Crataegus Oxyacantha (Hawthorn) Fruit – Histopathological Safety Profile in "Brain, Bone and Joint Tissue" of Dyslipidemic Rats

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Abstract

Objective: To evaluate the safety profile of ethanolic extract of Crataegus oxyacantha fruit in comparison with Atorvastatin om histological samples of brain, bone, and joint tissues.

Materials & Methods: A 2-month experimental study was conducted from July to September 2023 at Animal House of Akhtar Saeed Medical & Dental College on 48 male albino Wistar rats (aged 6 weeks) divided into 8 groups with 6 rats each, weighing 180-200 grams. Group 1 (Healthy Control) received a normal rat diet and 2 ml of Normal Saline for 2 months. Group 2 (Disease Control) was given a High-Fat Diet (HFD) and 2 ml of Normal Saline for 1 month, while 3-5 (Prophylactic Groups) received HFD along with Crataegus oxyacantha fruit's ethanolic extract, atorvastatin, and combination of them in doses of 40 mg/kg OD, 80 mg/kg OD and 20+40 mg/kg OD orally respectively for 1 month. Groups 6 to 8 (Therapeutic Groups) were given Crataegus oxyacantha fruit's ethanolic extract, atorvastatin, and in combination respectively after induction of dyslipidemia from the 30th to 60th day in the doses as mentioned above. Animals were sacrificed on the 30th day from Prophylactic Groups along with Group 2 and the remaining groups on the 60th day i.e., at the end of the study for the collection of brain, bone, and joint tissues. The histopathological results were analyzed by assigning percentages.

Results: Our study concludes that Crataegus oxyacantha which has hypolipidemic potential does not cause any organ damage in bone, joint, and brain tissue when compared to Atorvastatin.

Conclusion: Results show that Crataegus oxyacantha can be used as a safe alternative in treating hyperlipidemia in comparison with Atorvastatin.

Keywords: Histopathological evaluation of Bone, Joint and Brain Tissue, Crataegus oxyacantha, Atorvastatin

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Introduction

Dyslipidemia results in significant morbidity and mortality around the world. Genetic predisposition, sedentary lifestyle, or high caloric intake are the underlying causes. The absence of lipoprotein lipase activity or deficiency of apoprotein CII31 is the basis of underlying pathogenesis due to which defective lipid metabolism occurs.[1] The oxidative stress results in vascular lining injury and consequently enhanced atherosclerotic plaque formation. This results in stroke, angina, and other ischemic cardiovascular diseases such as myocardial infarction.[2] Metabolic diseases such as diabetes mellitus etc., are associated with dyslipidemia.[3] Dyslipidemia also causes a major hepatic pathology i.e., Non-Alcoholic Fatty Liver Disease (NAFLD).[4] Dyslipidemia should be prevented by dietary and life-

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style modifications first. The medical therapy includes anti-hyperlipidemics such as statins, bile acid binding resins, sterols, fibrates, and niacin. These drugs especially statins (HMG Co-A Reductase Inhibitors) are considered effective in treatment as well as the prevention of cardiovascular diseases in cases of dyslipidemia. Atorvastatin is given around the world due to its hypolipidemic effects. It acts as a competitive inhibitor of hydroxymethylglutaryl-coenzyme a reductase (rate-determining) enzyme in cholesterol synthesis through the mevalonate pathway. Atorvastatin also increases hepatic LDL receptor expression thus reducing LDL-C levels. It acts mainly in the liver and decreases hepatic cholesterol levels leading to increased hepatic cholesterol uptake and reduction of cholesterol levels in plasma.[5]

Prophylactic use of statins in cases of familial hyperlipidemia has already been established by researchers but adverse effects such as myalgia result in poor compliance thus dyslipidemia and its consequences remain untreated. Amongst all the known adverse effects, statin-induced myopathy (rhabdomyolysis) is the worst which has even led to the death of patients in the past[6]. Muscle pain can sometimes be confused with bone and joint pain. To confirm the underlying cause of pain, bone, and joint biopsies can be taken for histopathological assessment. Negative side effects of statins on bones are not confirmed yet, rather the opposite has been suggested i.e., an increase in bone mass density and reduced fracture risk.[7]

Neurodegenerative disorders like amyotrophic lateral sclerosis and psychiatric illnesses are associated with statins[8] and can be evaluated by brain tissue biopsy. Decreases in serum lipid levels have been suggested to affect the development of neuronal membranes, nerve synapses, and myelin sheath. This contributes to stunted serotonin activity that alters psychological behavior and impairs neurocognition.[9]

This is a serious issue that should not be ignored, different treatment strategies should be considered for dyslipidemia. Many phytochemicals alone or in combinations have been utilized in dyslipidemia treatment. Crataegus oxyacantha (Hawthorn), because to its antioxidant characteristics due to the presence of Flavonoids, is considered to have hypolipidemic potential.[10] Keeping in mind, this research was designed to assess to effects of Crataegus oxyacantha fruit on brain, bone and joint tissues in comparison to atorvastatin.

Materials & Methods

A 60-day study was conducted on 48 male albino Wistar rats (aged 6 weeks) divided into 8 groups with 6 rats each weighing 180-200 grams. Group 1 (Healthy Control) received a normal rat diet and 2 ml of Normal Saline for 2 months. Group 2 (Disease Control) was given a High-Fat Diet (HFD) and 2 ml of Normal Saline for 1 month, while 3-5 (Prophylactic Groups) received HFD along with Crataegus oxyacantha fruit's ethanolic extract, atorvastatin, and combination of them in doses of 40 mg/kg OD, 80 mg/kg OD and 20+40 mg/kg OD orally respectively for 1 month. Groups 6 to 8 (Therapeutic Groups) were given Crataegus oxyacantha fruit's ethanolic extract, atorvastatin, and in combination respectively after induction of dyslipidemia from the 30th to 60th day in the doses as mentioned above. Animals were sacrificed on the 30th day from Prophylactic Groups along with Group 2 and the remaining groups on the 60th day i.e., at the end of the study for the collection of brain, bone, and joint tissues. The samples were stored in formalin and then histopathological slides were made for their examination. The histopathological results were analyzed by assigning percentages. Percentages were assigned to histopathological findings using the Chi-Square test.

Results

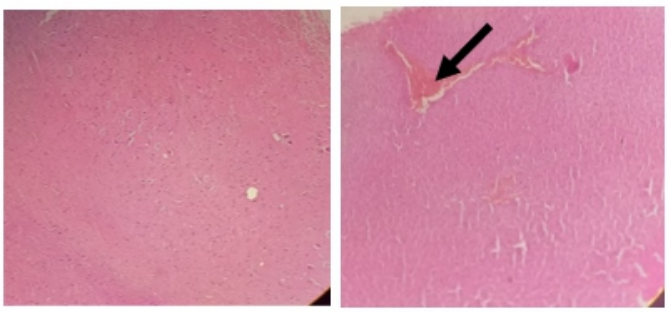
- Group 1 = Healthy Control (sacrificed at day 60 of study)
- Group 2 = Disease control (sacrificed at day 30 of study)
- Group 3= Crataegus oxyacantha 40 mg/kg/day, Group 4 = Atorvastatin 80 mg/kg/day
- Group 5 = Combination (Crataegus oxyacantha 20 mg/kg/day + Atorvastatin 40 mg/kg/day) along with high-fat diet (to evaluate prophylactic effects – sacrificed at day 30 of study)
- Group 6 = Crataegus oxyacantha 40 mg/kg/day, Group 7 = Atorvastatin 80 mg/kg/day
- Group 8 = Combination (Crataegus oxyacantha 20 mg/kg/day + Atorvastatin 40 mg/kg/day) after inducing hyperlipidemia (to evaluate therapeutic effects – sacrificed at day 60 of study).

Table 1: *Brain - Hemorrhage*

Brain Hemorrhage	Group 1 (n=6) n (%)	Group 2 (n=6) n (%)	Group 3 (n=6) n (%)	Group 4 (n=6) n (%)	Group 5 (n=6) n (%)	Group 6 (n=6) n (%)	Group 7 (n=6) n (%)	Group 8 (n=6) n (%)
Mild	-	-	-	-	-	-	-	-
Moderate	-	-	-	-	-	-	-	-
Severe	-	-	-	1 (12.5%)	-	-	2 (25.0%)	-

Table 2: *Bones & Joints – Hypocellularity*

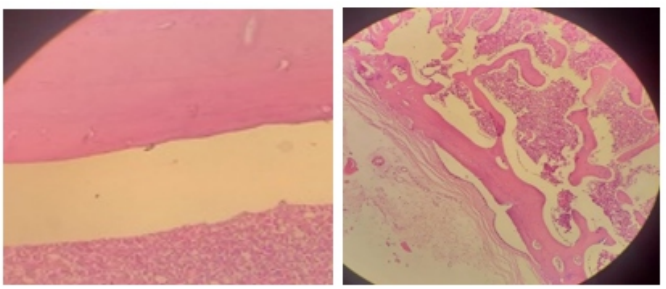
Hypocellularity in Bones & Joints	Group 1 (n=6) n (%)	Group 2 (n=6) n (%)	Group 3 (n=6) n (%)	Group 4 (n=6) n (%)	Group 5 (n=6) n (%)	Group 6 (n=6) n (%)	Group 7 (n=6) n (%)	Group 8 (n=6) n (%)
Mild	-	-	-	-	-	-	-	-
Moderate	-	-	-	-	6 (100.0%	-	-	6(100.0%)
Severe	-	-	-	6(100.0%)	-	-	6(100.0%)	



(a) **(b)**

Figure: 1 (a): *Photomicrograph (10×10; H&E) showing a longitudinal section of normal brain in G-1, G-2, G-3, and G-6 showing glial cells, neurons, and vessels.*

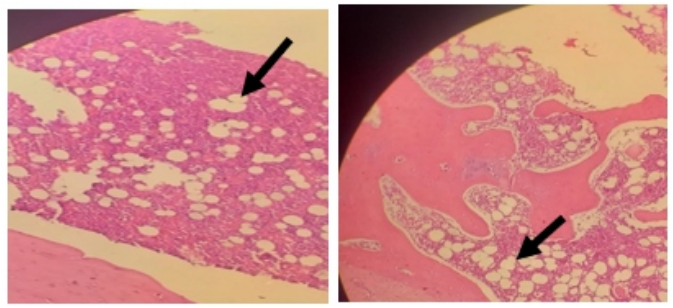
Figure: 1 (b): *Photomicrograph (10 x 10; H&E) showing a longitudinal section of the brain in G-4, G-5, G-6, and G-7 showing brain hemorrhage (black arrow).*



(a) **(b)**

Figure: 2 *(a) – Photomicrograph (10 x 10; H&E) showing longitudinal section of normal bone in G-1, G-2, G-3, and G-6 showing osteocytes, osteoclasts, compact bone, and marrow.*

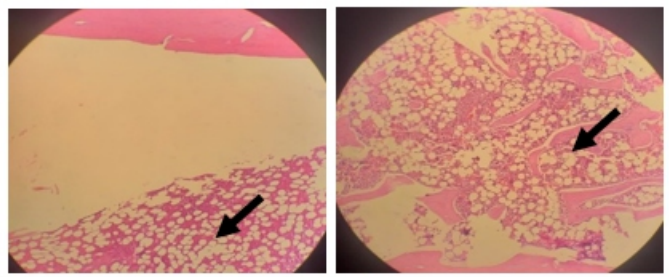
Figure: 2 *(b) – Photomicrograph (10 x 10; H&E) showing a longitudinal section of the normal joint in G-1, G-3, and G-6 trabecula and periosteum along with osteocytes, osteoclasts, compact bone, and marrow.*



(a) **(b)**

Figure: 3 *(a) – Photomicrograph (10 x 10; H&E) showing longitudinal section of bone in G-5 and G-8 showing moderate hypocellularity (black arrow).*

Figure: 3 *(b) – Photomicrograph (10 x 10; H&E) showing a longitudinal section of the joint in G-5 and G-8 showing moderate hypocellularity (black arrow).*



(a) **(b)**

Figure: 4 *(a) – Photomicrograph (10 x 10; H&E) showing longitudinal section of bone in G-4 and G-7*

showing severe hypocellularity (black arrow).

Figure: 4 *(b) – Photomicrograph (10 x 10; H&E) showing longitudinal section of joint in G-4 and G-7 showing severe hypocellularity (black arrow).*

Discussion

Atorvastatin, like other statins, can cause serious musculoskeletal and hepatorenal adverse effects. Therefore, an alternative such as Crataegus oxyacantha (Hawthorn), which has hypolipidemic potential, was tested on rats at the animal house of ASMDC in Lahore.

To differentiate the muscle pain from that of bone and joint, biopsies were taken for histopathological assessment to confirm the underlying cause of pain. Histological examination of bones and joints of rats in our study may indicate a toxic potential of Atorvastatin in G-5 & 8 showing moderate hypocellularity while in G-4 & 7, severe hypocellularity in bones as well as joint tissues. Healthy and disease control groups (G-1 and G-2), as well as Crataegus oxyacantha treated groups (G-3 and G-6), showed no changes in bones and joints as the longitudinal section of the bone showed normal osteocytes, osteoclasts, compact bone, and marrow while joint showed normal trabecula indicating its bone and joint safety potential.

Some studies conducted support our results while others contradict them. Animal studies related to the protective role of Crataegus oxyacantha on bones supported our findings.[11] Regarding the effects of Atorvastatin, in some patients, the development of arthralgias and tendinopathies have been reported with their use while other studies have suggested improvement in bone metabolism i.e., increase in bone mass density and reduced fracture risk.[12] During a study conducted on the effects of statins on bone metabolism and treatment of bone catabolic diseases, it was observed that statins, when administered in oral therapeutic doses, had minimal efficacy in treating osteoporosis. However, they were found to be safe and effective in treating bone or its inflammation, especially in the case of osseous deficiencies. When applied locally, statins also showed promise in treating periodontitis by targeting accessible bony defects.[13] Researchers developed an animal model to promote the healing of critical bone size defects using local statin application which showed an apparent osteogenic and angiogenic effect.[14] Another research in mice has shown that atorvastatin can increase bone mass and promote osteogenesis, thus boosting bone formation.[15]

In our study, histopathology of brain tissue only showed hemorrhage in 12.5% rats of G-4 and 25% rats of G-7 which were given 80 mg/kg/day of Atorvastatin alone. While the longitudinal section of the brain in G-1, G-2, G-3 G-5, G-6, and G-8 showed normal glial cells, neurons, and vessels. So, in our study Crataegus oxyacantha alone as well as in combination with Atorvastatin was able to prevent brain pathology. This protective potential of Crataegus oxyacantha on the brain is also supported by other studies.[16,17]

Other studies also support our data related to adverse effects of Atorvastatin on brain tissue as meta-analysis confirmed an increase in the risk of hemorrhagic stroke, especially with the use of atorvastatin.[18] Some studies showed contradicting results to this study. The researchers investigated the impact of statins on memory, cognition, and brain volume in the elderly. Their research indicated that there was no difference in the rate of decline in memory or overall cognition between those who used statins and those who did not.[19] One study showed the effectiveness of statin treatment in the prevention or improvement of symptoms of several brain disorders[23]. There are contrasting results shown by different studies about the effects of Atorvastatin on the above-mentioned tissues but Crataegus oxyacantha has shown no adverse effects rather protective role of Crataegus oxyacantha can be assumed.[20]

Therefore, in totality, our multiorgan safety profile suggests that one month-prophylactic or therapeutic treatment with Crataegus oxyacantha alone in doses of 40 mg/kg OD didn't cause any histopathological damage in skeletal muscle, cardiac, hepato-renal, brain, and bone tissue. In comparison, Atorvastatin alone (80 mg/kg) caused brain hemorrhage, and marked hypocellularity in bone and joint tissue. However, its low dose combination with Crataegus oxyacantha (40 mg/kg +20 mg/kg respectively) prevented severe damage causing only moderate changes, which were possibly due to either low dose administration of Atorvastatin, protective effects of Crataegus oxyacantha, or both.

Conclusion

Our research concludes that Crataegus oxyacantha, which has a known hypolipidemic potential, does not cause any histopathological changes. No organ damage was observed in bone, joint and brain tissues when compared to atorvastatin. Atorvastatin caused significant histopathological changes such as marked hypocellularity in bone and joint tissues and brain hemorrhage.

Therefore, we suggest that Crataegus oxyacantha is a safer alternative for treating dyslipidemia, without posing any significant threat to bone, joint or brain tissues.

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Authors Contribution

SMNZ: Conceptualization of Project
SABB: Data Collection
SAFB: Literature Search
SZ: Statistical Analysis
NY: Drafting, Revision
MIP: Writing of Manuscript