Abelmoschus esculentus (Okra) leaf modulates some iron profile and inflammatory parameters in Sprague Dawley rats

Austin Iroghama Aruomaren (Iroghama.aruomaren@uniben.edu)
University of Benin
Akhere Akuekegbe Omonkhua
University of Benin
Evarista Osime
University of Benin

Research Article

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Abstract

Background

Okra is a multipurpose plant which can be consumed freshly or dried. Okra contains iron, β-carotene and other phenolic compounds with important biological properties like flavonol and isoflavonoid derivatives which may possess anti-inflammatory properties. The objective of this study was to determine the effect of okra leaf on some iron regulatory proteins and its anti-inflammatory properties.

Methodology:

Fifty (50) rats were grouped into 10 groups with 5 rats per group and each group were fed with a pre-formulated diet of *Abelmoschus esculentus* leaf or the standard phytoestrogen diadzein. Hepcidin, ferroportin, ferritin, IL-6, IL-2 and MIP 1β were analysed using sandwich ELISA kits from Elabscience Biotechnology, Wuhan, China. Full blood count was analysed using Sysmex haemotology autoanlyser. Serum iron was also analysed spectrophotometrically.

Results

The results showed that, in male rats, 40% okra leaf-fed group had a significantly higher RBC count ($p = 0.0419$), haemoglobin concentration (HB) ($p = 0.0054$), haeamtocrit (HCT) ($p = 0.012$) and mean cell haemoglobin (MCH) ($p = 0.0064$) when compared to control rats. Serum iron, total iron binding capacity (TIBC), transferrin saturation, ferroportin, ferritin were all significantly higher ($p < 0.05$) in the experimental groups when compared to the controls. However, in female rats fed with 40% okra leaf, there was significantly lower hepcidin concentration ($p < 0.001$) in control group when compared to 10% Okra leaf fed group. Furthermore, the 10% okra leaf-fed group also had a significantly higher hepicidin concentration when compared to the 40% okra leaf-fed groups. IL-6 levels were significantly lower ($p < 0.01$) in female rats fed with 40% okra leaf when compared to the 10% okra leaf-fed group. Macrophage inflammatory protein 1 beta (MIP-1β) in male rats showed that the groups fed with 10%, 20% and 40% okra leaf ($p < 0.001$) had significantly higher levels when compared to control and diadzein administered group.

Conclusion

Our data indicated feed formulated from *Abelmoschus esculentus* leaf is a rich source of non-haem iron. *Abelmoschus esculentus* leaf also significantly imparts iron metabolism through its action on ferroportin and hepcidin. Also this study indicates that continuous consumption of *Abelmoschus esculentus* leaf may help act as an anti-inflammatory agent.

INTRODUCTION

Studies have shown that phytoestrogens inhibit endogenous estrogen production in the ovary leading to disturbances in follicle development and lack of estrous [1]. Animal and human studies evaluating phytoestrogen effects on the adult hypothalamic–pituitary–gonadal (HPG) axis following adult exposures have been fairly

consistent and reveal the potential for suppression [1, 2]. This implies that phytoestrogen can have delimiting effect on the ovaries although this may take place over time. Intake of phytoestrogen have also been reported to cause polycystic ovary-like (PCOS) condition in animals [3, 4]. Subjects with polycystic ovarian syndrome (PCOS) have shown decreased circulating hepcidin levels [5]. Yang *et al*. [6] also described a mechanism by which estradiol could increase iron absorption and iron release from storage cells. Similarly, phytoestrogens have been reported to indirectly stimulate erythropoiesis through their action on androgen [7]. In addition, phytoestrogens from soy beans have been reported to trigger PCOS like symptoms [8]. Many food and vegetables, contain phytoestrogens [9].

Okra is a vegetable originally thought to have its origin from South East Asia, however, it is now widely cultivated in tropical and subtropical regions [10]. In West Africa, the wide varieties of okra cultivars are of two morphologically distinct species, the common okra, (*Abelmoschus esculentus*) and West African okra (*Abelmoschus caillei*) [11]. Okra is known as 'ilasa' in south-west Nigeria, In Igbo-Ora, Oyo state, south-west Nigeria, the specific variety of okra it is called "ilasa kekere" and has been identified as *A. esculentus* [12]. Some vegetables and foods including okra have been reported to contain phytoestrogen which may influence fertility [9].

Furthermore, little is known of the effect of okra leaf on hepcidin, ferroportin serum iron and erythropoeisis in general. Iron is an essential trace element and required for human life and it is tightly regulated in the plasma [13]. Iron homeostasis is maintained by altering the absorption of iron from the gut and the release of iron from its storage sites in hepatocytes and macrophages. A key regulator of these processes is hepcidin, a peptide hormone produced by the liver to inhibit iron transport by binding and inhibiting the cellular iron exporter, ferroportin. The levels of hepcidin are regulated in a homeostatic manner so that high iron levels raise hepcidin to decrease iron absorption and lower the rate of release from storage cells [13]. Okra have also been reported to elicit some anti-inflammatory effects, however, there is scarcity of information on its direct effect on inflammatory cytokines such as IL-6 and IL-2. Given the particularly interesting phenomenon of high dizygotic twinning in Igbo-Ora, south-west Nigeria and the high consumption of okra leaf; as well as the association between phytoestrogens and iron metabolism, this study explored the effects of okra leaf fortified feed on. The aim of this study, was to assess the effect of okra leaf on some iron profile parameters and some inflammatory cytokines in Sprague Dawley rats.

Materials and Methods

Study Population and Animal care

This study used animal (rats) model. Fifty (50) Sprague Dawley rats, 25 male and 25 female, were purchased from the animal holdings of the College of Medicine, University of Lagos, Nigeria. Rats within the experimental groups were fed with a predetermined concentration of *Abelmoschus esculentus* (okra)leaf. The rats were housed at TRIGAS (Tetfund Research for *Irvingia gabonesis* Antidiabetic Study) Research Laboratory, University of Benin. Animals were exposed to 12 h dark and light cycles with access to feed and water *ad libitum*. The rats were allowed to acclimatize for two weeks before the commencement of the study.

Ethical Approval

Institutional review board (IRB) approval was obtained from the Ethics Committee, Faculty of Pharmacy, University of Benin, Nigeria with reference number - EC/FP/019/18

Feed Preparation

Fresh leaves of *Abelmoschus esculentus* (okra)were obtained from a local market in Igbo-Ora community, Oyo State, Nigeria. The air dried leaves were pulverized into fine powder using an electric blender and stored at room temperature in air tight polythene bag prior to use. The okra leaves were formulated into feed (at three different concentrations of 10%, 20%, and 40% weight/weight of feed). Steps in feed formulation involves the balancing between crude protein and energy levels as previously described by Roush *et al.* [14]. The next phase was to determine the levels of indispensable amino acids in the formulation to be sure the dietary levels meet the requirements of the rats to be fed. All requirements for the amino acid was expressed as a percentage of the diet. For this study, the indispensable amino acids used were lysine and methionine.

Experimental design

Okra leaves were purchased from Igbo-Ora, Oyo State, Nigeria and were identified and authenticated in the Department of Plant Science and Biotechnology, University of Benin, Nigeria. Herbarium specimen for the okra leaf, with voucher number UBH-442 was deposited at the University of Benin Herbarium. The okra leaf feed formulation was done at the Department of Animal Science, Faculty of Agriculture, University of Benin, Nigeria**.** This study had 10 groups, of 5 rats per group. The rats were then fed for two months with the formulated feed. The study had a positive control group that was administered 25mg of diadzein (table 1).

Laboratory Analysis

The serum from the rats were analyzed for hepcidin, transferrin saturation, ferroportin 1, ferritin, IL-2, IL-6 and rat macrophage inflammatory protein 1 beta (MIP-1β). All assays were analyzed using sandwich ELISA kits from Elabscience Biotechnology, Wuhan, China. The samples were analysed by adding test and control samples to appropriate ELISA microwell plates. Then a biotinylated detection antibody specific to the different analytes and avidin-Horseradish peroxidase (HRP) conjugate was then added to all appropriate wells. Free components are washed away. Then a substrate solution is added to each wells. There after the enzyme-substrate reaction is terminated by adding a stop solution. The OD is measured at 450nm wavelength. The OD value is proportional to the concentration of the different analyte analyzed. Serum Iron and UIBC were estimated colorimetrically using TECO Diagnostics, California, USA and TIBC calculated. Complete blood count was analysed using 3 part haematology autoanalyzer.

Statistical Analysis

Data obtained were analyzed by GraphPad Prism 8.0.1 (San Deigo, Califonia, USA), the differences between two groups were analyzed using the Independent t-test. While for data with more than two variables (groups), differences among groups were analyzed by one-way analysis of variance (ANOVA). Inter-group comparisons were done by the turkey's post hoc test. A value of $p<0.05$ was accepted as significant

Results

Complete Blood Count in Sprague Dawley rats fed with Okra leaf

The role of diet in haemopoiesis cannot be overemphasized, this study evaluated the effect of okra leaf formulated feeds on haemopoiesis. In male rats, 40% okra leaf fed group had a significantly higher RBC count ($p=0.0419$), haemoglobin concentration (HB) ($p=0.0054$), haeamocrit (HCT) ($p=0.012$) and mean cell haemoglobin (MCH) ($p=0.0064$) when compared to control. HCT, HB and MCH were also significantly higher in male rats fed

with 40% okra leaf when compared to diadzein administered and 10% okra leaf fed groups (table 2). Monocyte count was also significantly higher in 40% okra leaf fed group compared to 10% okra fed group. Similarly parameters in female rats showed that RBC count, HB, MCH and MCHC were all significantly higher in the group fed with 40% okra leaf when compared to control, diadzein administered, 10% okra leaf and 20% okra leaf fed groups (Table 3).

Iron Profile parameters in Sprague Dawley rats fed with Okra leaf

To evaluate the role of okra leaf on iron metabolism; serum iron, total iron binding capacity (TIBC), transferrin saturation, hepcidin, ferroportin and ferritin were analysed. Figure 1 (Panel A and B) shows the effect of okra leaf on serum iron levels both in male and female rats. Female rats fed with 10% okra leaf, 20% okra leaf and 40% okra leaf significantly had high levels of serum iron ($p = 0.001$), total iron binding capacity (TIBC) ($p<0.001$) and transferrin saturation ($p<0.001$) when compared to control and diadzein administered groups. Furthermore similar results were also observed in male rats; serum iron ($p<0.01$), TIBC ($p<0.01$) and transferrin saturation ($p<0.001$) were significantly higher in diadzein administered, 10% okra leaf, 20% okra leaf and 40% okra leaf groups when compared to the control.

Another objective of this study was to determine the effect of okra leaf on some iron regulatory proteins (Figure 1). Hepicidin, ferroportin and ferritin levels were determined in Sprague Dawley rats after the consumption of different concentration of feeds formulated from okra leaf. In male rats, ferroportin levels was significantly higher ($p<0.01$) in group fed with 40% okra leaf, 20% okra leaf and diadzein administered groups when compared to the control. Also groups fed with 20% okra leaf and 40% okra leaf ($p<0.001$) also had significantly higher ferroportin levels when compared to 10% okra leaf fed and diadzein administered group. Ferroportin levels ($p<0.001$) were significantly higher in female rats fed with 10% okra leaf, 20% okra leaf and 40% okra leaf when compared to controls and diadzein administered groups (Figure 2; Panel A and B). Female rats fed with okra leaf showed (Figure 2; Panel D) significantly lower hepcidin concentration ($p<0.001$) in control group when compared to 10% Okra leaf fed group. Furthermore, 10% okra leaf fed group also had a significantly higher hepicidin concentration when compared to 40% okra leaf fed group. Results from male rats showed (Figure 2; Panel C) that control and diadzein administered groups had significantly lower hepicidin concentration ($p<0.001$) when compared to 10% okra leaf fed group. In addition, 40% okra leaf fed group also had a significantly lower hepcidin concentration compared to 10% okra leaf and 20% okra leaf fed groups. There was a significant increase (Figure 2; Panel E) in ferritin concentration ($p<0.01$) in male rats fed with 20% and 40% okra leaf fed groups when compared to 10% okra leaf fed group. Figure 2; Panel F shows that there was a significant decrease in ferritin concentration ($p<0.01$) in control and diadzein administered group when compared to 10%, 20% and 40% okra leaf fed groups.

Some Inflammatory biomarkers in Sprague Dawley rats fed with Okra leaf

In order to determine inflammatory response as a result of consumption of okra leaf, IL -2, IL-6 and MIP 1β were analysed. IL-6 levels in female rats were significantly lower ($p<0.01$) in rats fed with 40% okra leaf when compared to 10% okra leaf fed group as shown in Figure 3; Panel B. However in male rats, there was no significant difference in IL-6 results. IL-2 in male rats were significantly lower in rats administered with diadzein (25mg) and 40% okra leaf fed groups when compared to controls ($p<0.001$). Furthermore, 10% okra leaf fed group also had significantly higher IL-2 levels when compared to 40% okra leaf fed group (Figure 3; Panel C). Similarly, in female rats, IL-2 was significantly lower in the group administered diadzein ($p<0.001$) when compared to 10%, 20% and 40% okra leaf fed groups (Figure 2; Panel D). Macrophage inflammatory protein 1 beta (MIP-1β) in male rats showed that the

groups fed with 10%, 20% and 40% okra leaf had significantly higher levels when compared to control and diadzein administered group (Figure 3; Panel E). Results from female rats, shows that groups fed with 40% okra leaf had significantly higher MIP-1β levels when compared to control ($p<0.001$), diadzein ($p<0.001$), 10% ($p<0.05$) and 20% ($p<0.05$) okra leaf fed groups (Figure 3; Panel F).

Discussion

Okra is a multipurpose plant which can be consumed freshly or dried. The pod and vegetable has been used for salads, stew and soup [15]. Kahlon *et al*. [16] and Varmudy, [17] reported that 100g of okra leaf can contain 0.70mg of iron and 385.00 µg of β-carotene. Okra is also rich in phenolic compounds such as flavonol and some these flavonol derivatives and isoflavonoid have been reported to be rutin and quercetin [15]. Furthermore, Okra has shown promising but not confirmed anti-inflammatory properties [18].

The objective of this study was to determine the effect of *Abelmoschus esculentus* leaf on haematological parameters, iron profile parameters and inflammatory markers in Sprague Dawley rats. Serum iron, TIBC, transferrin saturation, ferritin, hepcidin and ferroportin, were all significantly high in all groups fed with okra leaf. The result suggests a high iron content in okra leaf used for this study. In developing countries, iron deficiency is usually a consequence of absorption disorders, loss of blood, or the intake of a restricted diet (vegan diet). Foods present different forms of iron that differ in their bioavailability, depending on the source. Hemic iron is present mainly in animal proteins such as hemoglobin and myoglobin, which has a higher bioavailability; these proteins are present in meat, fish, and shellfish [19].

Non-hemic iron is present in different chemical forms, which significantly affects its absorption. This type of iron is present in both organic and inorganic forms. The most common sources of non-hemic iron present in foods are low-molecular-weight compounds such as ferric citrate, phosphate, phytate, oxalate, hydroxide and in high-molecular-weight compounds such as ferritin. The best sources of non-hemic iron are seeds, grains, nuts, and the green leaf of vegetables [19]. Likewise, the absorption of iron depends to a large extent on the concentration of iron present in the body and enhancers such as ascorbic acid and some muscle tissue proteins. Although, Okra (*Abelmoschus esculentus)* has been reported to contain iron [20], reported that iron-rich vegetable diet did not necessarily translate into high iron absorption rate and stated that other factors such as vitamin C and β –carotene should be investigated. The reported presence of vitamin C and β –carotene in okra leaf [21] may have contributed to high serum iron observed in this study.

One of the objectives of this study was to evaluate some iron associated proteins involved in iron metabolism. Most dietary iron is in the ferric form (Fe^{2+}) which are converted to ferrous form (Fe^{3+}) by duodenal ferric reductase and then transported by divalent metal transporter 1 (DMT-1) [22]. Hepcidin is a hormone secreted by the liver which regulates iron homeostasis. It is the master regulator of systemic iron homeostasis, coordinating the use and storage of iron with iron acquisition [23]. In this study, hepicidin concentration was lower in the groups fed with higher concentrations of okra leaf. This is probably due to high iron levels which induce the synthesis of ferroportin which in turn causes a negative feedback mechanism in hepcidin activity. The hepcidin results from this study suggest that hepcidin regulation is probably a negative feedback mechanism to high serum iron content. Another mechanism could probably be due to the fact that estrogen down regulates hepcidin expression through the estrogen responsive elements [24]. Kuhnle *et al*. [25] using LC-MS reported that okra contains a high concentration of phytoestrogens and these natural compounds have been shown to elicit estrogen like properties

[26]. Young *et al*. [27] had also previously reported increased serum hepcidin levels in women who had iron supplements from food sources.

The role of hepcidin in the regulation of the bioavailability of dietary iron is associated with its interaction with transmembrane protein, ferroportin (Fpn) [28]. In this study, ferroportin levels were higher in the treatment groups which is indicative of iron been pooled into circulation from the labile pool. Ferroportin is both the hepcidin receptor and the only known cellular iron exporter in vertebrates [29]. Ferroportin is expressed on duodenal enterocytes absorbing dietary iron, macrophages in liver and spleen recycling old erythrocytes, hepatocytes storing iron and placental trophoblasts transferring iron to the fetus during pregnancy [30]. The biological role of ferroportin is the transportation of iron from cells to blood vessels. Hepcidin regulates the expression of ferroportin on a post-translational level [31]. It can directly bind to ferroportin molecules, with subsequent internalization of the resultant hepcidin-ferroportin complex. Ferroportin undergoes lysosomal degradation inside the endosome. Its loss from the cellular surface induces a secondary decrease in the release of iron from the cell [29, 31]. Consequently, the interaction between hepcidin and ferroportin inhibits the efflux of iron ions from enterocytes to blood vessels [32]. Whenever the systemic deposits of iron are either sufficient or too high, the liver synthesis of hepcidin is enhanced; the hormone is released into circulation, reaches the enterocytes, and binds to ferroportin, thereby inducing its endocytosis and degradation [33]. Ferroportin is also expressed in erythroid precursor cells, and it has been proposed that its presence enhances the sensitivity of precursors to systemic iron levels and helps determine their commitment to expansion and differentiation [34]. Furthermore, enhanced hepcidin expression will exert a negative feedback regulation on iron absorption which will culminate in the degradation of ferroportin over time. The results from this study is suggestive that lower concentration of hepcidin correlates with high concentration of ferroportin, which resulted in the high serum iron observed in this study.

Serum ferritin which is the main storage protein for iron was significantly high in all test groups compared to controls. Similar result was reported by Nyakundi *et al*. [35], where they stated that serum ferritin levels directly correlates with consumption of iron and ascorbate-rich foods. Although, high serum ferritin level does not necessary connote high iron level because it can also be a marker for an inflammatory process [36], the result from this study with the high serum iron and TIBC suggest that the increase in serum ferritin is probably due to increase iron levels. In this study, there was also a significant increase in transferrin saturation in groups fed with okra leaf. This suggest over saturation of iron and ferritin within the entire metabolic process thereby increasing the capacity for the body to transport more iron. Intracellular iron levels regulate ferritin biosynthesis and transferrin expression affinity [37]. This regulation is mediated by the interaction of iron responsive elements (IREs) with cytosol RNA-binding proteins called iron regulatory proteins (IRPs). A careful balance between the IREs and IRPs, swings the pendulum of iron transportation (transferrin levels) and iron storage (increased ferritin levels) [37]. The binding of IRPs to IREs lead to changes in expression of iron-regulated genes such as hepcidin, with subsequent alterations in intracellular iron uptake, use and storage. When iron levels are high, the IREs do not bind to IRPs and the translation of ferritin mRNA occurs normally.

The study showed a significant increase in RBC count, haemoglobin concentration and haematocrit in the experimental groups when compared to control. This indicates that okra leaf can be used to help boost red cells and haemoglobin concentration. Okra has been previously reported to be rich in iron and folate [10, 38] which may also contribute to the increase in red cell parameters observed in this study.

The rapid evolution of molecular information about iron transport and homeostasis has uncovered a comprehensive understanding of the complex mechanisms involved in this process. It has long been recognized

that iron levels must be tightly regulated to provide an essential nutrient that is involved in oxygen delivery, metabolism and redox regulation while guarding against excessive levels of a primary toxicant that can generate reactive oxygen species (ROS) to produce cellular damage and death. Unlike other essential minerals, the delicate balance between iron nutrition and toxicity is maintained by systemic control mechanisms that drive iron conservation and limit uptake until needs are presented [39]. Excessive inflammation and oxidative stress is closely linked to the development of many human chronic illnesses. This study also investigated the effects of okra leaf on some anti-inflammatory and pro-inflammatory cytokines. The results shows that IL- 6 and IL- 2 which are pro-inflammatory cytokines were all lower in groups fed with okra leaf. Although, the levels of IL-6 was not statistically significant in male rats but in female rats 40% okra leaf fed group had lower levels when compared to 10% okra leaf fed group. Inflammatory cytokines, in particular IL-6 have been reported to regulate hepcidin transcription via the JAK-STAT3 pathway [40]. In addition, data for MIP - 1β (anti-inflammatory cytokine) showed a significant increase in the groups fed with okra leaf. This result indicates the anti-inflammatory effect of okra. This results corroborate the reports by other researchers, where they reported a decrease in IL- 6 [18, 41, 42] in animals fed with okra. Some of the most abundant phytochemicals in okra leaf extracts have been identified as quercetin, quercitrin, rutin and kaempferol [43]. Some of these derivatives, in fact have been previously studied for their anti-inflammatory activities [44, 45].

Conclusion

Data from this study shows that feed formulated from *Abelmoschus esculentus* leaf significantly increased serum iron, TIBC, ferroportin and serum ferritin levels. Serum hepcidin concentration was lower in groups fed with 40% okra leaf. This indicates that continuous consumption of okra leaf increases iron metabolism and maybe a steady source of non-haem iron. The results also showed that okra leaf has anti-inflammatory properties.

Abbreviations

IL - Interleukins

MIP-1β - Macrophage inflammatory protein 1 beta

RBC - Red Blood Cell

ELISA – Enzyme Linked Immunoassorbent Assay

HB - Haemoglobin

HCT - Haematocrit

MCH - Mean cell haemoglobin

TIBC - Total iron binding capacity

UIBC - Unsaturated iron binding capacity

HPG - hypothalamic–pituitary–gonadal

PCOS - Polycystic ovarian syndrome

TRIGAS – Tetfund Research for *Irvingia gabonesis* Antidiabetic Study

HRP - Horseradish peroxidase

OD – Optical Density

IRE - Iron responsive elements

IRP - Iron regulatory proteins

Declarations

Ethics Approval

Institutional review board (IRB) approval was obtained from the Ethics Committee, Faculty of Pharmacy, University of Benin, Nigeria with reference number - EC/FP/019/18

Consent for Publication

All authors gave their consent to the publication of this article

Availability of data and material

The data and materials used for this study have been cited in the manuscript

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

This work was carried out and approved in collaboration between all the authors who takes responsibility for its intellectual contents, accuracy and integrity. AAI, OAA and OE designed the study; AAI and OAA sourced for funding; AAI and OE wrote the protocol; AAI and OAA contributed in literature search; AAI and OE participated in the Lab experiments; AAI did the statistical data analysis. All authors contributed in the discussions. AAI and OAA drafted the manuscript. All authors read and approved the final manuscript.

Authors' information

Corresponding Author: Aruomaren Austin Iroghama

Lecturer

Department of Medical Laboratory Science (Haematology Unit)

University of Benin.

Prof. Omonkhua, Akhere Akuekegbe

Head of Department

Medical Biochemistry

University of Benin

Osime Evarista

Head of Department

Department of Medical Laboratory Science.

University of Benin

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Tables

Table 1: Grouping and different feeds fed to different groups

S/N	Treatment	Number of Rats
1	Control (Male)	5
2	Control (Female)	5
3	Positive Control (25mg of Diadzein) Male	5
4	Positive Control (25mg of Diadzein) Female	5
Okra leaf		
5	10% okra leaf formulation (Male)	5
6	10% okra leaf formulation (Female)	5
7	20% okra leaf formulation (Male)	5
8	20% okra leaf formulation (Female)	5
9	40% okra leaf formulation (Male)	5
10	40% okra leaf formulation (Female)	5

Table 2: Haematological parameters of male rats fed with different concentrations of *Abelmoschus esculentus* leaf

Parameters	Control	Diadzein	10% okra Leaf	20% okra Leaf	40% okra Leaf	F value	P Value
RBC ($x10^9$/L)	6.83±0.34	7.31±0.25	7.65±0.03	8.92±0.87	$9.99±1.10^{a}$	3.471	0.0419
HCT (L/L)	45.37±1.78	46.33±0.89	47.6±1.24	49.83±0.70	$51.68±0.87^{ab}$	5.06	0.0127
HB (g/dL)	15.2±0.90	15.17±0.43	15.83±0.46	17.27±0.43	$18.5±0.59^{abc}$	6.406	0.0054
MCV (fL)	60.5±0.25	63.43±1.18	61.88±0.90	64.03±1.98	65.08±1.80	1.651	0.2255
MCH (pg)	20.27±0.31	20.73±0.14	21.73±0.28	20.7±0.55	$22.65±0.49^{abd}$	6.121	0.0064
MCHC (g/dL)	33.47±0.66	32.73±0.40	33.35±0.68	32.33±0.23	33.85±0.64	1.00	0.4449
TWBC ($x10^9$/L)	15.8±0.45	$9.03±1.59^{a}$	$10.9±0.80^{a}$	$10.87±0.52^{a}$	13.35±1.19	5.987	0.0069
Monocyte (%)	8.2±0.73	8.46±0.75	7.67±0.67	9.93±0.14	$11.03±0.70^{c}$	4.681	0.0165

Results presented in mean±SEM. Superscript “a” represents significance with control, “b” significance with diadzein, “c” significance with 10% okra leaf, “d” significance with 20% okra leaf

Table 3: Haematological parameters of female rats fed with different concentrations of *Abelmoschus esculentus* leaf

Parameters	Control	Diadzein	10% okra Leaf	20% okra Leaf	40% okra Leaf	F value	P Value
RBC ($x10^9$/L)	6.62±0.11	6.85±0.21	6.88±0.09	7.61±0.54	$10.34±0.26^{abcd}$	18.67	0.0001
HCT (L/L)	40.87±0.87	42.08±1.13	40.6±0.40	42.83±0.89	43.43±0.69	1.662	0.223
HB (g/dL)	14.6±0.15	14.9±0.29	14.73±0.17	14.98±0.36	$16.87±0.34^{abcd}$	8.659	0.0016
MCV (fL)	62.97±3.28	61.48±1.47	61.7±1.40	65.18±1.59	67.03±0.36	1.686	0.2176
MCH (pg)	22.07±0.41	21.78±0.46	21.43±0.26	22.05±0.21	$23.83±0.36^{abcd}$	5.931	0.0072
MCHC (g/dL)	33.27±0.61	$35.43±0.31^{a}$	$36.3±0.46^{a}$	$36.88±0.45^{a}$	$37.53±0.48^{ab}$	11.76	0.0004
TWBC ($x10^9$/L)	9.1±2.055	16.48±2.75	16.07±0.58	12.33±1.96	10.77±1.92	2.228	0.127
Monocyte (%)	4.5±0.11	8.37±1.44	9.33±1.62	9.32±1.98	9.16±1.11	1.663	0.2226

Results presented in mean±SEM. Superscript “a” represents significance with control, “b” significance with diadzein, “c” significance with 10% okra leaf, “d” significance with 20% okra leaf

Figures

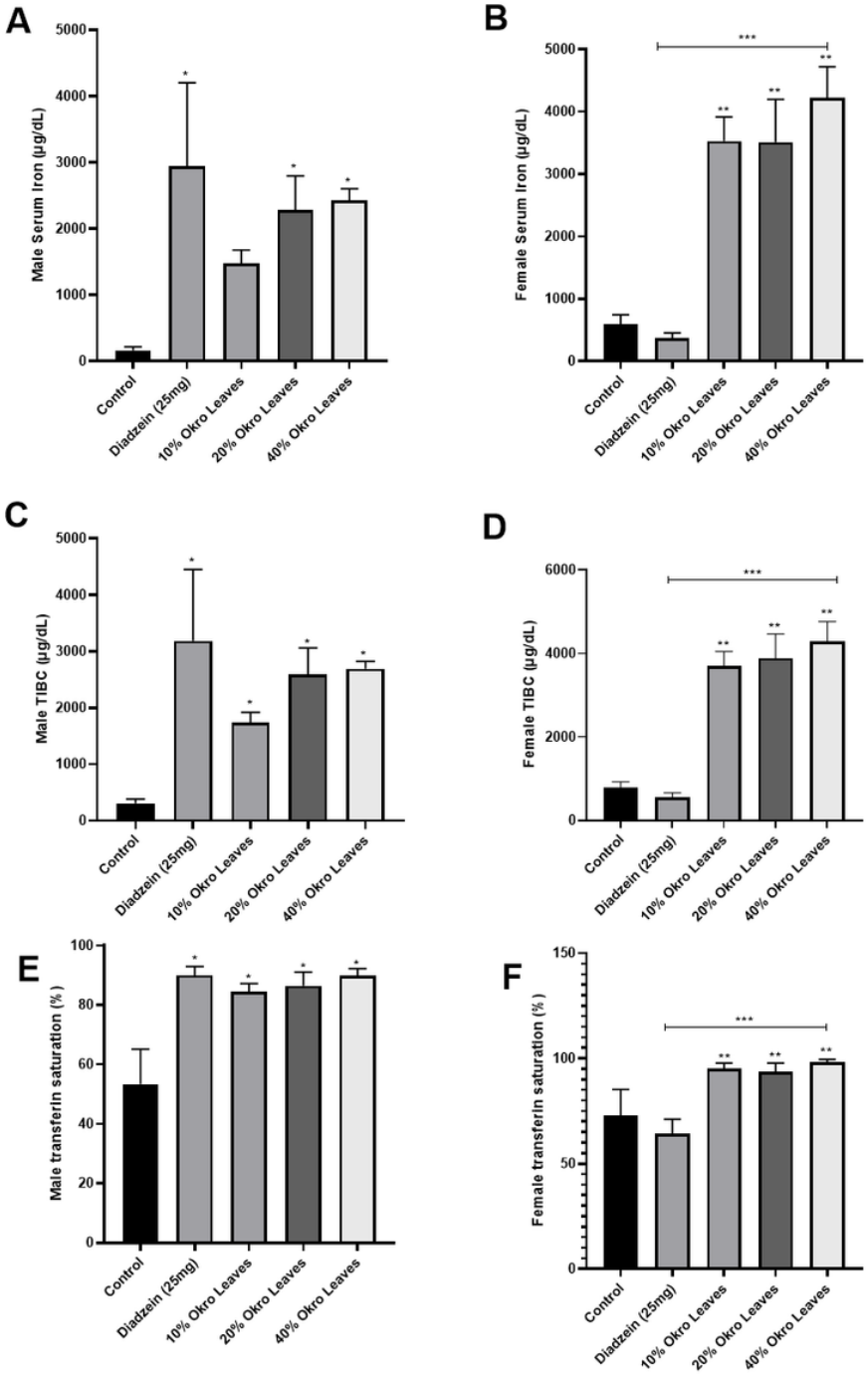

Figure 1

Effect of okra leaf feed on serum iron, TIBC and transferrin saturation. Panels A and B show the effect of okra leaf on serum iron in both male and female Sprague Dawley rats. Panels C and D show the effect of okra leaf on TIBC in both male and female Sprague Dawley rats. Panels E and F show the effect of okra leaf on transferrin saturation in both male and female Sprague Dawley rats. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001)

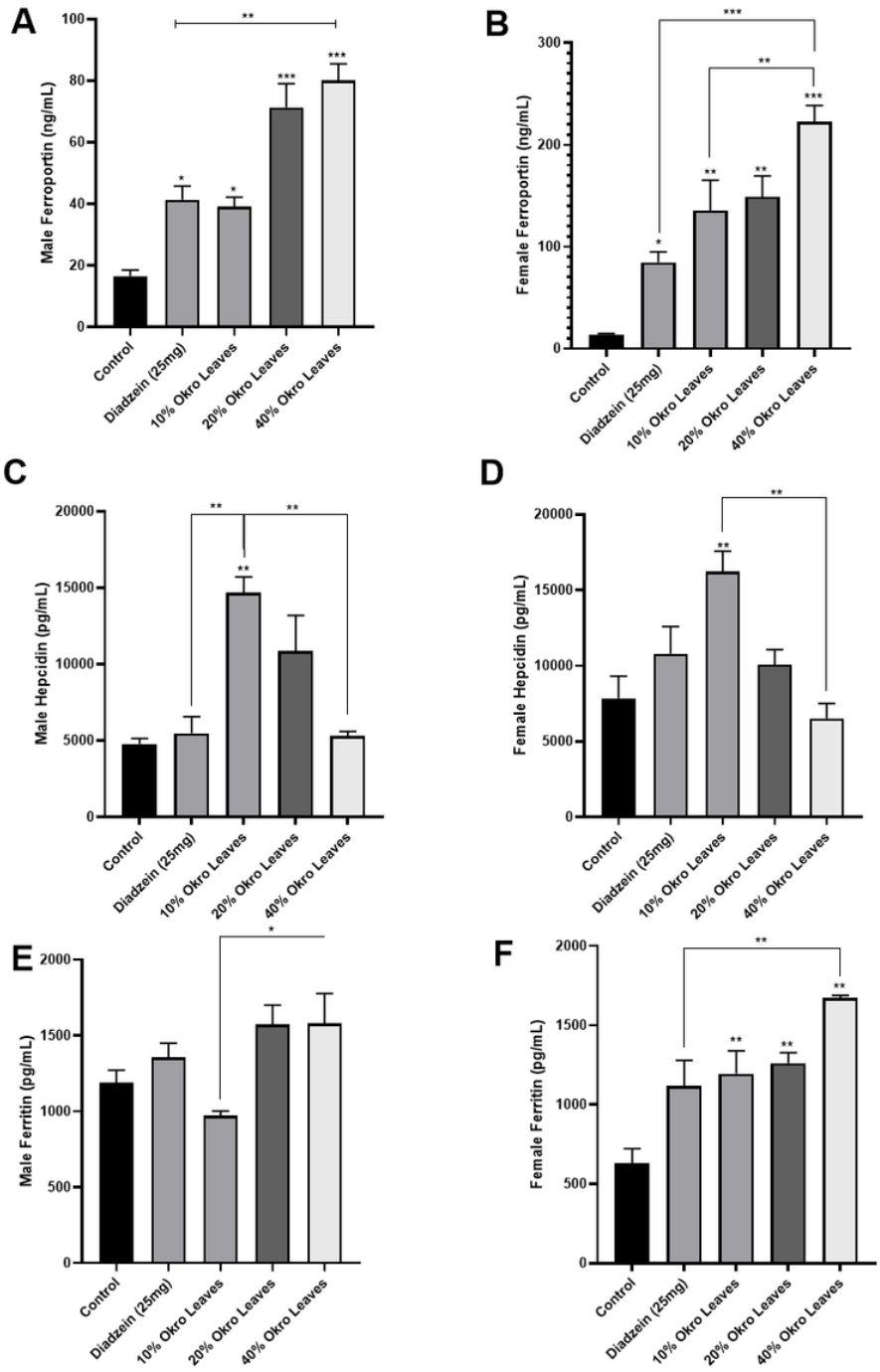

Figure 2

Effect of okra leaf feed on some iron homeostatic parameters. Panels A and B show the effect of okra leaf on ferroportin in both male and female Sprague Dawley rats. Panel C and D show the effect of okra leaf on hepcidin in both male and female Sprague Dawley rats. Panels E and F show the effect of okra leaf on serum ferritin in both male and female Sprague Dawley rats. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001)

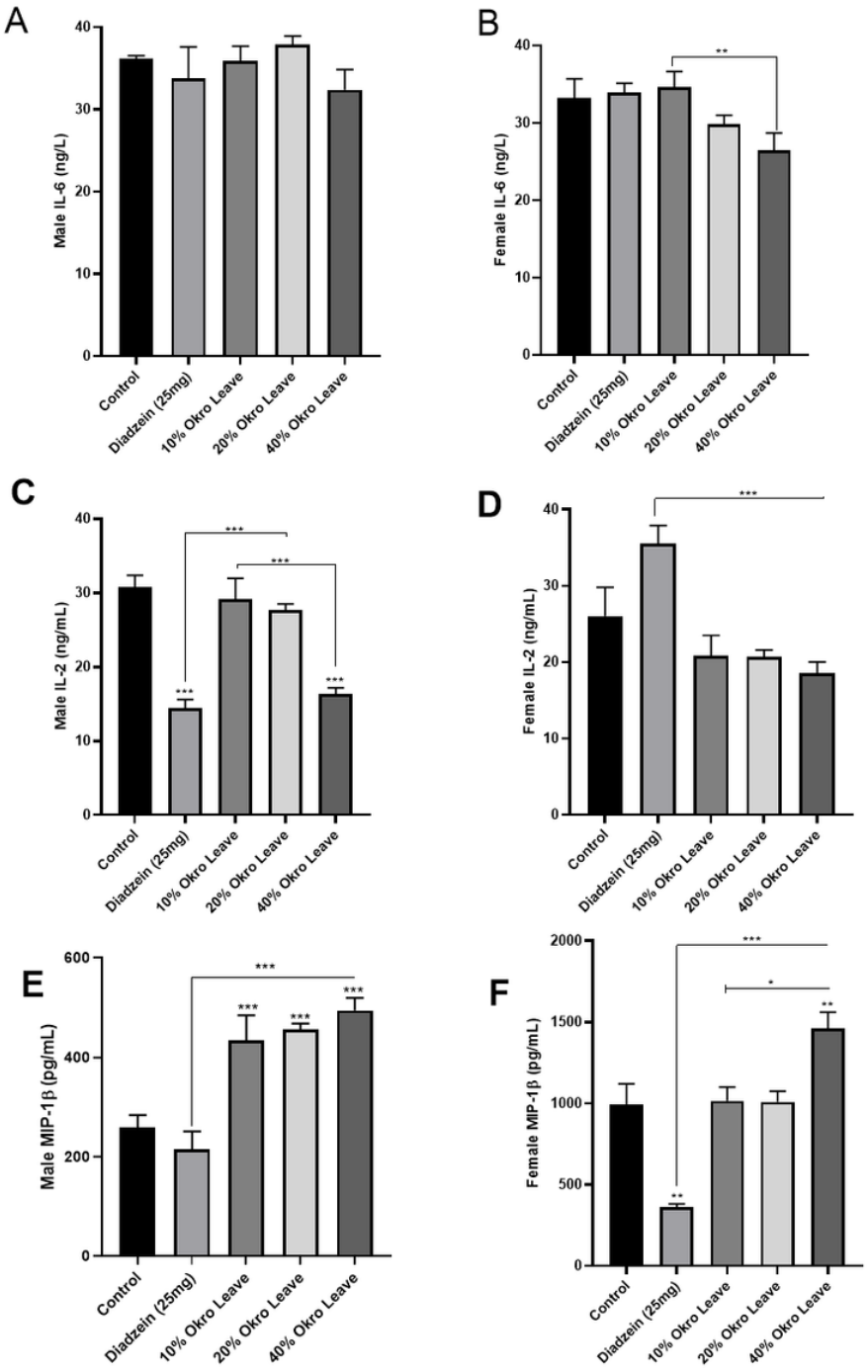

Figure 3

Effect of okra leaf feed on some inflammatory cytokines. Panels A and B show the effect of okra leaf on IL-6 in both male and female Sprague Dawley rats. Panels C and D show the effect of okra leaf on IL-2 in both male and female Sprague Dawley rats. Panels E and F show the effect of okra leaf on transferrin MIP-1β in both male and female Sprague Dawley rats. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001