of *C. occidentalis* leaves and stem was higher than 5.0g/kg (Mirtes *et al.*, 2011). It was reported by (Kennedy *et al.*, 1986) that substances have LD₅₀ higher than 5.0 g/kg through oral route. This may be measured practically as non-lethal, demonstrating that the acute toxicity of *C. occidentalis* leaves and stem was almost zero. Leaves suspension at the rate of 250mg/kg, given to iron overloaded rabbits were found to have no side effects. Our findings were in agreement with the findings of (Mirtes *et al.* 2011) who suggested that *C. occidentalis* extract did not induce any damage to the kidneys and liver at the dose of 250mg/kg body weight.

Mild diarrhea was seen in rabbits throughout the experimental period with *C. occidentalis*. This result was

in agreement with the findings of (Aragão *et al.*, 2009 and Elujoba *et al.* 1999) who described the laxative effects of *C. occidentalis* in rats at the dose of 250mg/kg and 500mg/kg. (Akomolafe *et al.*, 2003) identified that Anthraquinone derivative was the chemical constituent of *Cassia* species which was responsible for laxative and purgative effect of suspension under study.

In the present study, we observed that *C. occidentalis* leaves suspension was significantly effective in reducing the iron level from serum and organs under study. It reduced 64.33% iron from kidneys, 51.6% from liver and 39.5% from heart of the iron overloaded rabbits suggesting that *C. occidentalis* had antioxidant effect (Yen *et al.*, 1998) and radical scavenging ability. This result was in line with the finding of (Arya *et al.*, 2010) who reported that leaves of extract of *C. occidentalis* were rich in phenolics and were responsible for antioxidant activities. They also reported that *C. occidentalis* leaves extracts were also responsible for Nitric oxide, ferric and hydroxyl oxide scavenging abilities.

Conclusion: The present work has shown that the plant *C. occidentalis* can be of great importance in reducing the secondary iron overloading in thalassaemia patients. Deferoxamine and deferiprone both iron chelators are used now a days but they are very expensive and difficult to use and have many side effects. This plant being orally active and cheap which may prove to be suitable replacement for standard iron chelators. However, a considerable degree of more work is required on this plant before it is recommended for use in thalassaemic patients.

REFERENCES

- Abetz, L., J. F. Baladi, P. Jones, and D. Rofail, The impact of iron overload and its treatment on quality of life. *Health and Quality of Life Outcomes.*, 4: 1–6 (2006).
- Abida, S., and Azizzullah. Elimination of excessive iron by Terminalia chebula in the iron overloaded rabbits. *Biol.*, (Pakistan), 55: 13-19. (2009).
- Abolghasemi, H., A. Amid, S., Zeinali, H. Radfar, P. Eshghi, S. Rahiminejad, A. Ehsani, H. Najmabadi, T. Akbari, A. Adams, P. C., Y. Deugnier, R. Moirand, P. Brissot, The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. *Hepatology*; 25:162-166. (1997).
- Adams P.C., Y. Deugnier, R. Moirand, P. Brissot, The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. *Hepatology* 25:162-166. (1997).

- Akomolafe, R.O., I.O.Adeoshun1, A.A. Elujoba, E.O. Iwalewa and A.O. Ayoka, Effects of Cassia Sieberiana Leaf Extracts on the intestinal motility of Rat African Journal of Biomedical Research, 6; 141 – 145 (2003).
- Amarowicz, R., B. Pegg, P. M. Rahimi, B. Barl, and J.A. Weil, Free radical scavenging capacity and antioxidant activity of selected plant species from Canadian prairies. Food Chem., 84: 551–562 (2004).
- Aragão, P., A. Lyra, B. Silva, A. Andrade, A. Ferreira, F. Ortega, D. Silva, P. Silva, A. Fraga, G. Wanderley, and L. Lafayette, Toxicological reproductive study of Cassia occidentalis L. in female Wistar rats. J Ethnopharma.,123: 163–166 (2009).
- Arya, V., S. Yadav, S. Kumar, and J.P. Yadav, Antimicrobial activity of Cassia occidentalis L. (leaf) against various human pathogenic microbes. LSMR., 9: 1–11 (2010).
- Bassett, M., J. Halliday, and L. Powell, Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. Hepatology 6, 24-28 (1986).
- Bonkovsky, H., Iron and the liver. American Journal of Medical Science 301, 32-43. (1991).
- Borsari, M., E. Gabbi. F. Chelfi, R. Grandi, M. Saladins, and S. Severi, Silybin a new chelating agent. J-ingorg-biochem, 85:123-9 (2001).
- Brown, Jr., R. Dubach and C.V. Moore, Studies in iron transportation and metabolism Long term iron overload in dogs, J. Lab. &clin. Med. 50:862-893 (1957).
- Brugnara C., Hemoglobin content to diagnose iron efficiency in children, IAMA, 281(23):2225-2230,(1999).
- Cappellini, M. D. and A. Taher, Deferasirox (Exjade R) for the treatment of iron overload. Acta Haemat., 122: 165–173 (2009).
- Elujoba, A., T. Abere, and A. Adelusi, Laxative activities of Cassia pods source from Nigeria. Nig J Nat Prod Med., 3: 51–53 (1999).
- Gomella, L. G., and S. A. Haist, Clinician's pocket reference (11th Ed.). New York: McGraw-Hill. (2007).
- Haraguchi, M, M. L. Dagli, P. C. Raspantini, S. L. Gorniak, The effects of low doses of Sennaoccidentalis seeds on broiler chickens. Vet Res Commun; 27:321-8 (2003).
- Harris, C., Quantitative chemical analysis. 6th ed. pp. 258-261 (2003).
- Hoffbrand A.V., Iron Chelation therapy. Curr-opin-haematol, 12: 153-8 (1995).
- Kennedy, L., L. Ferenz, and A. Burgess, Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD50. J ApplToxicol., 6: 145–148 (1986).
- Kushner, J. P., J. P. Porter, and N. F. Olivieri, Secondary iron overload. Hematology American Society of Hematology Education Program, pp. 47–61 (2001).
- Kwiatkowski, L., and A. R. Cohen, Iron chelation therapy in sickle-cell disease and other transfusion-dependent anemias. Hemato Clin NAméri., 18: 1355–1377 (2004).
- Lewis, S., M. Bain, B.J. Bates, I. Dacie and Lewis, Practical haematology 10th Ed. Churchill Livingstone, 481-522, (2006).
- Link G, A. Pinson and C. Hershko, Heart cells in culture: a model of myocardial iron overload and chelation. J. Lab. Clin. Med. 106: 147-153 (1985).
- Mathew, S., and E. Abraham, Studies on the antioxidant activities of cinnamon (Cinnamomumverum) bark extracts through in vitro models. Food Chem., 94: 520–528 (2006).
- McDonald, J.H., Handbook of Biological Statistics Sparky House Publishing, Baltimore, (2008).
- Mirtes, B., P. Ticiana, B. Carlos, A. Pablo, A. Burno, Andrade., A. Igor, H. Joao, G. Almir Wanderley, and L. Simone, Acute and subacute toxicity of Cassia occidentalis L. stem and leaf in Wistar rats. J Ethnopharmaco., 136:341-346 (2011).
- Nilsson, U. A., M. Bassen, K. Savman, and I. Kiehlmer, A simple rapid method for the determination of “free” iron in biological fluids, Free Radical Res. 36, 677–684 (2002)
- Ozsoy, N., A. Can, R. Yanardag, and N. Akev, Antioxidant activity of Smilax excels leaf extracts. Food Chem., 110: 571–583 (2008).
- Pietrangelo A, Y. Deugnier and J. Dooley, EASL clinical practice guidelines for HFE haemochromatosis. European Association for the Study of the Liver. J Hepatol., 53: 3–22 (2010).
- Pignatti, C., and R. Galanello, Thalassaemias and related disorders: quantitative disorders of hemoglobin synthesis. In: John P(ed) Wintrobe's clinical hematology, 12th ed. Lippincott Williams and Wilkins, Philadelphia, pp. 1083–1131 (2009).
- Porter J., R. Galanello and G. Saglio, Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): a 1-year prospective study. Eur J Haematol., 80:168–176 (2008).
- Porter, B., Practical management of iron overload: Review. Br J Haemat., 115: 239–252 (2001).
- Shaheen, S., M. Ahmad, S. Khalid, M. Zafar, A. Aftab and S. Sultana, Systematic and phytochemical evaluation of selected herbs of Family

- fabaceae *The Journal of Animal & Plant Sciences*, 23(3): 872-881 (2013).
- Sharma, N., P. Trikha, M. Athar, and S. Raisuddin, In vitro inhibition of carcinogen-induced mutagenicity by *Cassia occidentalis* and *Emblica officinalis*. *Drug Chem Toxicol.*, 23: 477-84 (2000).
- Telfer, T., E. Prestcott, S. Holden, M. Walker, V. Hoffbrand, and B. Wonke, Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassemia major. *Br. J. Haemat.*, 110: 971-977 (2000).
- Vichinsky, E., Consensus document for transfusion-related iron overload. *Seminars in Hematology.*, 38: 2-4 (2001).
- Waldmeier, F., J. Bruin, and U. Glaenzel, Pharmacokinetics, metabolism, and disposition of deferasirox in beta-thalassemic patients with transfusion-dependent iron overload who are at pharmacokinetic steady state. *Drug Metab. Dispos.*, 38:808-16 (2010).
- Yen, G., H. Chen, and P. Duh, Extraction and identification of an antioxidative component from *Cassia tora* L. *J. Agric. Food Chem.*, pp.820-824 (1998).
- Zafar, M., A. A. Anjum, M. F. Qamar, M. I. Najeeb and A. Maqbool. Role of herbal polysaccharides as growth promoters in broilers. *Sci. Int. (Lahore)*, 23(2), 157-159, (2011).