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ORIGINAL ARTICLE

Comparative Effects of Nigella Sativa and Cassia Senna in Iron Overloaded Mice

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ABSTRACT

Background: Iron overload-related complications are the main concern in patients who must often receive blood transfusions. The only way to treat iron overload in these patients is by chelating agents. Owing to the high cost and adverse effects of chelating agents use of naturally present chelators is under study.
Aim: To compare the protective effects of two commonly used herbs Nigella Sativa and Cassia Senna in iron-overloaded mice.
Study Design: Experimental randomized controlled trial.
Setting: Zoology department of Govt College University Lahore during six months period.
Method: A total of forty-eight mice were divided into four groups (n = 12). Group one was normal to control while group 2, 3, and 4 were overloaded with iron by intravenous injection of iron dextran (0.1 ml/kg body weight) daily for fifteen days. After 15 days iron overload was confirmed by blood testing. For additional 15 days, group three mice were provided with Cassia Senna (100 mg/Kg body weight) while group four mice were permitted to feed on Nigella sativa (200 mg/Kg body weight). Animals were sacrificed on day 32 and organs were preserved to check the iron levels. Blood sampling was done on days 0, 15, and 31 to analyze serum iron levels.
Results: The protective effect of Nigella Sativa was more marked than cassia senna in iron 0verloaded mice.
Conclusion: The addition of Nigella Sativa instead of cassia senna in iron-laden patients as an adjunct therapy can be more beneficial in preventing the damaging effects of iron overload.
Keywords: iron overload, Nigella Sativa, Cassia Senna

INTRODUCTION

Iron plays an important role in several cellular processes, such as oxygen transport, respiration, lipid metabolism, replication, and gene regulation, and its levels in the human body are monitored only by absorption[1]. From a nutritional viewpoint, both excess and declined levels of serum iron have immense epidemiological importance and remarkable penalty[2]. Hemochromatosis, also known as iron overload, is a condition that is characterized by excessive iron accumulation in the functional parts of body organs, resulting in organ damage and failure. Human bodies store iron, mainly in the form of ferritin. Limited content of ferritin is secreted into bloodstreams and the blood ferritin concentration is checked physiologically. However, there is a lack of a mechanism to remove excess iron load from the human body caused by blood transfusion. Each transfused unit of packed red blood cells contains 200-250 mg of elemental iron. For example, transfusion iron normally amounts to 03 to 0.6 mg/kg per day for transfusion-dependent thalassemia patients, with two to four units/month. This excessive iron causes irreversible damage and leads to the distraction of major vital organ functioning[3,4]. Iron overload mainly affects the liver, heart, and kidneys by increasing the production of reactive oxygen species, and oxygen-free radicals[5,6]. For the elimination of surplus iron various chelating agents are in clinical use, which form the firm solvable multiplexes with iron, and are then removed from the body with urine or feces[7]. Chelating agents are natural molecules that acquire specialized ligands with high attractiveness for metals like iron. Deferoxamine is one of the commonly used chelators, it is costly and linked with various adverse effects. Despite its efficacy, these disadvantages are troublesome especially for the patients of developing countries[8].

The above-mentioned shortcomings of standard iron chelators are referring to natural chelators in managing iron overload, especially in developing countries[9]. Nigella sativa and Cassia Senna are amazing herbaceous plants with significant importance in the traditional medicinal system. These are fascinating sources of sennosides, glycosides, and other nutrients that can offer a solution to multiple health-related problems including constipation, diabetes, obesity, and free radicle-induced organ damage. They have anti-microbial, anti-fungal, cytotoxic, thrombolytic, and antioxidant properties[10,11]. These are polyphenol-enriched medicinal herbs that may also prevent or minimize the negative properties of iron overload due to their chelating and antioxidant properties[11,12].

The chelating properties of Nigella Sativa and Cassia Senna are studied individually earlier, but according to the best of our knowledge, the comparative effects of Nigella Sativa and Cassia Senna on iron overload-induced organ damage have still not been explored. So, we aimed to explore the comparative role of these herbs against iron overload-induced organ damage.

METHODOLOGY

After getting approval from the ethical review board of the university (letter-number GCU-11B-537), the experiment was started with 40 healthy male albino mice weighing 25+5 grams. Animals were attained and retained in the animal house of Government College University Lahore. Fresh water and a salable pelleted diet were provided on daily basis. A well-ventilated room with a constant temperature of 22+ 2°C, moisture 50 + 10%, and a 12-hour light/dark cycle was provided to these mice[13].

The seeds of Nigella sativa and Cassia Senna leaves were brought and licensed by Botany Department, Government College University, Lahore, through proper taxonomical rules. Seeds were crushed into powder form and the calculated form of the powder was mixed with mice feed. The leaves were rinsed, dried up, and powdered with the help of an electric blender[10]. Iron dextran injections were bought from the local market.

Mice were divided into Group 1 (normal control), Group 2 (disease control), and Groups 3 & 4 (experimental groups), each comprising ten mice. Throughout the experiment, Group-1 mice were fed on a normal diet with no intervention while Groups 2, 3, & 4 were fed on a normal diet and injected intravenously with 0.1 mL/g bodyweight iron dextran (25 mg/mL) daily for 15 days to achieve iron overload[7]. After the confirmation (by blood tests), group 2 remains untreated while group 3 on Cassia Senna (100 mg/Kg body weight) mixed feed, and group 4 mice were fed on Nigella sativa (200 mg/Kg body weight), for a further 15 days[7,10].

Blood samples were collected from all mice on days 0, 15, and 30 through the intracardiac technique under chloroform anesthesia. The blood was then centrifuged at 5,000 rpm, and serum was collected and stored at -8 ºC in Eppendorf tubes. Mice

were sacrificed on day 30; the heart, kidneys, and liver were taken and stored at -86 degrees centigrade for iron assessment. Serum Frozen organs were first treated in aqua regia for the approximation of iron levels. After adding the aqua regia (1 ml/sample), organs and serum were kept overnight at room temperature (to dissolve them in aqua regia). After that, the mixture was boiled for 2–3 minutes, and 1 ml of distilled water was added. The mixture was purified to remove any contaminations. To determine the amount of accumulated iron in serum and other tissues atomic absorption spectrophotometer was used. SPSS version 22 was used to evaluate the individual parameters in different groups. The student's t-test was employed and a p-value less than 0.05 was regarded as significant[7].

RESULTS

On day 15, a significant increase in the iron levels of serum, liver, heart, and kidney of Group 2, 3, & 4 mice was observed compared to the control group indicated by a p-value of less than 0.05 as revealed in table 1.

Table 1: Comparison of iron levels among the groups on day 15.

Groups	Serum iron Concentration (mg/dl)	Liver iron Concentration (mg/dl)	Kidney's iron Concentration (mg/dl)	Heart iron Concentration (mg/dl)
Normal control group (G 1)	0.003295 ±0.002145	0.00260 ±0.00078	0.00269 ±0.00069	0.00317 ±0.00113
Disease control group (G 2)	0.017601 ±0.001141	0.02287 ±0.00143	0.01896 ±0.00043	0.03010 ±0.00111
p-value	0.05	0.02	0.000	0.000
Group 3	0.017300 ±0.00111	0.02278 ±0.00132	0.01887 ±0.00043	0.03020 ±0.00111
p-value	0.04	0.02	0.01	0.000
Group 4	0.017599 ±0.001122	0.022861 ±0.00133	0.01879 ±0.00043	0.03110 ±0.00111
p-value	0.05	0.03	0.01	0.000

Although our findings on day 30 showed that both Nigella Sativa and Cassia Senna decreased these iron levels to significant levels (Figure-1).

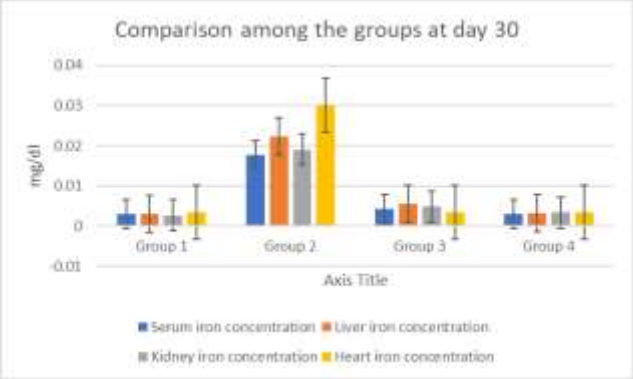

Figure 1: Comparison of mean iron concentrations in serum, liver, kidneys, and heart on day 30.

The comparison of Nigella Sativa and Cassia Senna's treated groups proved that Nigella Sativa (G 4) is more efficient in lowering iron concentrations (Figure 2).

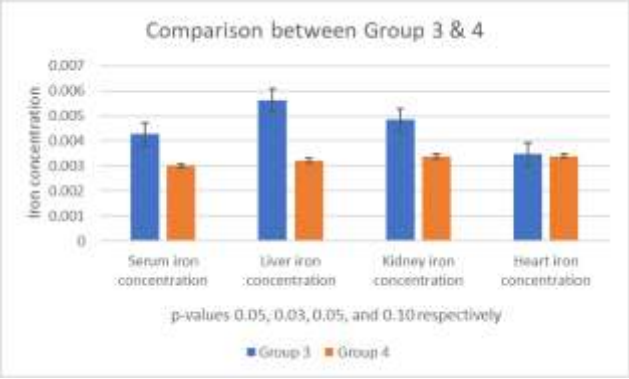

Figure 2: Comparison of mean iron concentrations among groups 3 & 4 at day 30.

DISCUSSION

This study aimed to compare the protective effects of the Nigella Sativa and Cassia Senna herbs on the excess iron present in serum, heart, liver, and kidneys because these vital organs are mainly influenced by iron overload[14]. In this experimental study, we artificially iron-overloaded the mice with the help of iron dextran injection and confirmed it after 15 days by comparing disease control and experimental groups with the control group (p-value less than 0.05). Iron dextran was also used by Shendge and his colleagues to overload iron in mice and to check the oxidative stress induced by this iron overload in 2021[13]. Das et al likewise used iron dextran injection to overload iron in mice and serum iron concentration was increased to double the amount of iron in the serum of normal mice[15]. We treated group 3 with cassia senna from day 16 to 30 and the iron levels declined to the control values in serum, kidney, liver, and heart (p-value less than 0.05). This herb was also used by Wang et al to reverse the iron-induced hepatic damage, he reversed CdCl2-induced hepatotoxicity by decreasing levels of ALT, and AST[16]. Some other herbs like Medicago sativa and Allium porrum were also used previously to reduce the iron concentration in serum, liver, and heart to significant levels[17]. In a recent study, the Nigella Sativa was also premeditated in iron overloaded mice, in that experiment iron concentration of serum, kidney, liver, and heart were decreased to a significant level with the use of this herb[7]. We also use this herb in our experimental group 4, Nigella Sativa treatment significantly decreased the iron level of the liver. Danladi et al also observed the effect of Nigella Sativa on the liver antioxidant enzyme activities which were decreased by CCl4 treatment[18]. The plant extracts of Tetracarpidium ionophore and T. conophorum also showed chelating effects in a previous study[19]. The favorable effects of a Chinese folk herb M. nigra were studied by Figueredo et al, to control the intermediaries of inflammation and to lessen iron overload in several tissues[20].

CONCLUSION

In the present study, both Nigella Sativa and Cassia Senna decrease the iron levels in serum, liver, kidney, and heart of iron-overloaded mice. But the effects of Nigella Sativa were more significant (p-value less than 0.05) as compared to Cassia Senna except in the heart. Further studies are recommended to reproduce these in patients receiving blood multiple blood transfusions.

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