

# Comparative liver function assessment of natural and available drug (Ferrous sulfate) for iron-deficiency anemia in rat model

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**Abstract:** To assess the comparative effect of anti-anaemic drug (ferrous sulfate) with naturally occurring anti-anaemic compound (*Illicium verum* commonly called star anise) on liver in rat model. Model and both test groups were made anaemic. Ferrous sulfate was given to T1 group of rats as 30mg/kg body weight (b.w) and *Illicium verum* to T2 group of rats with dose of 80mg/kg b.w for six weeks. *Illicium verum* treated group (T2 rats) produced depression, decreased anxiety and enhanced short-term memory, whereas ferrous sulfate treated group (T1 rats) enhanced long term memory. The liver function test of T2 rats showed that the total bilirubin was in normal range, but direct bilirubin, SGPT, ALP and GGT were significantly decreased in T2 rats in comparison with T1 and also from model group of rats. It was concluded in this study that by comparing the effect of ferrous sulfate with naturally occurring *Illicium verum* on iron-deficiency anaemia, *illicium verum* produces same effects and can be used to treat iron-deficiency anaemia without affecting liver function.

**Keywords:** Iron-deficiency anaemia, ferrous sulfate, *Illicium verum*, behavioural activity.

## INTRODUCTION

Most common form of anaemia is called iron deficiency anaemia (IDA) produced by less intake of iron or loss of iron. The anaemia is common in Pakistan because of less iron in diet. The prevalence of anaemia in Pakistan was 83% in pregnant women, 78% in lactating mother, 58% in girls and 82.9% in children (Akhtar *et al.*, 2013). Ferrous sulfate is commonly used drug of choice to treat anaemia since 19<sup>th</sup> century (Nadir *et al.*, 2015). According to World Health Organization (WHO) anaemia affected 43% children globally (Cancelo-Hidalgo *et al.*, 2013). Oral iron therapy produces common side effects on gastrointestinal tract reported in previous studies such as nausea, abdominal pain, flatulence, constipation, black stool and diarrhoea (Tolkien *et al.*, 2015).

Iron is always found in bound state like hemosiderin, transferrin and ferritin (Tietz *et al.*, 1990). Iron sulfate is important part of our diet; it produces free radicals (cause tissue damage). These free radicals cause lipid peroxidation (Mladenka *et al.*, 2006). Ferrous sulfate (FeSO<sub>4</sub>) act as a toxin for liver, found in numerous home based pharmaceutical formulations. The overdose occurs in children either accidentally or intentionally (Anandakumar *et al.*, 2014). The cell uptakes only those iron which is bound with transferrin Fe<sup>3+</sup> with the help of transferrin receptors. Iron is essential for erythropoiesis process and biosynthesis of enzymes that contain heme portion such as cytochromes remaining iron is stored in hepatocytes (McKie *et al.*, 2000).

Disorder of liver is dangerous issue for health. Herbal remedies were used to manage liver problems in India (Abdel-Reheim *et al.*, 2017). Liver is an organ which maintains homeostasis of body, detoxification, biotransformation, and excretes different compound like end products of medicines. Hepatotoxicity due to different drugs is main cause of iatrogenic illness in all hospital admissions (Dossing *et al.*, 1993). *Illicium verum* commonly called star anise, its tree is evergreen and produces fragrance and found in Asian countries like Pakistan and china (Asif *et al.*, 2016). *Illicium verum* is used in both medicine and food and the ministry of health of people republic of china in March 2002 suggesting it as nontoxic to humans (Li *et al.*, 2016). It is usually used in different therapeutic purposes, the fruit of *Illicium verum* is used to cure rheumatic pain, stomach aches, skin inflammation, insomnia and vomiting (Sung *et al.*, 2012). The crude extract of *Illicium verum* and its compound show a broad variety of pharmacological activities like sedative anti-oxidant, insecticidal, anti-microbial, analgesic and anti-fungal activity (Wang *et al.*, 2011). *Illicium verum* also act as an anticancer agent (Kausar *et al.*, 2017).

Therefore, the aim of this study was to decrease the side effects of iron supplementation by replacing it with *Illicium verum* which is available in every kitchen, used to add aroma in food. The main objective of this study is to assess the comparative effect of ferrous sulfate with *Illicium verum* as an anti-anaemic drug on liver in animal model.

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## MATERIALS AND METHODS

### Quality of animals

Previously reported methodology was adopted for the study (Moldawer *et al.*, 1989) (Crowe *et al.*, 1995).

Briefly, Male albino Wister rats of weight between 180 to 200gm purchased from animal house of Aga Khan University (AKU), were used during the experiment. The ethical approval was obtained by ethical committee of FUUAST. These rats were individually kept in special cage. The floor covered with saw dust in a silent room, and allowed them to access for normal rat's food and water for at least 3 to 4 days before initiating experiment. So, the rats habituate in new environment. Total 24 rats were used, divided into 4 groups, each group contained six rats. Group 1 called control treated with saline. Group 2 called model, group 3 and 4 were used as test 1 and 2 respectively. Model and both test groups were made anaemic by pricking on their tail and drawn 1-2 drops of blood daily for one week. After making them anaemic model remained untreated till the end of the experiment. While, test 1 group of rats was treated with the marketed available anti-anaemic drug called ferrous sulphate for 6 weeks and test 2 treated with naturally occurring anti-anaemic *Illicium verum* extract.

### Mean weight of animals before treatment

Control group (C) =180gm  
Model group (M) = 195gm  
Drug treated group (T1) =190gm  
*Illicium verum* extract treated group (T2) =190gm

### Mean weight of animals after making anaemic

Control group (C) =183gm  
Model group (M) = 141gm  
Drug treated group (T1) =144gm  
*Illicium verum* extract treated group (T2) =152gm

### Preparation of saline

For the preparation of 0.9% solution of NaCl, 4.5gm NaCl was taken and dissolved in 500ml water then freeze it to obtain cool saline.

### Preparation of marketed available drug

Ferrous Sulfate 200mg tablet was purchased from General Medical Store and crushed into powdered form and was given to T1 rats as 30mg/kg b.w, after dissolving in 0.9% solution of NaCl for six weeks (Waris *et al.*, 2017).

### Preparation of plant fruit extract

*Illicium verum* purchased from store was crushed in biochemistry lab of FUUAST and extract was prepared in ethanol for two weeks (Kausar *et al.*, 2017). Ethanol was filtered through Whatmann No 42 (125mm) filter paper twice and remaining ethanol was evaporated with the help of hot plate magnetic stirrer and precipitates were liquefied in saline, given to T2 rats in dose of 80mg/ kg b.w for six weeks.

### Decapitation of rats and blood collection

Rats were decapitated after performing all activities. For this study the blood was collected from neck wound in the gel containing tubes for LFT.

### Mean weight of animals before decapitation

Control group (C) =184gm  
Model group (M) = 146gm  
Drug treated group (T1) =151gm  
*Illicium verum* extract treated group (T2) =177gm

### Biochemical test

All biochemical tests were performed at Liaquat National Hospital, Karachi, Pakistan. These tests include direct bilirubin (DB), total bilirubin (TB), gamma glutamyl transpeptidase (GGT), serum glutamic pyruvic transaminase (SGPT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). All materials and quality controls were provided by Roche, Pakistan. The principle of this system is photometry.

## STATISTICAL ANALYSIS

Statistical analysis was done using statistical package for social science (SPSS Version. 20). Results presented in terms of mean  $\pm$  SD (n=6). Significant dissimilarity calculated by Tukey HSD and Duncan test P value ( $p < 0.05$ ) following one-way ANOVA, considered significant. Significant difference was determined in T1 and T2 as compare to M and C group.

## RESULTS

Results of behavioural activities shown in fig. 1(a) to 1(h) remained same to 1<sup>st</sup> phase of this study (Baqar *et al.*, 2018). fig. 2 (a) Effects of *Illicium verum* extract treatment on total bilirubin (TB) showed effects on control, model and test groups of rats. Statistically analyzed by one-way ANOVA (df6, 23) (F=9.3111) ( $*p < 0.05$ ) disclose that after 6 weeks of treatment T.B was markedly reduced in test 1 group of rats assessed with control and model, while increased in test 2 group of rats.

Fig. 2 (b). Effects of *Illicium verum* extract treatment on direct bilirubin (DB) statistically analyzed by one-way ANOVA (df6, 23) (F=8.1872) ( $*p < 0.05$ ) indicates that following 6 weeks of treatment D.B was markedly fallen in rats of test 2 group as compared to control, model and test 1 group of rats. (c) Statistically analyzed by one-way ANOVA (df6,23) (F=11.9254) ( $*p < 0.05$ ) illustrated that value of SGPT was also decreased in test 2 group of rats but increases in test 1 group of rats.

Fig. 2 (d) ALP statically analyzed by one-way ANOVA (df6,23) (F=56.342) ( $*p < 0.05$ ) illustrated that ALP significantly decreases as compare to model, but slightly

**Table 1:** Mean  $\pm$  S.D (Standard Deviation) of Liver function test compared between control (C), Model treated (M) Ferrous sulfate treated (T1), *Illicium Verum* extract treated (T2)

Liver function test	Mean $\pm$ S.D of Control (C)	Mean $\pm$ S.D of Model treated (M)	Mean $\pm$ S.D of Ferrous sulfate treated (T1)	Mean $\pm$ S.D of <i>Illicium Verum</i> extract treated (T2)	f- value
Total Bilirubin (T.B) in mg/dl	0.86 $\pm$ 2.14	0.3 $\pm$ 3.14	*0.56 $\pm$ 1.18	0.81 $\pm$ 4.87	9.3111
Direct Bilirubin (D.B) in mg/dl	0.225 $\pm$ 3.03	0.11 $\pm$ 2.78	0.21 $\pm$ 1.12	*0.086 $\pm$ 2.91	8.1872
Serum glutamic pyruvic transaminase (SGPT) in U/L	308.5 $\pm$ 5.13	187 $\pm$ 4.52	267 $\pm$ 6.15	*142 $\pm$ 6.56	11.9254
Alkaline phosphatase (ALP) in U/L	2 $\pm$ 17.36	1.78 $\pm$ 10.23	2.6 $\pm$ 18.11	1.66 $\pm$ 11.44	56.342
Gamma glutamyl transpeptidase (GGT) in U/L	0.225 $\pm$ 9.26	1.2 $\pm$ 3.65	0.56 $\pm$ 8.76	*0.09 $\pm$ 6.75	3.98120

\* show result is significant

decreases as compared to control in test 2 group of rats while increases in test 1 group of rats. (e) GGT statically analyzed by one-way ANOVA (df6,23) (F=3.98120) (\*P<0.05) illustrated that GGT decreases in both test groups as compare to model group of rats but in test 1 increases as associated with control group of rats.

Fig. 1 comparison of behavioural study results in Control (C), Model (M), Test 1 (T1) and Test 2 (T2). Values are mean  $\pm$  SD (n=6) significant difference by student t-test p<0.05 level between T1 and T2 as compared to M and C. Behavioural studies including: (a) Forced swimming activity (time of locomotor activity) (b) Open field activity (no. of square crossing) (c) Light and dark activity (time spend in the light box) (d) Light and dark activity (no. of entries in the light box) (e) Water maze test (time for habituation) (f) Water maze test (for short term memory) (g) Water maze test (for long term memory) (h) Home cage activity (no. of cage crossing).

Fig. 2 comparison of biochemical results in Control (C), Model (M), Test 1 (T1) and Test 2 (T2). Results were represented as mean,  $\pm$  SD (n= 6) significant difference by Tukey HSD and Duncan test p<0.05 level from C, M, T1 and T2 following one-way ANOVA. (a) Total bilirubin (mg/ dl) (b) Direct bilirubin (mg/dl) (c) Serum glutamic pyruvic transaminase (U/L) (d) Alkaline phosphatase (U/L) (e) Gamma glutamyl transpeptidase (U/L).

## DISCUSSION

In this comparative study of ferrous sulfate and *Illicium verum*, TB was significantly decreased in T1 (ferrous sulfate) treated group of rats as compared to T2 (*Illicium verum*) treated group of rats. Meanwhile, DB, SGPT, ALP and GGT were significantly decreased in T2 group of rats. As, in our previous study, it was concluded that *Illicium verum* extract similar to common medicine Ferrous sulfate produces same effect to treat anaemia in animal

model (Baqa et al., 2018). Similarly, in our study, better results were observed on liver in T2 rats as compared to T1 group of rats showing the effectiveness of natural remedy.

In our study, weights of M, T1 and T2 rats were decreased due to iron-deficiency anaemia, but after treatment before decapitation weight of T2 group was increased as compared to T1 and M groups (Aktas et al., 2014). In previous study, *Illicium verum* was also used for treating other purposes such as liver cancer but no Glutathione S-transferases (GST) activity was improving and no change in Glutathione (GHS) was noticed (Yadav et al., 2007). To observe *Illicium verum* effect on liver function is the strength of our study.

As compared to natural remedy ferrous sulfate commonly causes injury on gastric mucosa (Nadir et al., 2007). In this study ferrous sulfate increases SGPT, ALP and DB similarly. These results are also consistent to another published study that shows the significant increase in serum concentration of bilirubin, LDH, ALT, ALP, AST and GGT (Abdel-Reheim et al., 2017). Other study reported that ferrous sulfate causes hepatotoxicity by high level of AST and ALT in serum (Pari et al., 2015). The membrane of liver was damaged and releases all enzymes present in intracellular content into blood circulation.

Our results are supported by a study that *Ocimum sanctum* leaves in alcoholic extract produce significant results for hepatoprotective activity (Chattopadhyay et al., 1992). Similarly, *Ventilago madraspatana Gaertn* with its active compound emodin reduces toxicity of liver (Bhadauria 2010). Curcumin inhibits GST in cytosol in the liver of rat (Oetari et al., 1996). The level of blood cells (erythrocyte, leukocyte) and haemoglobin concentration in rat's blood increases by aqueous leaf extract of pumpkin in previously reported study similar to *Illicium verum* seed extract (Toma 2015).

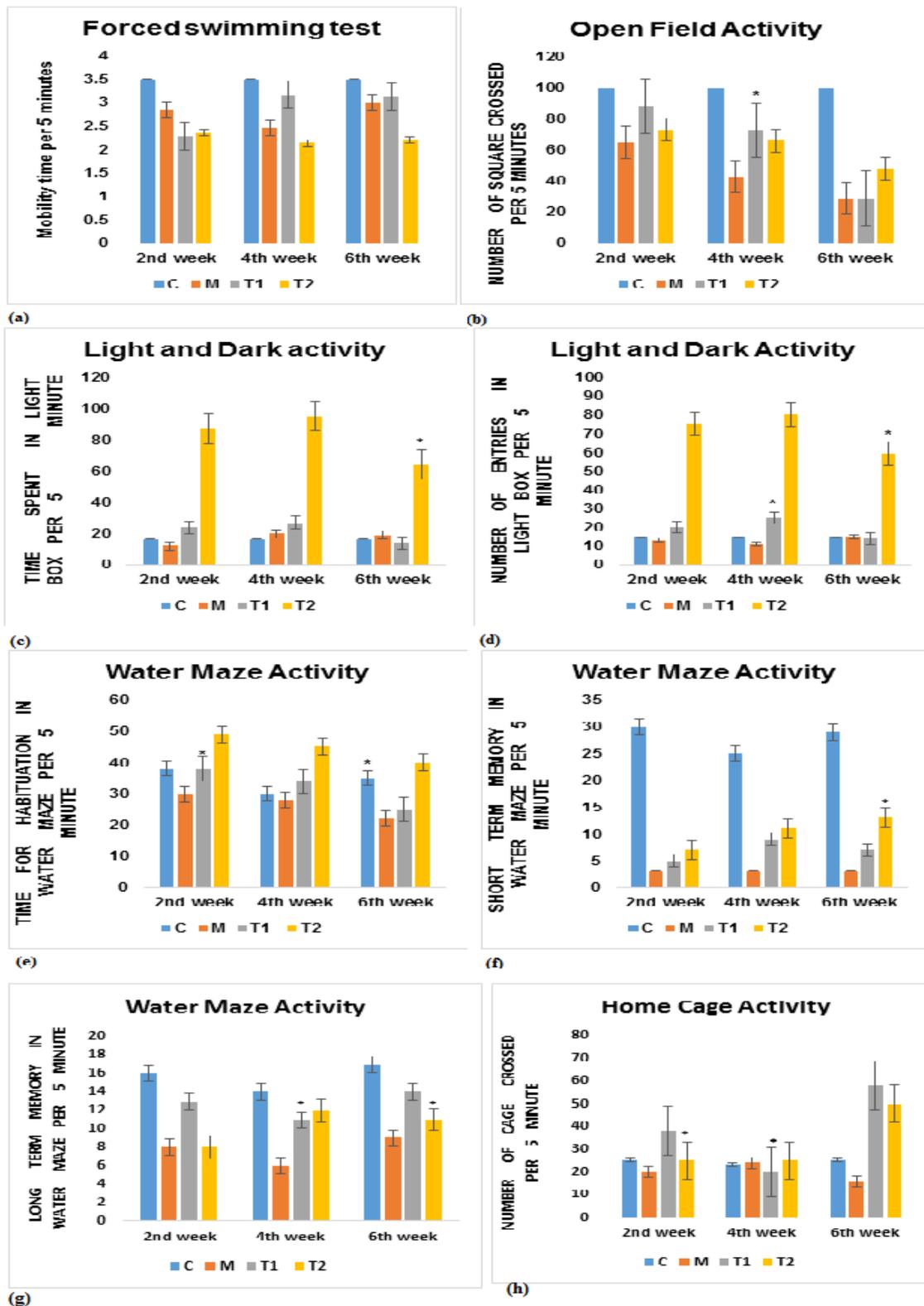
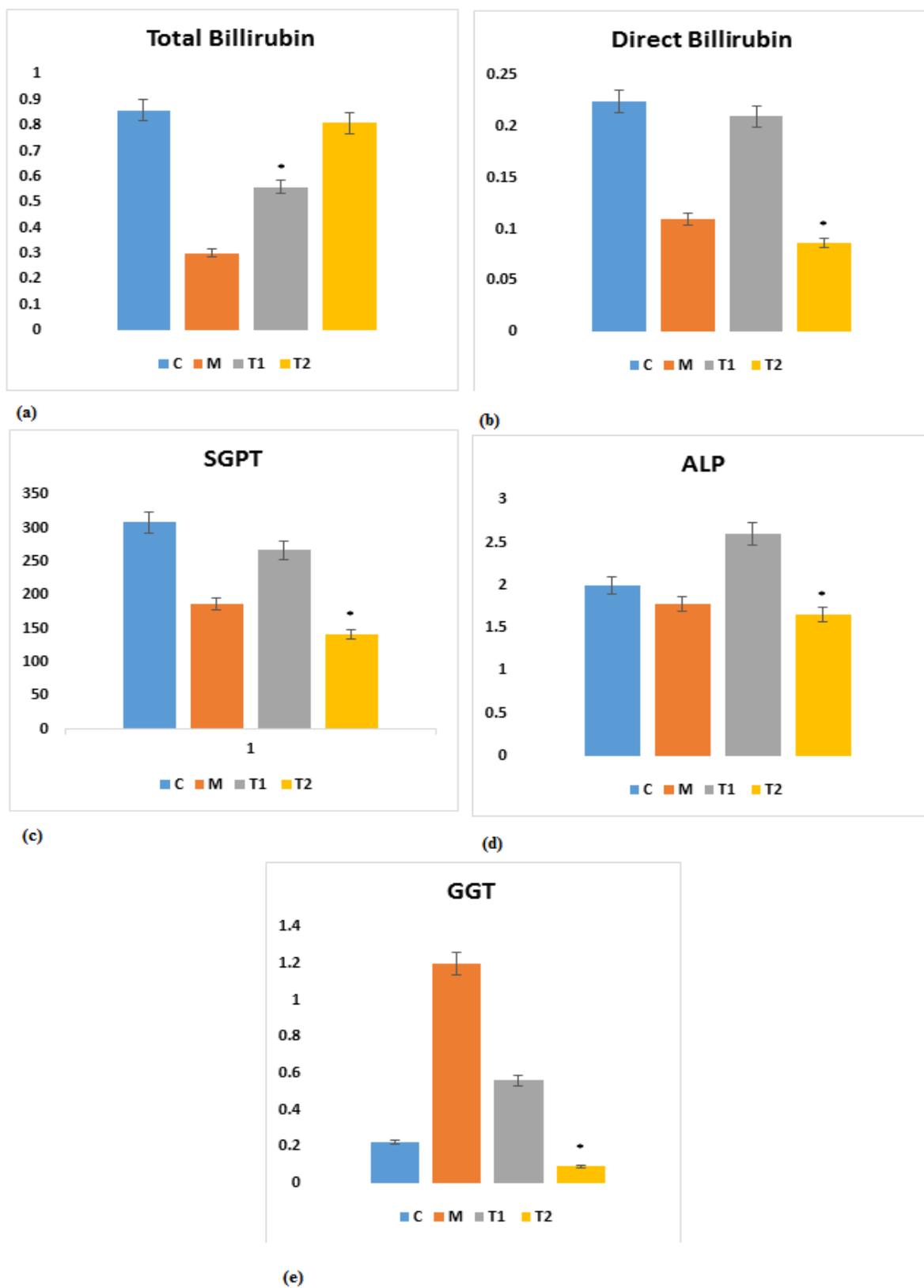


Fig. 1: Comparison of Behavioural results in Control (C), Model (M), Test 1 (T1) and Test 2 (T2).



**Fig. 2:** Comparison of Biochemical results in Control (C), Model (M), Test 1 (T1) and Test 2 (T2).

## CONCLUSION

On comparative assessment of common market available drug ferrous sulfate and natural compound, *illicium verum* it was concluded that naturally occurring *Illicium verum* (star anise) produces same effects and can be used to treat iron-deficiency anaemia with decreased effects on liver function. However, further large prospective study is still required for this purpose.

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