Antioxidant Impact of Green Synthetized Magnetic Nanoparticle of Nigella Sativa Seed Alcoholic Extract in Male Rats

Zahraa Mohammed Habeeb[1*], Salim Hussein Jari[2]

Abstract

That experience was conducted for the purpose of known the impact antioxidant nanoparticles of *Nigella sativa* agonist oxidative stress of the Nanoparticles the iron in rats. The experience was conducted in veterinary medicine Al-Qadisiya university at the animal house. This study was carried out during a period from 20.11.2020 to 20.04.2021 (64) sixty four mature rat 90 days age. Wister rat average body weight (100-120) gram will be used in the preset study the rat will be kept under controlled hygienic condition with free access to food and water for two week before starting the experiment. The animal will be randomly assigned into 4equal groups 16 each and treated as follow. The following objective will be studied. Gene expression examination in 14 and 28 days. added Evaluation of liver alanine aminotransferase ALT and the alkaline phosphatase ALP, AST aspartate amino transferase gene expression level, And GSS (glutathione) and Sod 1(superoxide dismutase) gene expression. When gave mix groups (Nigella sativa and Iron) to male rats significant decrease fold change gene expression level compared with control groups. While gave *Nigella sativa* alone to male rats significant decease fold change (gene expression level) compared with control groups. Iron alone gave to male rats increase significant e fold change gene expression level compared with control group. Gene expression (fold change) (glutathione) and Sod 1(superoxide dismutase) gene expression. Examination in 14 and 28 days gave significant increase in mix group and *Nigella sativa*, significant decrease in Iron group.

Key Words: Gss, sod1, AL, AST, ALT.

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Introduction

The term Nanotechnology has been extremely developed field all over the world, producing various nanomaterial with alteration in many different physicochemical and physical properties including crystalline nature, size, shape, as well as interaction with many biological systems (1) (2)(3). Nigella is a variety of around 14 types of creature plant in the family Ranunculaceae, local to southern Europe, North Africa and southwest Asia. Normal names applied to individuals from this sort are Fiend in-a-shrubbery or love in a fog. The plant develops to 20-90 cm tall, with finely partitioned leaves, the leaf sections barely direct to threadlike. The blossoms are white, yellow, pink, light blue or pale purple, whit 5-10 petals. The natural product is a container made out of a few of joined follicles, each containing various seed (4).

The Nigella sativa seed contain a lot of fixed and unpredictable oils (5), protein, alkaloids, and saponins (6)(7)(8).

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The oil and the seeds constituents, I specific thymoquinone, have shown likely therapeutic properties in conventional medication (9). It has been shown that both unrefined alkaloid extricate and watery concentrate of the seed were compelling against an assortment of organic entities even those were safe of anti-microbial (10)(11). Current preliminaries have demonstrated that its seeds alone or in mix with different medications are exceptionally viable in diabetes mellitus (12)(13) and further develops lipid profile through expanding HDL and diminishing LDL and fatty oils (8)(13).

Material and Method [Experimental Design]

1. Frist group (IO+NSMNP): Will be orally administered with combination of IONPs (10mg\kg\day) and NSMNPs (25mg\kg\day) for 28days.
2. Second group (NSMNP): Will be orally administered with NSMNPSs (25mg\kg\day) for 28 days.
3. Third group (IONP): Will be orally administered with IONPs (10 mg\kg\day) for 28 days [14].
4. Forth group (control): Will be kept without treatment as negative control.

Preparation of Black Seeds Extract

Methanolic extract has been obtained according to (15) using Sox let apparatus as follow Preparation alcoholic extract *Nigella sativa* seeds will be purchase from the local market. Alcoholic extract will be prepared by as described by (16) seeds will be washed with distal water several times, dried at 50 c and crushed in a matter with pestle. 150 ml distal water 70%concentration ethanol 350 ml, the with 45 gram Nigella sativa and preparation sox let apparatus in 2021\3\9 time 9:00 am to 02:00pm.

Biosynthesis of Magnetic Nanoparticles of Nigella Sativa Seed Alcoholic Extract

A portion of the Nigella sativa concentrate will be added drop wise with 50ml - 0.1 M Fe cl3.6H2O.solution in 1:1..ration at room temperature following this 1M NaoH Will be added till the PH become 11the outcome combination will be blended involving attractive stirrer for 30 mint and arrangement of extraordinary dark variety arrangement will be affirmed amalgamation Iron oxide nanoparticles the NPs will be isolated by centrifugation 800 rpm for 20 mint and purified by resulting washing with ethanol and water 2-3 mint.the NPs will be at last dried in hit broiler 80 c for 3h and will be put away in seal light holder further usr(17).

Results

That gene expression (fold change) in the 14 and 28 days adding fold change to the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The results Iron group significant increase to the gene expression compared with control. *Nigella sativa* decrease to the gene expression compared with control group. The adding past result when treatment mix (Iron and *Nigella sativa*) significant decrease group compared with control group. In the fold change 14days figures(8)(9)(10) and fold change28 days figures(14)(15)(16). Statistical analysis figures(3)(4)(5).

sacrificed before treatment 14 and 28 days of treatment. At the liver Gss and sod1 genes expression levels (fold changes). Iron group shown significant (decrease level) compared with control group. liver Gss and sod1 genes expression levels (fold changes) significant increase shown in mix group (Iron and Nigella sativa) and Nigella sativa group pest compared control group. the fold change in14days figures(6),(7)and fold change 28 days figures(12), (13). figures Statistical (1),(2). End of every period, assessment of liver mRNA articulation levels of Gss and sod1 qualities has been performed utilizing qRT-PCR method based. blend bunch (IRON and Nigella sativa) treated liver examples recorded critical high absolute RNA focuses contrasted and control. Critical height of both contrasted and control, superoxide dismutase and glutathione even in flawless male killer rodents.

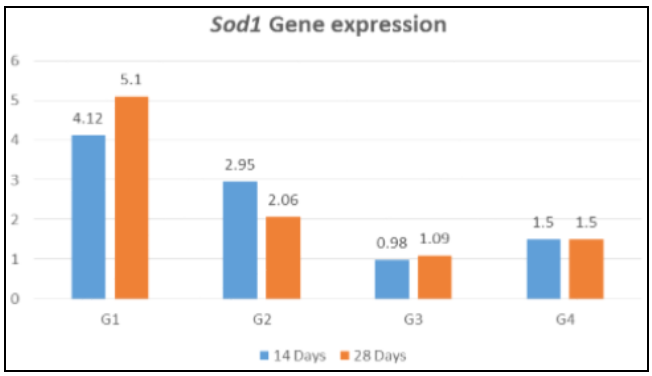

Figure 1. LSD0.05=For group=0.169preiod=0.119Interaction=0.239

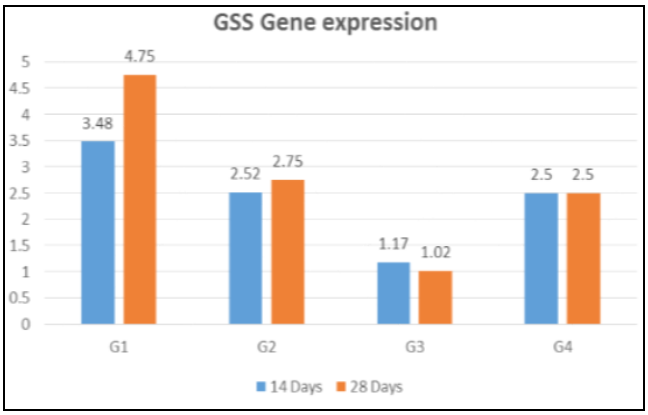

Figure 2. LSD0.05=for group=0.1146. Preiod=0.088. Interaction=0.176

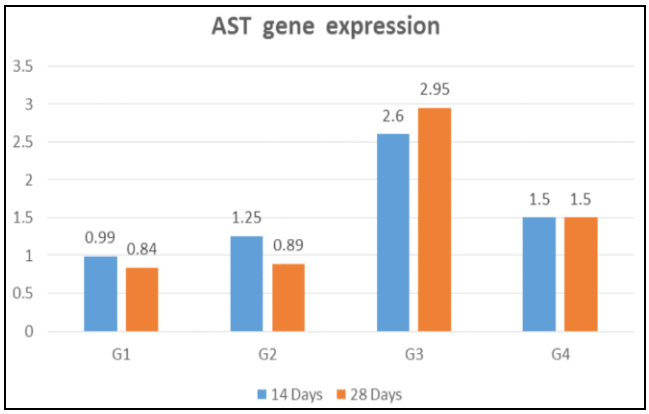

Figure 4. LSD0.05=for group=0.116. Period =0.082. Interaction=0.165

G1=first group mixed = will be orally administered IRONPs (10 mg\kg\day) will be orally administered NSMNPs (25 mg\kg\days) rats for 28 days.G2=second group Nm = will be orally administered NSMNPs (25 mg\ kg\days) on rat for 28 days. G3=third group Fe = will be orally administered IRONPs (10 mg\kg\day) rats for 28 days. G4= Forth group Control = rats without treatment.

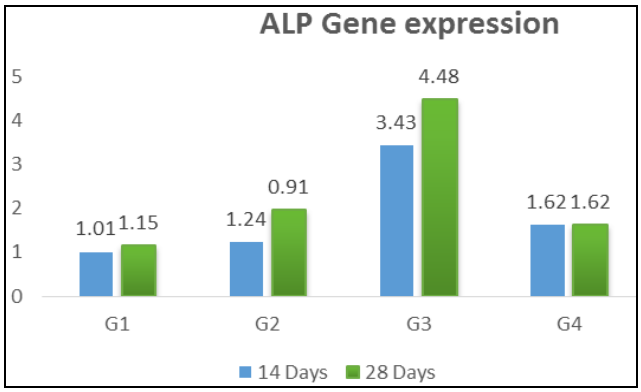

Figure 5. LSD0.05=for group=0.182. Period=0.129.Interactions=0.258

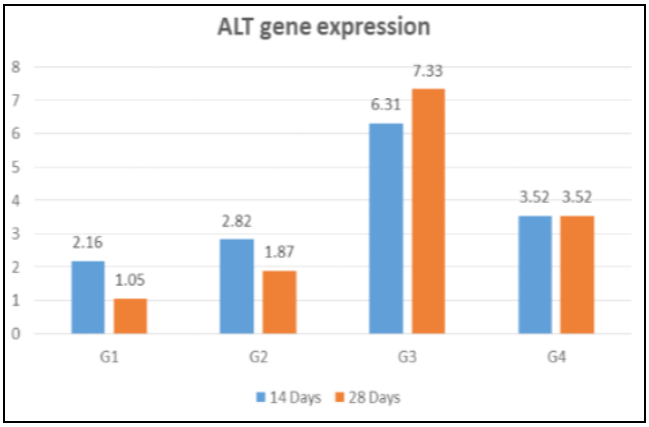

Figure 3. LSD0.05=for group=0.258. Period =0.182. Interaction=0.385

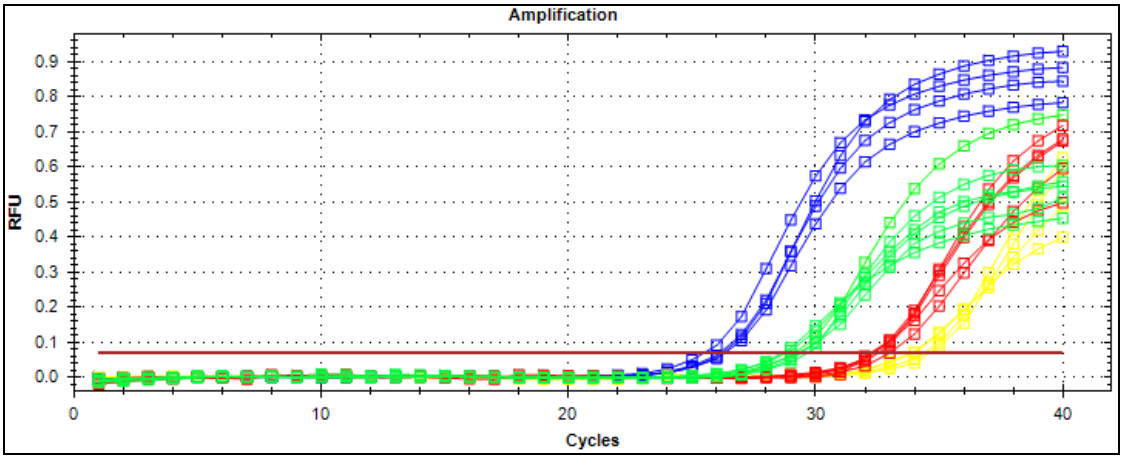

Figure 6. Real Time PCR plots of *SOD1* gene in Liver of experimental rats of treatment and control group. (day 14) showed different in threshold cycle number (ct value) between treatment and control group

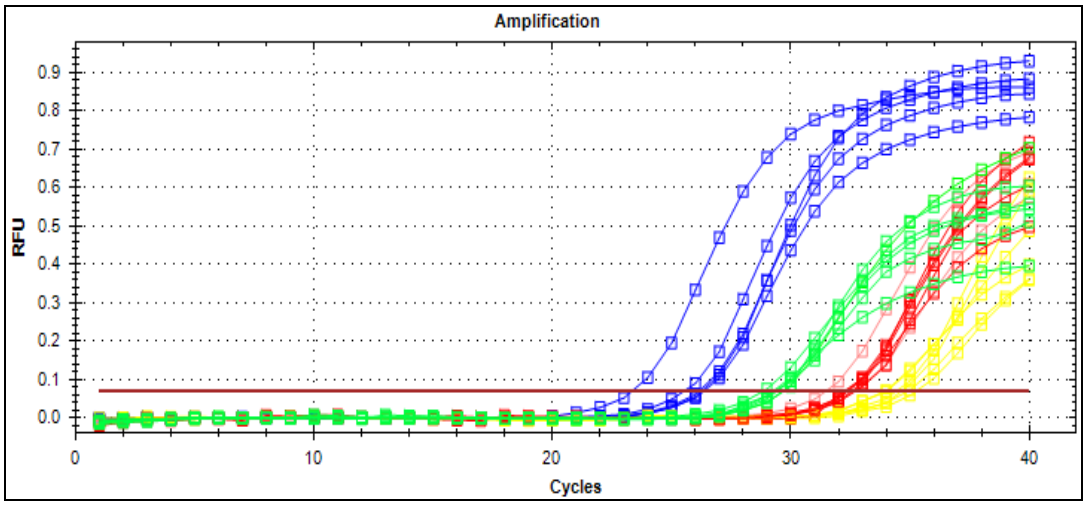

Figure 7. Real Time PCR plots of *Gss* gene in Liver of experimental rats of treatment and control group

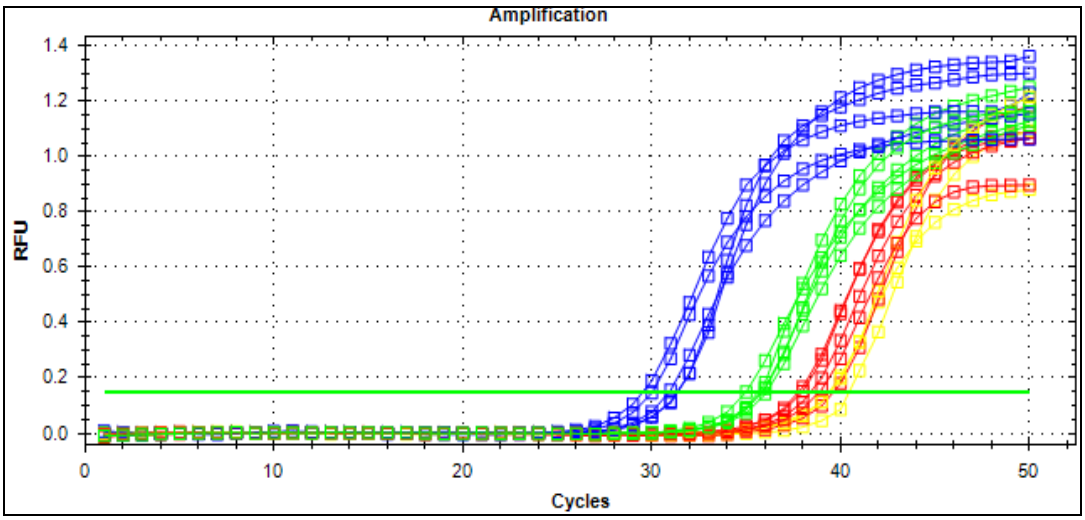

Figure 8. Real Time PCR plots of ALT gene in Liver tissue of experimental rats of treatment and control group

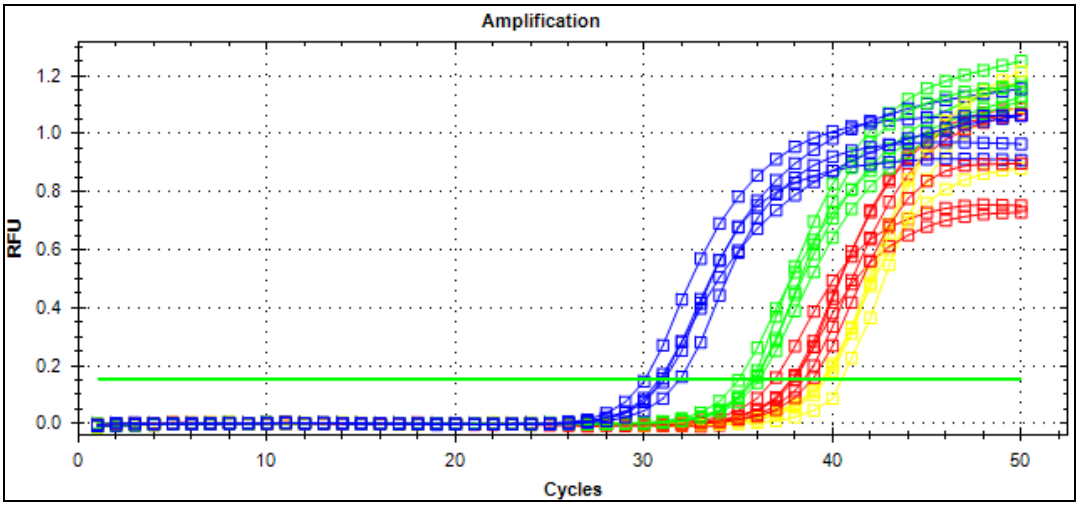

Figure 9. Real Time PCR plots of AST gene in Liver tissue of experimental rats of treatment and control group

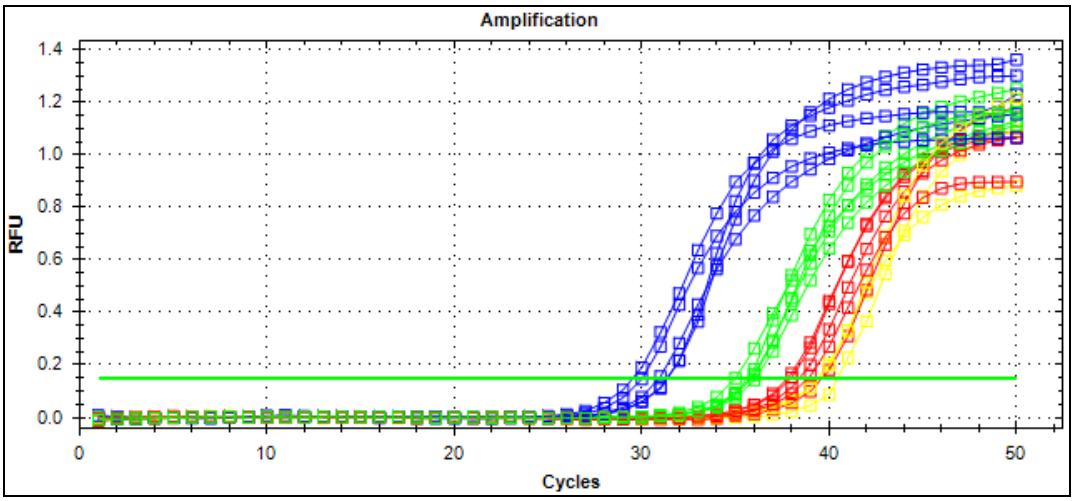

Figure 10. Real Time PCR plots of ALP gene in Liver tissue of experimental rats of treatment and control group

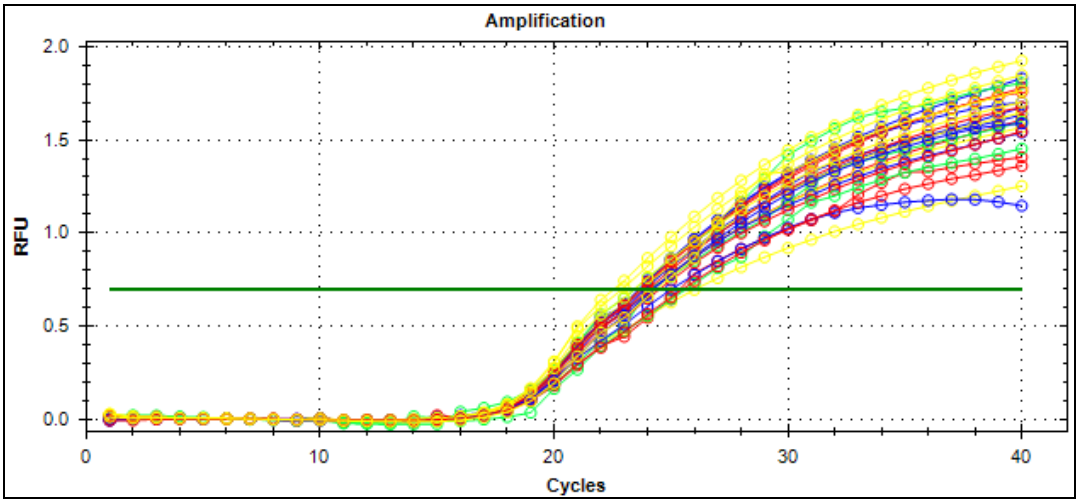

Figure 11. Real Time PCR plots of housekeeping GAPDH gene in of experimental rats of treatment and control group

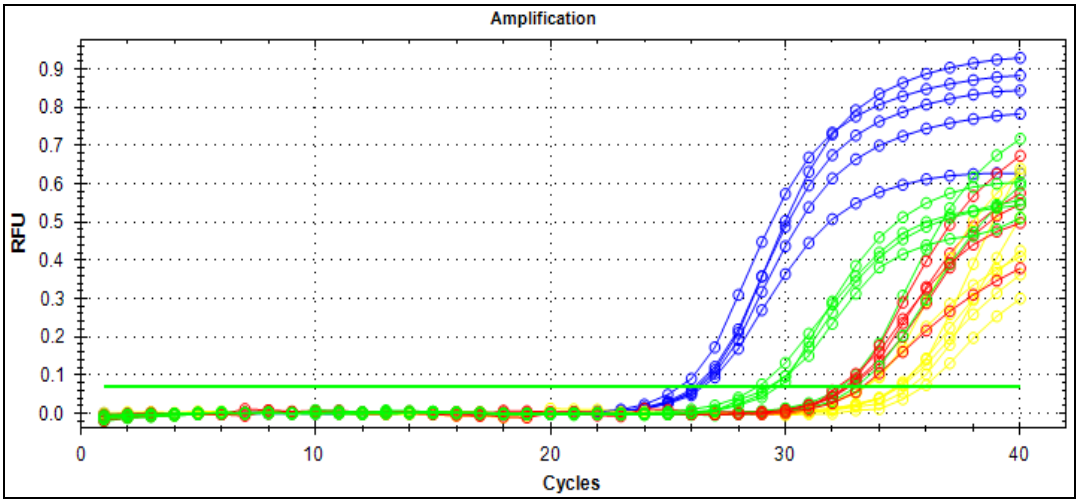

Figure 12. Real Time PCR plots of *SOD1* gene in Liver of experimental rats of treatment and control group 28 day

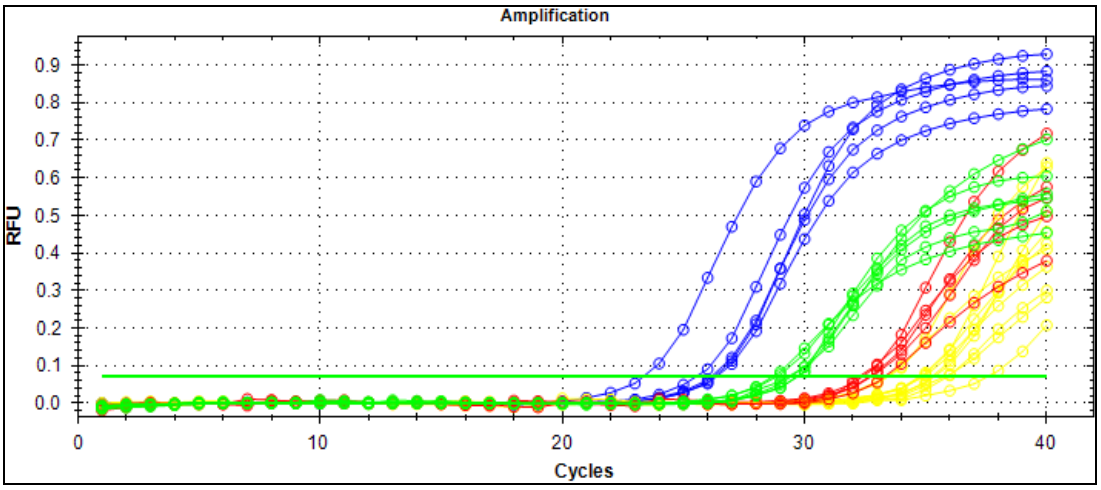

Figure 13. Real Time PCR plots of Gss gene in Liver of experimental rats of treatment and control group

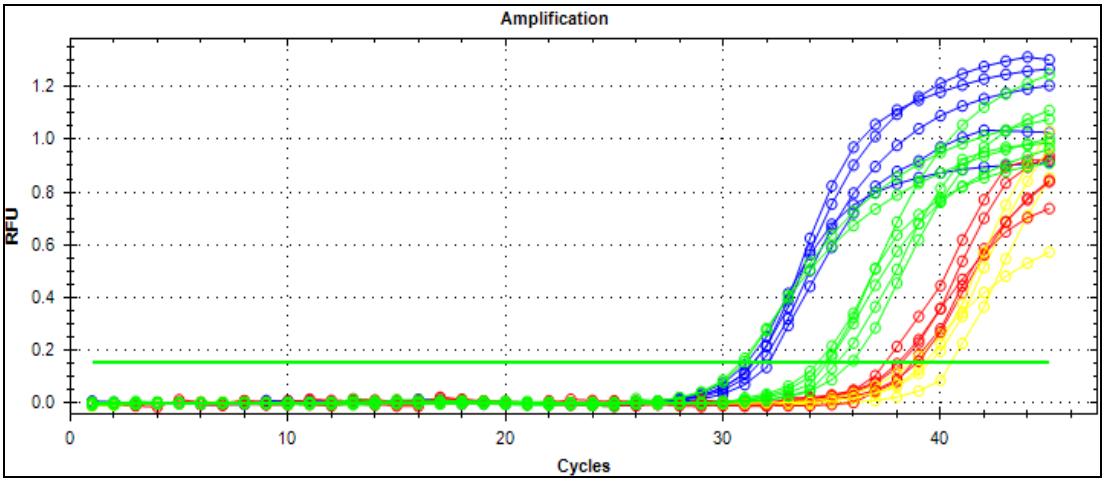

Figure 14. Real Time PCR plots of AST gene in Liver tissue of experimental rats of treatment and control group

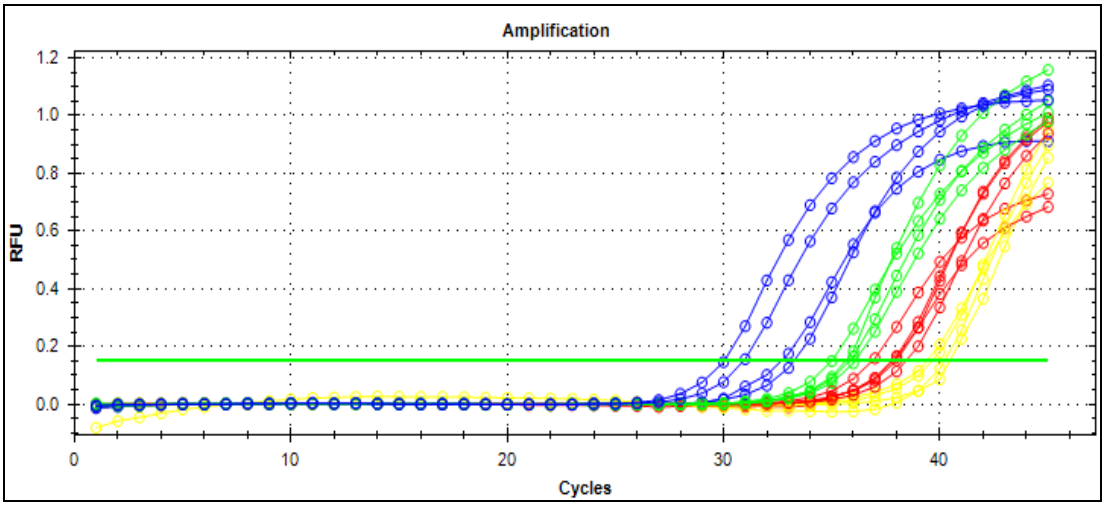

Figure 15. Real Time PCR plots of ALT gene in Liver tissue of experimental rats of treatment and control group

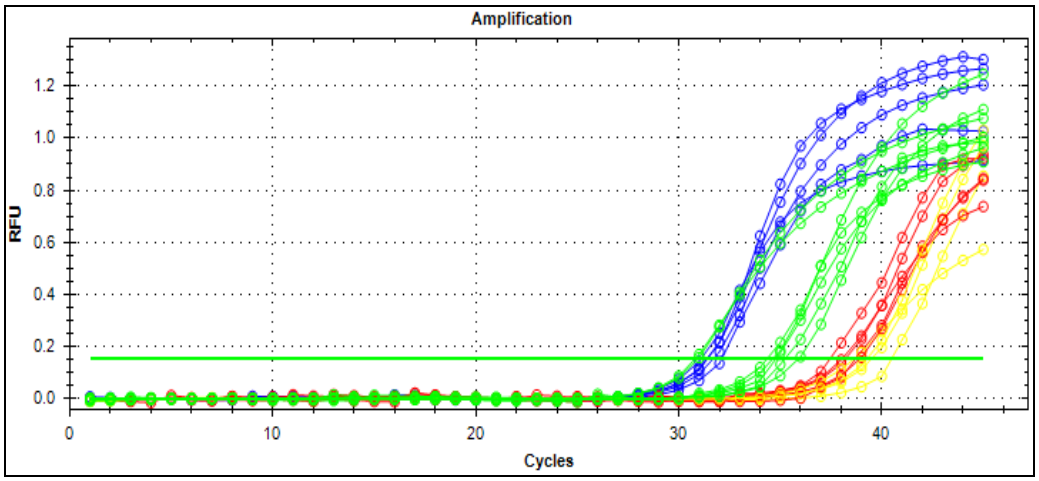

Figure 16. Real Time PCR plots of ALP gene in Liver tissue of experimental rats of treatment and control group

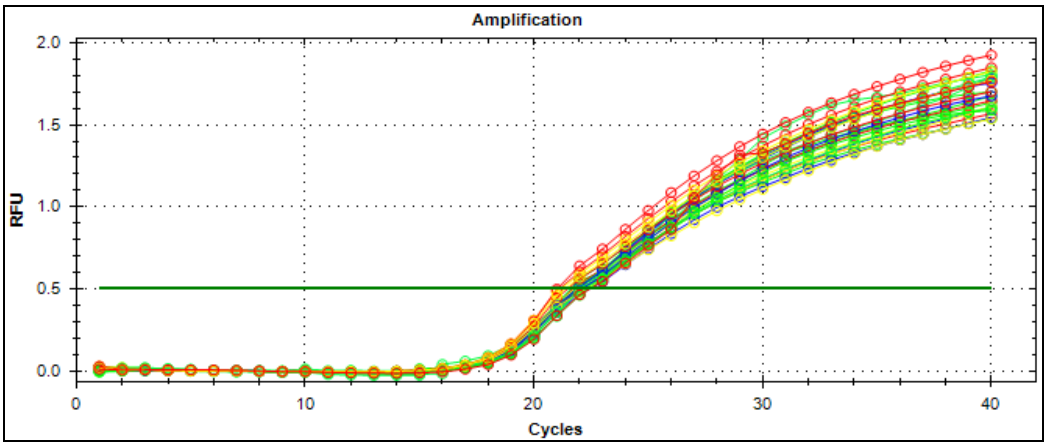

Figure 17. Real Time PCR plots of housekeeping GAPDH gene in of experimental rats of treatment and control group

Discussion

That experience was conducted for the purpose to known the impact antioxidant nanoparticles of *Nigella sativa* agonist oxidative stress of the Nanoparticles of iron in rats, to studied gene expression ALT, ALP, ALS. Gss, Sod 1. G1 first group mixed will be orally administered IRONPs (10 mg\kg\day) will be orally administered NSMNPs (25 mg\kg\days) rats for 28 days. G2 second group Nm would be orally administered NSMNPs (25 mg\ kg\days) for 28 days. G3=third group Fe = will be orally administered IRONPs (10 mg\kg\day) rats for 28 days. G4= Forth group Control = rats without treatment *Nigella sativa* seed extract provide nutritional support (18) as it is rich in nutritional values protein to fatty acids (19) Consumption of N. sativa seed oil as supplement decreased liver enzymes (AST and ALT). (20) and other building blocks for the body such as carbohydrate, proteins, vitamin and minerals (21). It has been shown that the nutritional and medicinal value of *Nigella sativa* effected in improving digestion and providing energy [22]. Thus our results revealed slight increase in body weight under normal environment. It is evident that administration *Nigella sativa* extract 28 days appear increase organs weights liver, kidney, spleen, brain)in rats (23)(24)(25).

Gene expression levels liver tissue during 14 and 28 days (ALT, AST, ALP). When mix groups (Nigella sativa and Iron) to male rats decease gene expression level compared with control groups and other groups. Indicative role *Nigella sativa* protection liver from infection, the work antioxidant, to healthy liver. While give *Nigella sativa* alone to male rats significant decease gene expression level compared with control groups. Increase resistance liver infection (virus, bacterial, fungi) compared with control groups. Iron alone give to male rats significant increase gene expression level compared with control groups increase oxidative stress on liver. (26), Antioxidant Status The effects of dietary black seed supplementation on the oxidative stress and

antioxidant enzymes activities in all the studied tissues.

Gss (glutathione) and Sod1 (superoxide dismutase) gene expression. Our experiment showed that m RNA expression level of liver tissue was healthy male rats compared with control male rats. (Iron treated male rats with methanolic extracts of *Nigella sativa* seed) significant simple increase or like control in mRNA gene expression level liver tissue the pest group mix compared with control male rats. These findings are indicative to the antioxidant and protective role of (*Nigella sativa* and Iron) gene expression level showed increased rapidly and several fold change. Raised GGT isn't indicative of liquor misuse, with research showing it stays high in previous consumers as well as ebb and flow consumers. In men, the most significant levels of GGT happen in the people who drink everyday. In ladies, gorge consumers and those drinking liquor without food will have particularly undeniable levels. The degree of GGT is freely portion dependant, with those in the main two quartiles of liquor admission having the most elevated titres. (27) The list items these sentiments. What's more, settled upon. the gatherings Nigella sativa organization huge reduction quality articulation of (ALT, AST, ALP), and critical increment quality articulation (Gss, Grass 1) compared with control.

Remedial utilization of Nigella sativa as a multipurpose "drug" has been boundless since its starting point in the old Center East through skin or oral treatment. TQ is the dynamic compound of NS and is dependable its calming, cell reinforcement, antibacterial, and anticancer properties (28). Likewise, the degree of dietary iron essentially impacts iron retention. One more significant component in managing iron retention connects with the type of iron present in an eating regimen. Heme and non-heme iron are the two significant wellsprings of iron. Heme iron, principally found in meat, fish, and poultry, is more really assimilated than non-heme iron because of its relationship with porphyrin ring (29).(30) In another review, supplementation with the 1 g N. sativa oil case double a day for a very long time diminished essentially body weight, weight record, AST and ALT levels (31). What's more, N. sativa forestalled liver harm in an assortment of clinical research facility studies. Hepatoprotective and immunepotentiating impacts. The last pharmacological properties seem, by all accounts, to be associated with the beneficial (32) impacts of Nigella sativa Cancer prevention agent and hepatoprotective impacts of Nigella sativa Wellbeing food. The indexed lists these assessments. Also, settled upon. the gatherings Iron organization huge increment quality articulation of (ALT, AST, ALP), and critical lessening quality articulation (Gss, Turf 1)compared with control.

Conclusion

1. Results from the present study provided important information concerning the possible therapeutic use *Nigella sativa* seed methaonlic alcohol extract improving antioxidant of the mammals lives climate such as Iraq.
2. Expand study on the nanoparticle methanonli extract Nigella sativa in treatment anemia.
3. The Nigella sativa increase anti- oxidative enzymes (Sod1-Gss).

Recommendations

1. The using *Nigella sativa* seed methaonlic alcohol extract to treatment liver infection (acute or chronic infection) in mammals.
2. The using *Nigella sativa* seed methaonlic alcohol extract to increase body weight in mammals.
3. The effect thymoquinonemethaonlic extract *Nigella sativa* on hepatocyte regeneration and antitoxic (hepatotoxic male rats).

References

Han, X., Li, S., Peng, Z., Al-yuobi, A.S.O., El-Shahawi,M.S. and Leblanc, R.M. (2016). Interactions between Carbon Nano materials and Biomolecules. *J. Oleo Sci.,* 65: 1-7.

Raza, M.A, Z, Rauf, A., Sabi, A.N., Rias, S., Naseem, (2016). Size and Shape Dependent Antibacterial of Silver Nano (4): 74.

Cacciotti, I., Chronopoulou, L., Palocci, C., Amalfitano, A., Cantiani Cordaro, M, Lajolo, C., Calla, Boninsegena, A., Lucchetti, D., Gallenzi, P. (2018). Controlled release of 18-B glycyrrhetic acid by nano delivery systems increase cytotoxicity on oral carcinoma cell line. *Nanotechnology,* 29(28): 285101.

Dwivedi, S.N. (2004). Herbal remedies among triples of siddhi district of Madhya Pradesh, *J Econ. Tax.,* 28(3): 675-686.

Nickavar, B.; Mojab, F., Javidnia, K.; Amoli, M.A. (2003). Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from *Iran. Z. Naturforsch C.* 58(9-10): 629-631.

Ali, B.H., & Blunden, G., (2003). Pharmacological and toxicological properties of *Nigella sativa*. phytother Res 17. 299-305.

Al-Ghandi, M.S. (2003). Protective effect of *Nigella sativa* seeds against carbon tetrachloride -induced liver damage *Am J. Chin.. Med.,* 31(5): 721-728.

Kalus U, Pruss A, Bystron J, Jureck M, Smekalova A, Lichius J and kiesewetter H. (2003). Effect of *Nigella sativa* (black seed subjective Feeling in patients with allergicn disease. *J. Phytother Res* 17(10): 1209-1214.

Salem M.L. (2005). Immunomdulatory and therapeutic properties of the *Nigella sativa* L. Seed; *Internet Immunopharmacol* 5: 1749 - 1770.

Morsi, N. (2000). Antimicrobial effect of crude extracts of Nigella sativa o on multiple antibiotics resistant bacteria *J. Acta M I Microbiological Colonic.* 49(1): 63 -74.

Riaz M, Syed M and Chaudlhary FM. Chemistry of medicinal plants plan genus Nigella (Family –Ranuculaceae). *J Hand medicus.* 1996; 39(2): 40- 45.

Musa, D.; Duisiz, N.; Gumushan, H.; Ulakoglu, G., and Muharrem, (2004). Antitumor activity of on ethanol extract of *Nigella sativa* seed; *J. Bologia Bratislava.* 59: 635-670.

Al - Rowais N A. Herbal medicine in treatment of diabetes mellitus. *Saundi Med. J.,* 2002,

Zaoui I, Cherrah Y, Mahassini N, Alaoui K, Amar Ouch H, and Hassarssue M. (2002). Acute and chronic toxicity of *Nigella sativa* fixed oil; *J. Phytomedicine.* 9(1): 3-6.

Szalay B, Tatrai E, Nyiro G, VezerT,and DuraG. Potential toxic effects effect iron oxide nanoparticles in vivo and vitro experiment.J. Appl. Toxical., 2017,32(6):446 453. https://doi.org//10.1002/jat.177

Harboron, H.J.; (1984). Phytochemical methods : A Guide to Mode Techniques of plant Analysis.Chapman and Hall, London, UK., 1-34.

Awan, MA, Akhter, S, Husna, AU, Ansari, MS, Rakha BA Azam m AA, Qadeer, S. (2018). Antioxidant activity of *Nigella sativa* Seeds Aqueous Extract and its use for cryopreservation of buffalo spermatozoa. *Andrologia,* 50(6):e13020-e1 3026.

Amooaghaie, R., Saeri, M.R. and Azizi, M. (2015) Synthesis, Characterization and Biocompatibility of Silver Nanoparticles Synthesized from Nigella sativa Leaf Extract in Comparison with Chemical Silver Nanoparticles. *Ecotoxicology and Environmental Safety,* 120, 400-408. https://doi.org/10.1016/j.ecoenv.2015.06.025

Ahmed, A., (2004). Activation of the immune system of Anopheles Gambiae Against Malaria parasite: Acomparisson between bacterial injection and Botanical Extract. *International Conference on Biological sciences* 3(1): 122-141.

Rashidmayvan M, Mohammadshahi M, Seyedian SS, Haghighizadeh MH. The effect of Nigella sativa oil on serum levels of inflammatory markers, liver enzymes, lipid profile, insulin and fasting blood sugar in patients with non-alcoholic fatty liver. *J Diabetes Metab Disord* 2019; 18: 453-9.

Ramadan, M., and Morsel, J. (2003). Analysis of glycolipids from black cumin (Nigella sativa L.), Conriander (coriandrum sativum L). and niger (guizotia abyssinica cass.) oil Seeds. *Food chemistry,* 80: 197-204

Swami, S.M., Tan, B.K. (2000). Cytotoxic and immunopotentiating effect of ethanolic extract of *Nigella sativa* L. seeds*.; mJ. Etno-Etnopharmacol.* 70: 1-7.

Salem, M., and Hossein, M. (2000). Protective Effect of black seed oil ffrom *Nigella sativa* against murine Cytomegalic virus Infection. *J. Immunopharmacology,* 22(9): 729-740.

Andaloussi, A., Elimadi, A., Settaf, A, Cherrah, Y., Haddad, P (2004). The effect of *Nigella sativa* in the body weight. *Ethno - pharmacology.* 94: 251-259.

Kanter, M.; Coskun, O., and Gurel, A., (2005). Effect of black Cumin (*Nigella sativa*) on Cadmium induced oxidative in the blood of the rats. *J. Biol. Trace. Elem. Res.* 107(3): 277-278.

AItan, M.; Kanter. M.; Donmez, S.; Kartal.; M., and Buyukbas, S (2007). Combination therapy of *Nigella sativa* and human parathyroid hormone on bone mass, biomechanical behavior and structure streptozotocin –induced diabetic rats, *Acts His to chemical*. (4): 304-314.

Maria Latif, Mehwish Faheem, Asmatullah, Seyed Hossein Hoseinifar and Hien Van Doan Animals 2021, 11, 48. https://doi.org/10.3390/ani11010048

Stranges S, Freudenheim JL, Muti P, Farinaro E, Russell M, Moon Nochajski TH et al. Differential effects of alcohol drinking to pattern on liver enzymes in men and women. *Alcohol Clin to Exp Res.*, 2004.; 28(6): 949-56.

Ijaz, H.; Tulain, U.R.; Qureshi, J.; Danish, Z.; Musayab, S.; Akhtar, M.F.; Saleem, A.; Khan, K.K.; Zaman, M.; Waheed, I.; et al. Nigella sativa (Prophetic Medicine): A Review. *Pak. J. Pharm. Sci.*, 2017, 30, 229–234.

Cook JD (1990). Adaptation in iron metabolism. *Am J Clin Nutr* 51: 301–308.

Hussain M, Tunio AG, Akhtar L, Shaikh GS. Effects of Nigella sativa on various parameters in patients of non-alcoholic fatty liver disease. *J Ayub Med Coll Abbottabad* 2017; 29: 403-7.

Mollazadeh H, Hosseinzadeh H. The protective effect of Nigella sativa against liver injury: a review. *Iran J Basic Med Sci* 2014; 17: 958-66.

Swamy, S.M., Tan, B.K., (2000). Cytotoxic and immunopotentiating effect of of ethanolic extract of Nigella sativa L. seeds.; mJ. *Etno- Etnopharmacol.* 70: 1-7.