Acute Toxicity Study of Iron-21 Syrup in Female Wistar Rats

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ABSTRACT

The iron-21 syrup is used for iron deficiency anaemia which supplies iron and calories a provide iron and calorie nutriment to recompense haemoglobin deprivation. The objective of this study is to determine acute oral toxicity of Iron-21 syrup in vivo in Wistar rats. Iron-21 Syrup formulation was given through the oral route. The syrup formulation was administered in three increasing doses of 3, 6 and 12 ml/kg body weight for concentration of 500, 1000 and 2000mg/kg respectively. The other group of rats designated as a control group was given the only vehicle orally. The test and control group contains five rats each. Tests, as well as control group rats, were sacrificed on the fifteenth day of treatment. The blood and tissue samples of test animals were sent for histopathological studies examination. Four parameters were observed throughout the study, and they are cage side observation, the effect to the body weight, haematological parameter and histopathology. All animals were survived till they sacrificed. No notable changes were found in behaviour, haematological and histopathology studies. The oral administration of Iron-21 Syrup is not shown any toxic effect in the animal at a given dose. Therefore, it is a safe remedy for human use.

INTRODUCTION

Herbal medicines prepared from medicinal plants used for prevention as well as treatment of various diseases. A third of the world’s population is suffering from anaemia. 50% of cases of anaemia are due to deficiency of iron (Lopez et al., 2016). In India and other developing countries, this is the most common malnourishment disease (Trivedi and Mishra, 2009). Iron deficiency is highly widespread malnutrition, affecting women and children. High iron demands during pregnancy and in infant growth, leads to severe malnutrition in young children, pregnant and postpartum women (Stoltzfus et al., 2004). This iron malnutrition causes certain chronic diseases such as kidney disease, heart failure, cancer, and inflammatory bowel disease. Anaemia is a result of iron and caloric deficiency due to excessive bleeding, malnutrition, hypermenorrhea, haemorrhage, Hookworm infestation, Hematemesis, liver
toxicity etc. Due to the lowering of haemoglobin in the blood, oxygen supply to various body organs becomes impaired, resulting in person becomes weak, suffers from body ache, muscular pain, giddiness, lethargy. A person is unable to perform normal physical and mental functions (IRON – 21 Syrup, 2016). Countless iron-containing idiopathic formulations are available in the market to use in iron deficiency anaemia (Sharma et al., 2007). The long term treatment with iron salts is associated with various side effects (Baliga et al., 2018). Herbal medicines are observed as less harmful to the human being (Alam et al., 2011). The crucial problem in the use of herbal drugs is the deficiency of standardization (Angell and Kassirer, 1998). However, Ayurvedic formulations may be regarded as safe, but at higher doses and long term use, they may show adverse effects. Some of the herbal formulations cause harmful effects at high doses and long term use of herbal formulation may lead to potential adverse effect (Matthews et al., 1999). Herbal formulations need to be tested for its safe use and to maximize its therapeutic activity (Kroes and Walker, 2004).

Iron 21 Syrup is a vitalizing tonic, containing appetizers, digestives, haematinics. It ensures better iron absorption and formation of blood. It is a combination of restorative herbal drugs which supplies Iron and Calorie nutrition to make up the loss of haemoglobin, and it mitigates all associated symptoms of anaemia (IRON – 21 Syrup, 2016). The drugs included in this formulation obtained from plant origin.

Therefore, toxicity study (oral route) of this syrup formulation was carried out in the experimental protocol to find out the safety of this polyherbal formulation. In this investigation, the acute toxicity of Iron-21 Syrup, a multiple herb formulation is tested on female Wistar rats. The composition of Iron-21 Syrup is given in Table 1.

MATERIALS AND METHODS

Drug

The supplier provided the test container containing the medicinal herb formulation, which is identified as Iron-21 Syrup. The herbal remedy Iron-21 Syrup, manufactured by Unijules Life Sciences ltd. Survey No. 338 (P-38), MIDC, Dist. Nagpur, Kalmeshwar-441501, (M.S.), India. The test substance was stored according to manufacturer storage condition at ambient temperature and out of the light. The analytical grade’s reagents were used to perform the study.

Test animals

Adult female Wistar albino rats weighing range of 130-170 gm. They procured from the Central preclinical research facility, DMIMS (Deemed to be University), Sawangi (Meghe), Wardha, M.S., India. Nulliparous non-pregnant female rats were selected for acute toxicity study. The animals were kept as five rats in each cage of dimension 37cm*23cm*16cm polypropylene cages. The experimental room was kept at a constant temperature. During the study, 12 hr light and 12 hr dark cycle was maintained. Food and water were provided ad libitum. Prior to the experiment, all rats were accustomed to the laboratory conditions (Kamal et al., 2012; Kumarnsit et al., 2006). The experiment was executed according to the ethical norms of institutional animal ethics committee registration number 571/02/a/CPCSEA, the Central preclinical research facility, Datta Meghe Institute of Medical Science (DU), Sawangi (M), Wardha M.S., India.

Acute toxicity study

As per OECD guidelines 420, female Wistar albino rats were selected for the acute toxicity test. Animals weighing the range of 130-170 g were used and maintained under standard laboratory conditions (OECD/OCDE, 2001). Healthy and matured 20 female rats were used for study and distributed into four groups. Each group contains five rats. Animals were kept for fasting overnight before drug administration by oral gavage. Food provided after 3 to 4 h of drug sample administration. All animals were observed individually one’s for the first 30 min after dosing and thereafter every 1 h for the next 4 h. The observation was daily for the next 14 d for every 24 h. Monitored for change in eyes, skin, fur and the mucus membrane, the rate of respiration, autonomic reactions like salivation, lacrimation, perspiration, piloerection, enuresis and defecation, a central nervous system effects like drowsiness, convulsions and seizures were reported (OECD/OCDE, 2001; den Heuvel, 1984).

Experimental design

Preparation of test substance

As the test drug was in the Syrup form, it was easy to administer by gavage tube.

Dosage of the test substance

Single-dose toxicity study was conducted as claimed by OECD recommendation no 420 (OECD/OCDE, 2001). Four groups of female Wistar rats were used for acute toxicity study. The animals fasted overnight prior to the test substance administration. All animals were weighed one day before the administration of medication. The volume per 100gm of
Table 1: Composition of Iron-21 Syrup

| Sr. No | Common Name | Biological Name          | Contain |
|--------|-------------|--------------------------|--------|
| 1      | Ajwain      | Trachyspermum ammi       | 20mg   |
| 2      | Vaividang   | Embelia ribes            | 20mg   |
| 3      | Nagarmotha  | Cyperus scariosus        | 20mg   |
| 4      | Chitrakmool | Plumbago zeylanica       | 20mg   |
| 5      | Shankhpushpi| Convolvulus pluricaulis  | 20mg   |
| 6      | Sonth       | Zingiber officinale      | 25mg   |
| 7      | Kalimirch   | Piper nigrum             | 25mg   |
| 8      | Pippali     | Piper longum             | 25mg   |
| 9      | Mulethi     | Glycyrrhiza glabra       | 25mg   |
| 10     | Hirda       | Terminalia chebula       | 50mg   |
| 11     | Baheda      | Terminalia belerica      | 50mg   |
| 12     | Amla        | Emblica officinalis      | 50mg   |
| 13     | Brahmi      | Bacopa monnieri          | 50mg   |
| 14     | Gokhru      | Tribulus terrestris      | 50mg   |
| 15     | Bala        | Sida cordifolia          | 50mg   |
| 16     | Bhangra     | Ecliptica alba           | 50mg   |
| 17     | Arjun       | Terminalia arjuna        | 50mg   |
| 18     | Shuddha hirakasis Bhasma | — | 50mg |
| 19     | Ashwagandha | Withania somnifera       | 100mg  |
| 20     | Shatavari   | Asparagus racemosus      | 100mg  |
| 21     | Manuka      | Vitis vinifera           | 100mg  |
| 22     | Syrup base  | —                        | q.s.   |

Each 5ml syrup contain

Table 2: Change in body weight at day one, eight and fifteen at a dose of 2000 mg/kg and control group

| Dose mg/Kg | Animal No. | Sex | Experiment day | Weight in Gram (g) | Mortality |
|------------|------------|-----|----------------|--------------------|----------|
|            |            |     | 1st | 8th | 15th | 15th-1st |            |
| 2000       | 1          | F   | 161 | 163 | 166  | 04       | NIL       |
|            | 2          | F   | 164 | 166 | 168  | 04       | NIL       |
|            | 3          | F   | 158 | 160 | 164  | 06       | NIL       |
|            | 4          | F   | 163 | 168 | 169  | 06       | NIL       |
|            | 5          | F   | 160 | 164 | 167  | 07       | NIL       |
| Mean       | -          |     | 161.2|164.2|166.8|5.4|NIL|
| SD         | -          |     | ±2.39|±3.03|±1.92|±1.34|NIL|
| Control    | 1          | F   | 158 | 160 | 162  | 04       | NIL       |
|            | 2          | F   | 156 | 157 | 160  | 04       | NIL       |
|            | 3          | F   | 159 | 163 | 165  | 06       | NIL       |
|            | 4          | F   | 161 | 162 | 165  | 04       | NIL       |
|            | 5          | F   | 158 | 161 | 164  | 06       | NIL       |
| Mean       | -          |     | 158.4|160.6|163.2|4.8|NIL|
| SD         | -          |     | ±1.82|±2.30|±2.17|±1.10|NIL|

Data are expressed as the mean ± S.E.M. n=5 All values were not significantly different from control at p>0.05.
### Table 3: Acute toxicity study with dosing of 2000mg/kg/d on female rats for fourteen days

| Sr No | Parameter               | Animal No |
|-------|-------------------------|-----------|
| 1     | Lacrimation             | No        |
| 2     | Salivation              | No        |
| 3     | Piloerection            | No        |
| 4     | Drowsiness              | No        |
| 5     | Tremors                 | No        |
| 6     | Convulsions             | No        |
| 7     | Fur                     | Normal    |
| 8     | Food consumption        | Normal    |
| 9     | Water consumption       | Normal    |
| 10    | Mortality               | No        |
| 11    | Histopathology          | Normal    |

**Parameter**
- Sr No: Serial number
- Parameter: Parameter name
- Animal No: Presence status (Yes or No)

### Table 4: Effects of Haematological parameters

| Sr. No. | Haematological parameters | Control       | 2000mg/kg dose |
|---------|----------------------------|---------------|----------------|
| 1       | Hb (g/dl)                  | 12.60±0.32    | 13.18±0.36     |
| 2       | PCV (%)                    | 38.06±0.35    | 41.62±1.12     |
| 3       | RBC (x 1012/L)             | 5.50±0.38     | 6.08±0.57      |
| 4       | WBC (x 109/L)              | 6.78±0.34     | 8.02±0.56      |
| 5       | MCV (FL)                   | 58.60±3.27    | 65.46±2.57     |
| 6       | MCH (pg)                   | 19.35±0.69    | 21.25±0.66     |
| 7       | MCHC (%)                   | 35.02±0.38    | 37.87±0.39     |
| 8       | Platelets (x 109/L)        | 806.38±29.91  | 722.16±30.74   |
| 9       | Haematocrit (%)            | 60.80±1.22    | 56.79±0.34     |
| 10      | Neutrophils (%)            | 43.04±0.35    | 48.05±0.61     |
| 11      | Lymphocytes (%)            | 70.98±0.73    | 68.23±1.01     |
| 12      | Monocytes (%)              | 0.77±0.13     | 0.64±0.14      |
| 13      | Eosinophils (%)            | 1.19±0.16     | 1.49±0.13      |

Data are expressed as the mean ± S.E.M. n=5 All values were not significantly different from control at p>0.05.

### Table 5: Effects of Iron-21 Syrup formulation on relative organ weights in female Wistar rats

| Organ   | Control   | 2000mg/kg |
|---------|-----------|-----------|
| Heart   | 0.51 ± 0.06 | 0.42 ± 0.04<sub><sup>ns</sup></sub> |
| Liver   | 2.99 ± 0.13 | 3.36 ± 0.16<sub><sup>ns</sup></sub> |
| Kidney  | 0.58 ± 0.03 | 0.80 ± 0.04<sub><sup>ns</sup></sub> |
| Brain   | 1.5 ± 0.11  | 1.62 ± 0.06<sub><sup>ns</sup></sub> |
| Lungs   | 0.76 ± 0.03 | 0.80 ± 0.02<sub><sup>ns</sup></sub> |

Data are expressed as the mean ± S.E.M. n=5 All values were not significantly different from control at p>0.05.
body weight, defined to 2 ml/100 g, the volume to administer was calculated for each rat. The herbal formulation was administered orally in a calculated single dose, with the aid of gavage having a syringe attached to fit cannula. After dosing, animals fasted for 3 to 4 h. The individual rat was treated with a limit unit oral dose of 2000 mg/kg of syrup in sequence at 48 h intervals.

The acute toxicity of Iron-21 syrup was studied at different dose concentration. Group-I was administered Syrup vehicle base, group II, III and IV were administered test syrup at a dose of 500, 1000 and 2000 mg/kg on an individual basis. Animals were kept without food, but with water overnight before the study. The test substance was administered after completing 24 h of fasting. After the ingestion of test formulation, fasting retained for 2-4 h (Kamal et al., 2012).

Observation and body weight measurement

After dosing, each animal was observed and particular attention was given. Each animal was observed after drug administration and special attention was given in the course of the initial 4 h and thereafter every 12 h daily, for the period of 14 d. Each and every result were observed, noted and continue for an individual animal.

Cage side monitoring includes the condition of fur, skin, breathing abnormalities, autonomic consequences like salivation, diarrhoea, urination and central nervous system consequences including seizures and tremors, the different level of activity, walk and body pose, handling response or sensory stimuli and change in muscle power (Kumarnsit et al., 2006; Ali et al., 2012).

Each rat body weight was counted and noted and thereafter test formulation is given for 2 weeks. Change in body weight of animals were computed and noted (OECD/OCDE, 2001).

Bodyweight

The difference in body weight was noted on the first day before dosing as well as on the seventh day and fifteenth day after dosing that is weekly (Kamal et al., 2012; Kumarnsit et al., 2006; Ali et al., 2012).

Haematology

The animals were kept deprived of food 12 hr before necropsy and blood sample collection. The blood sample was collected by cardiac puncture in vials containing EDTA. Send collected sample for the haematological study. Haematological parameters were performed by standard clinical procedures using haematological analyzer (Roche Integra, 400 Plus, Diagnostic Systems).

Histopathology

During experimentation, all animals were observed every day for any mortality. If any dead animal was found, do histopathology of that animal and observed for any abnormal sign on measure organs like kidney, liver, heart. After the complete study, all the female rats were sacrificed using anaesthesia and histopathology was performed, and results were recorded (Dacie and Lewis, 1991; Patrick-Iwuanyanwu et al., 2012).

RESULTS

Acute toxicity study

No death and morbidity or any toxicity or any behavioural changes observed in 14 d experimental studies. The external appearance like nose, eyes, fur and skin were normal. During the observation, no seizures, shock, salivation, diarrhoea, inactivity or abnormal behaviour like self-inflicted violence, walk backwards and so forth were observed; walk and pose, grip strength, sensory stimuli all are normal.

Effect on body weight

The weight variation between test groups and control groups were recorded. There was a slight increase in the weight of day one and day fifteen was noted. The change in body weight was recorded and reported in Table 2.

Clinical sign

There was no abnormal clinical sign observed throughout the acute toxicity study reported in Table 3.

Effect on haematological parameter

After the compilation of acute toxicity study, the blood sample was collected in heparinized vials. This sample was provided for determination haemoglobin, total RBC count, WBC count, platelets, neutrophils, basophils, eosinophils and monocytes count. The results were shown that is no significant change in blood cells counts. Effect of Haematological parameters reported in Table 4.

Histopathology

During the histopathological and microscopical study, no abnormal changes were found in control as well as test animals under study. Iron-21 Syrup formulation did not affect the histology of vital organs, like spleen, liver, kidneys, lungs and heart. Histopathological data reported in Table 5.

DISCUSSION

Herbal formulations require a robust and extensive pharmacological evaluation and safety issues due to
the large and growing use of natural-derived substances all over the world, which cannot rely only on the tradition or supposed millenarian beliefs; explanatory and pragmatic studies are useful and complementary in the acquisition of reliable data about herbal drugs. (Firenzuoli and Gori, 2007)

As time immemorial, the concept of herbal drug formulation with greater therapeutic efficacy and minimum side effects as compared to one plant extract has been the concept of classical phytotherapy using herbal drug combinations with superior efficacy and lesser side effects than a single plant extract or constituent has been often clinically and pharmacologically (Gupta et al., 2008).

As discussed in the introduction, the herbal formulation Iron-21 syrup is widely used for the treatment of iron deficiency anaemia; as its acute toxicity is not yet reported. So, study has been performed to evaluate acute toxicity of Iron-21 syrup. In this experiment, acute toxicity of herbal formulation was tested in Wistar rats (female) as a single dose study. The study was carried out at an oral dose of 2000mg/kg/d for fourteen days. No mortality and morbidity were noticed until fourteen days of study. From this result, it is proved that the drug could be safely administered by the oral route for acute treatments up to 2000 mg/kg/d.

During the experimental period, no exceptional changes were noticed in the behaviour of test animals, body weight and organ weight at all dose level when compared with a controlled group. It can be concluded that the test substance has no toxicity at a given dose. The blood testing of experimental rats can be used as risk evaluator in the human being. As the changes in the haematological system have a foretelling value for human toxicity of the test compound. In this study, all haematological parameter which was observed shows the normal value for animal blood sample. So we can predict that this herbal formulation is safe for human also.

CONCLUSIONS

From the experimental study and results obtained, it can be concluded that Iron-21 syrup was non-toxic and relatively safe for human consumption. There is a scope for chronic toxicity study for the safety profile of Iron-21 Syrup.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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