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Ameliorating Effects of Herbal Mixture for Dexamethasone Induced Histological Changes in Mice

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ABSTRACT

Organs of mammals are affected negatively by dexamethasone under the effect of different factors, which medicinal herbs can repair because of their unique properties as a rich source of bioactive phytochemicals that could lead to the creation of new medications. Most phytochemicals derived from plants are antioxidant agents that have improved health disorders. The objective of the current study is to detect the action of ethanol extract of three combination herbs (Hibiscus sabdariffa, Portulaca oleracea, and Eruca sativa) on the skeletal muscle liver, and testes of mice affected by dexamethasone drug. Eighteen white male mice equally divided into three groups. The first group received dexamethasone intramuscularly (IM) for 21 days. The second group was injected with dexamethasone IM and herbs mixture extract orally after one hour for 21 days. While the third group, which served as the control group, was injected with normal saline IM. The specimens were fixed and processed to evaluate the histological changes. Necrosis and degeneration appeared in the skeletal muscle and liver sections, while no histological changes observed in the dexamethasone-treated group's testes. We observed regeneration and repairing action in the organs studied of the herb in the combination-treated group. It was concluded that a combination of herbs extract plays a significant repairing action of organs affected by dexamethasone.

Key words: Polyhedral extract, *Hibiscus sabdariffa*, *Portulaca oleracea*, *Eruca sativa*, Dexamethasone

INTRODUCTION

Dexamethasone is a steroid that is about 30 times higher than cortisol in potency, possesses diverse anti-inflammatory properties, but basically involves suppressing inflammatory cells and limiting the inflammatory mediator expression (Zheng-rui et al. 2018).

The medicinal herbs contain bioactive phytochemical constituents, which are sorted into two categories according to their functions in the plant metabolism, primary and secondary constituents. Primary ingredients are the nutrient proteins, carbohydrates, amino acids, and chlorophyll while secondary ingredients, also called secondary metabolites (SMs), consist of phenolic compounds, alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, and many more which serve as defense system of the body by treating infections, health disorders or more precisely, to prevent diseases (Krishnaiah et al. 2007; Degla et al. 2022).

The calyces of *Hibiscus sabdariffa* (HS) L., family Malvaceae has been used as an ethnomedicinal cure for hypertension, hyperlipidemia, obesity, diabetes, jaundice, liver and urinary issues. It is an antihypertensive, hypolipidaemic, anti-obesity, and anti-hyperglycemic action that has been verified in humans and experimental animals (Kao et al. 2016). In addition to SMs that increase their therapeutic value, many studies have believed the main therapeutic effects of HS documented to anthocyanins, polyphenols, flavonoids, and organic acid. Protocatechuic acid and anthocyanins are among the antioxidants found in HS. Recent research has demonstrated that these antioxidants can protect the liver against chemically induced toxicity (Morales-Luna et al. 2018).

An Omega 3 fatty acid- rich plant is *Portulaca oleracea* (PO) from the Portulacaceae family, which is well known as Purslane, which has a broad spectrum of pharmacological actions, including antibacterial, analgesic, anti-inflammatory and wound-healing properties. Purslane extracts demonstrate an anti-diabetic effect (Singh and Kori 2014).

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Plant bio-active antioxidants evinced a great role and more vital functions in protecting humans against a variety disorders, including cardiovascular disease. Purslane has additionally been linked to a variety of biological effects, including hypoxia, hepatoprotective capabilities, and anti-inflammatory activities (Niharika and Sukumar 2016).

Rocket plant *Eruca sativa* (ES) is a member of Brassicaceae family that originated on the Mediterranean coast. The herb has traditionally been used as a tonic, rubefacient, astringent, digestive, laxative, emollient, stimulant, stomach, scurvy, and to promote sexual desire (aphrodisiacas) a result of its content of secondary metabolites and minerals with nutraceutical and organoleptic properties (Kishore et al. 2017).

The purpose of the present study is to determine the type of activity of these herbs in combination status in some organs affected by dexamethasone in mice.

MATERIALS AND METHODS

Animals

The study has been conducted in November 2019 on 18 male white mice at 4 weeks age weighing 25-29g, acquired from the animals' house of the College of Medicine/ University of Baghdad, Iraq. Animals were housed in a ventilated cages under an equal period of light/and dark cycle at 26±2°C and had free access to water and commercial feed (pellet).

Materials

Dexamethasone sodium phosphate ampoules of 8mg/2mL (H-tech international enterprises Co. Ltd) were used for inducing negative action in the mice organs at a dose of 1mg/kg of body weight intramuscularly daily for 21 days (Kamil et al. 2016). The constituents of the dried herb's combination of *Hibiscus sabdariffa* (HS), *Portulaca oleracea* (PO), and *Eruca sativa* (ES) were purchased from the local market in Baghdad, Iraq.

Preparation of Alcohol Extract

The extract was carried out in the Molecular Biology Department, College of the Medicine, University of Baghdad, Iraq. The dried herbs of HS, PO, and ES were grounded by electric grinder separately, 50g from each type of herbs were weighed and mixed with 450ml of 70% Ethyl alcohol in Erlyn Myer flask tightly sealed which was stirred overnight at 45°C on a magnetic stirrer. After 48hours, the sediments were filtered with gauze and sterilized using a 0.4µm sterile millipore filter and kept in deep freeze (-20°C) until use (Akram et al. 2016). The combination was collected as the following ratio 35, 35, and 30% from HS, PO, and ES respectively.

Design of Experiment

The experimental mice were divided into three groups, each separated group include 6 mice which were treated as the following:

First group was injected with dexamethasone intramuscularly (IM) at a dose 1mg/kg of body weight for 21 days. The second group was injected with dexamethasone 1mg/kg of body weight IM and 1mL of herbs mixture extract orally using gavages needle after one hour for 21days (Kamil et al. 2016). The third group served as the control group and was injected with 1ml normal saline IM. On the day 22 of the experiment, the animals were weighed and anesthetized by Ketamine/Xylazine.

The liver, skeletal muscles, and testes of each animal were extirpated and washed with normal saline solution and fixed by 10% formalin separately. These parts were processed and stained with hematoxylin and eosin (H&E) for histopathological examination.

RESULTS

The negative action of dexamethasone in the skeletal muscle histology appeared through the replacement of the myofibers with fat cells, a presence of hemorrhage, necrosis, and degeneration in the myofibers as well as there is the displacement between muscle fibers (Fig. 1). In addition to hyperpigmentation of the muscle tendon, a strong presence of Ca+2 in myofibers was also observed besides the distribution of fat droplets (Fig. 2) Furthermore, the changes of the liver affected by dexamethasone were detected by the accumulation of lipid on the hepatocytes and vacuolation in the cytoplasm with necrosis of liver plates (laminae) (Fig. 3).

In the treated group, these changes were replaced by normal muscle fiber (Fig. 4). Similarly, the changes of the liver were repaired through the appearance of Kupffer cells with carbon granules, arrangement of a sinusoid with interlobular connective tissues and repairing of bile canaliculi (Fig. 5). While no histological changes were apparent in the testes section affected by dexamethasone (Fig. 6).

DISCUSSION

Glucocorticoids administration leads to negative effects that involve oxidative stress in different tissues. The main negative effects of skeletal muscles are atrophy, tendon rupture by increased protein breakdown, and decrease protein synthesis. Muscle atrophy is a serious side effect of glucocorticoid in variable catabolic conditions. The acceptable mechanism, in case of muscle atrophy, is insulin resistance and spurt muscle calcium, which appears in the replacing myofiber of muscle with fat cells due to the buildup of muscle adipocyte (ACs) and intramuscular fat or intramyocellular (IMC) lipid. IMC lipid lowers insulin sensitivity leading to a decrease in the ability to protein synthesis in the muscle, thus decreasing muscle strength (Rivas et al. 2016). The histological changes in the current study confirm this side effect which agrees with Wang et al. (2021) and Nadia et al. (2019).

The repair action of the herbal compound for the targeted organs reveals the synergistic effect of the compound through its containment of phytochemical ingredients which are classified as exogenous antioxidants that involve vitamins and trace elements. Vitamin E, a well known distinguished antioxidant, has the potential role to prevent cell oxidation located mainly in the cell membrane through inhibition of lipid peroxidation and another radical-driven chain. It also coordinates with vitamin C to reduce radical tocopherol to non-radical ones. Vitamin E is very important in the cell membrane repair of skeletal muscle. Vitamin C in the form of ascorbate is water-soluble vitamin, located in extracellular spaces, has a particular

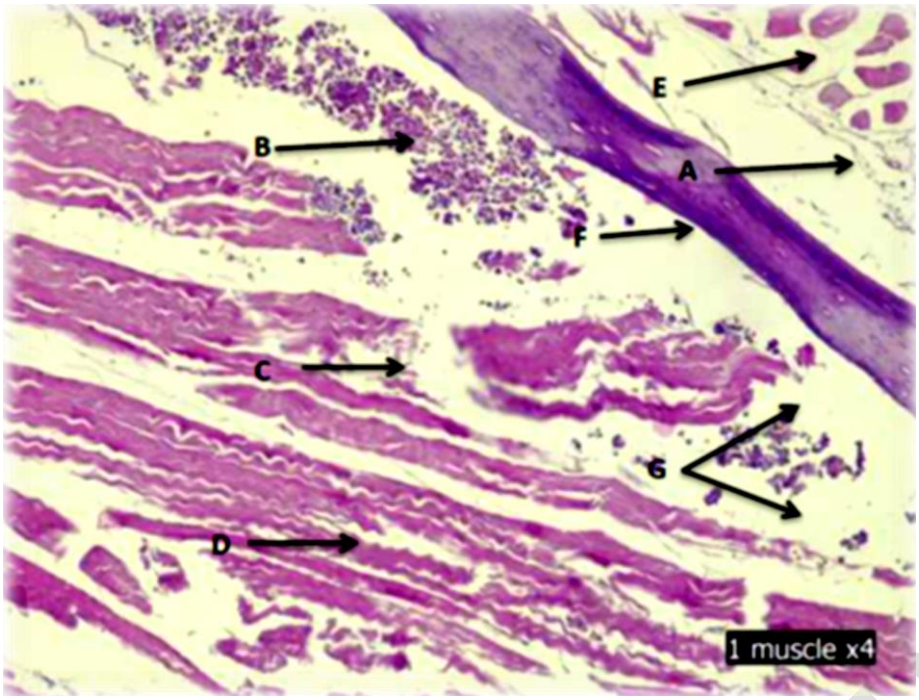

Fig. 1: Longitudinal section of mice muscles exposed to dexamethasone (DEX) for 22 days showed that: A) Myofibers are replaced with Fat cells. B) Hemorrhage in muscles fiber. C) Necrosis and degeneration. D) Destruction in muscle fiber. E) Displacement between muscle fiber. F) Hyperpigmentation of tendon in muscle. G) Loss of muscle fiber (H and E stain, 40 X).

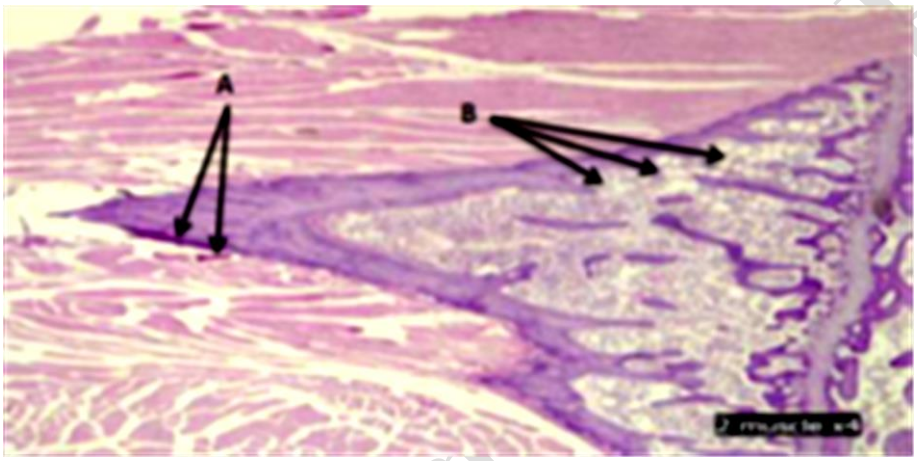

Fig. 2: A) Calcium (ca++) appearance strong positive. B) Droplet of lipid in the cytoplasm of myocell of mice muscles exposed to dexamethasone (H and E stain, 40 X)

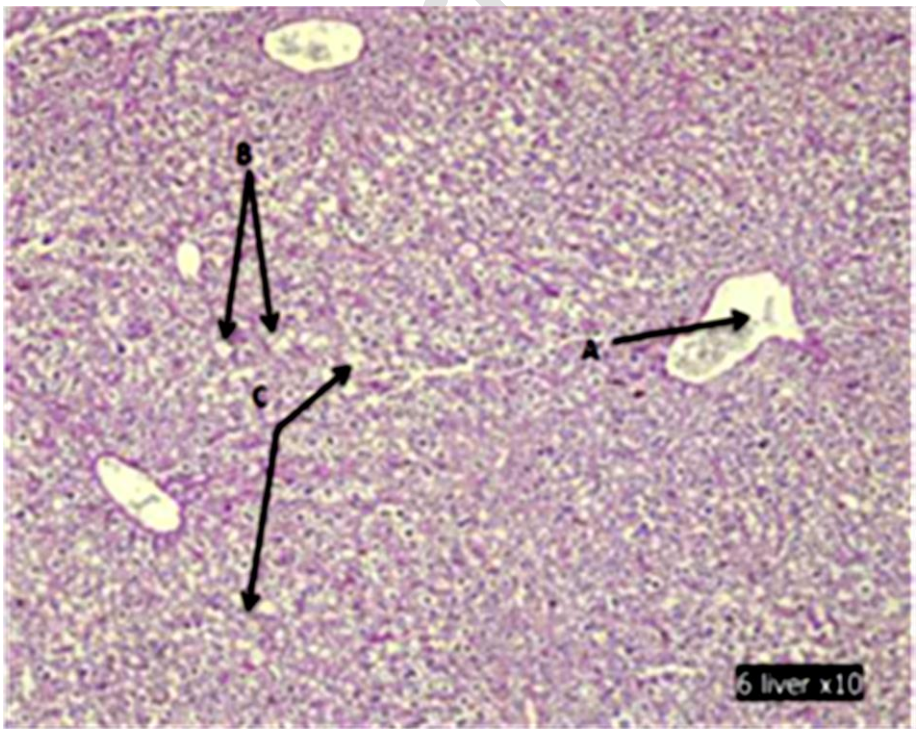

Fig. 3: Transverse section of mice liver of Dexamethasone group showed that: A) Central vein. B) Accumulations of lipid on hepatocytes. C) Cytoplasmic vacuolation in hepatocytes with necrosis in liver plates (laminae) (H & E stain 100x).

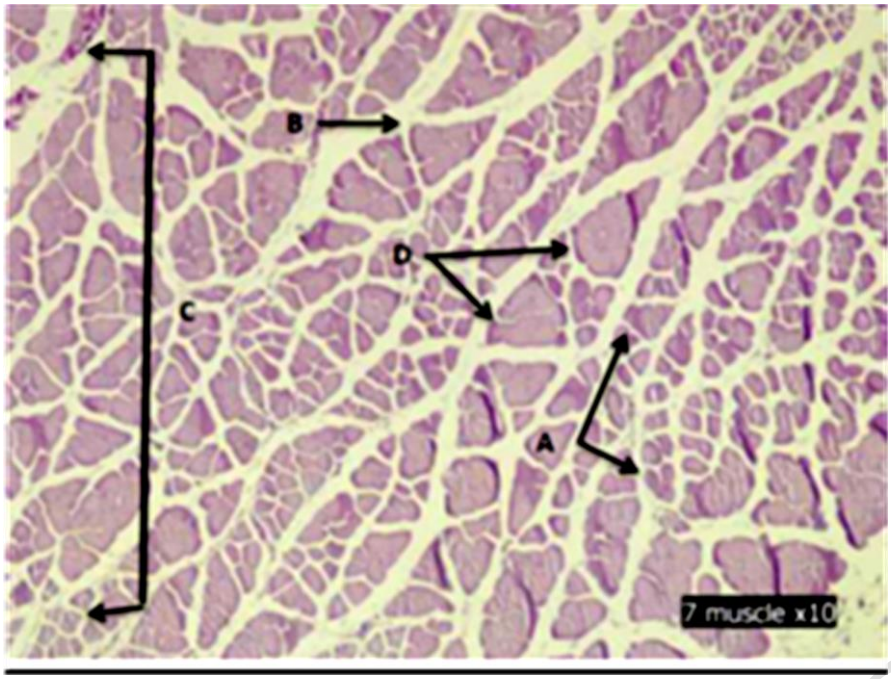

Fig. 4: Transverse section of mice muscles of herbal treated group showed that: A) Normal muscles fiber. B Normal interstitial of muscle. C) Normal shape of muscle fibers. D) Repaired endomysium in muscle fibers (H and E stain, 100x).

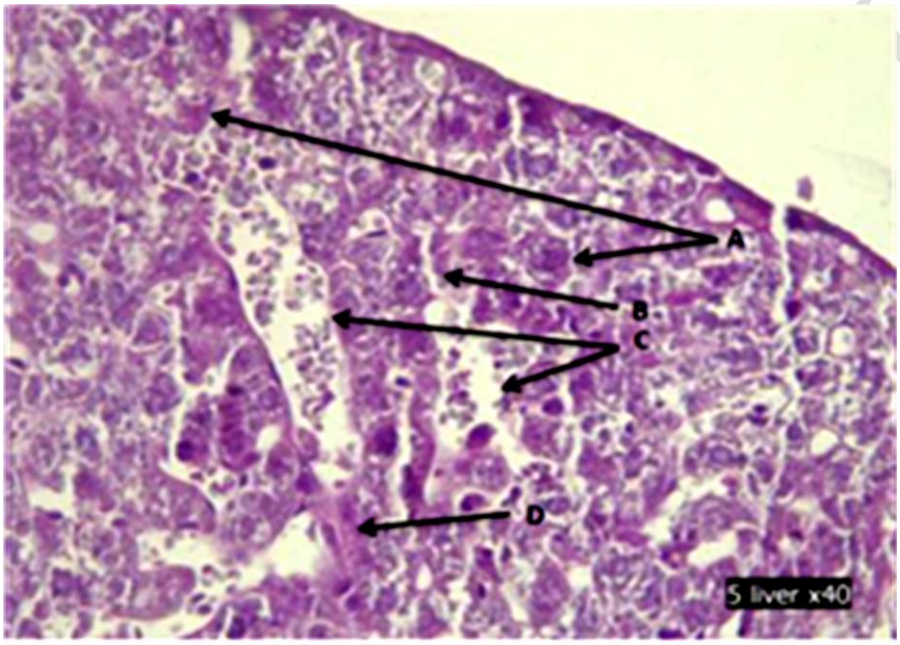

Fig. 5: Transverse section of mice liver of herbal treated group showed that: A) Repaired Kupffer cells with carbon granules. B) Sinusoid arrangement with interlobular connective tissues C) Central veins D) Repaired bile canaliculi (H and E stain, 400x).

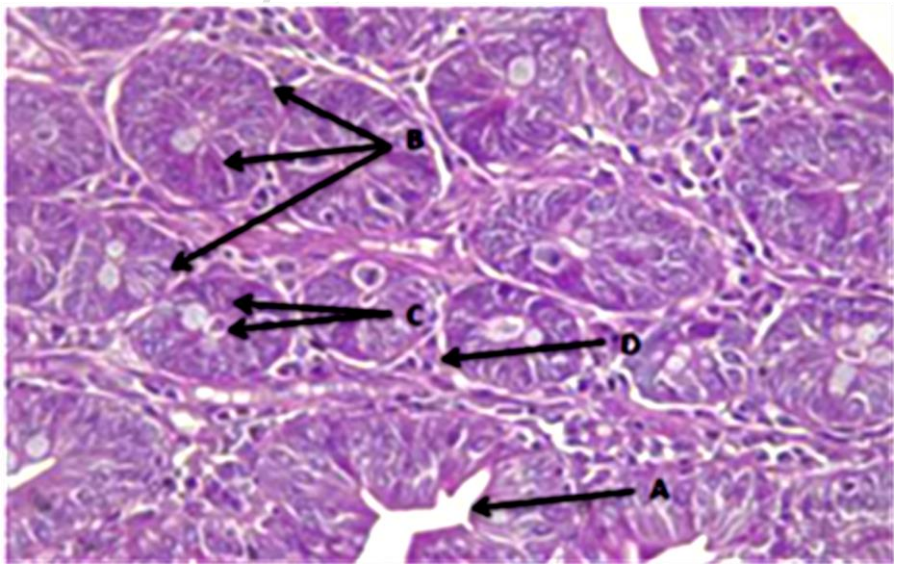

Fig. 6: Transverse section of mice mature testis treated with the dexamethasone showed that: A) Normal seminiferous tubules. B) Normal spermatogone and normal primary and secondary spermatocye C) Normal spermatid with spermatozoa D) Normal lydig cell (H & E stain, 400x).

role in the redox process, as a great antioxidant and enzyme cofactor, specifically of hydroxylases (Howard et al. 2011; Labazi et al. 2015). The trace elements found in the studying herbs, selenium, copper, manganese, and zinc act significantly in promoting antioxidant reactions and redox processes of different enzymes. Anthocyanins are classified as a flavonoid group, part of the polyphenol components. It is a natural pigment responsible for the purple color of HS and PO that has the potential effect on the regeneration process of skeletal muscle throughout redox control, elevated the level of protein synthesis, declines muscle collapse protein and it has anti-inflammatory properties. Also, an improvement of microbiota diversity of the gastrointestinal tract results in increased oxidative metabolism capacity, regulating the function of mitochondria and triggering the myogenesis (Astrid et al. 2020). The second organ that has been affected by the corticosteroid drug is the liver. Dexamethasone catalyzes protein breakdown, releasing amino acids, and lipolysis, releasing glycerol and fatty acids in the peripheral body organs, which act as gluconeogenesis substrates in the liver, causing degeneration and necrosis of the liver plate. Furthermore, glucocorticoids decrease glucose uptake from these organs contributing to the elevation of blood glucose. As a compensatory process, increase insulin secretion (Sapolsky et al. 2000) that suppress its action by glucocorticoid causing insulin resistance and hyperglycemia. The alteration in the glucose and insulin levels will increase glycogen deposition in the cytoplasm of the hepatocytes. The vacuolation in the cytoplasm, as well as accumulations of lipid on hepatocytes, are the result of protein breakdown and lipolysis induced by dexamethasone that acts as substrates for gluconeogenesis. This result agrees with Korićanac et al. (2006). The histological section revealed a normal structure in the testicular tissue of mice that disagree with Layasadat et al. (2013) because of insufficient dose or administration period process (Astrid et al. 2020). This activity may prove the significant antioxidant instance against oxidative stress of intensive training in skeletal muscle after being treated by HS (Sahil and Souravh 2014). Bhattacharjee et al. (2017) also reported that Protocatechuic acid (PCA) could trigger glucose metabolism in skeletal muscle that improves the level of glucose and lipid in the blood and reduce cytokines storm, on the other hand, the current study on the regenerative effect of the liver agrees with previous research that linked HS's hepato-repairing action to PCA and anthocyanins, which are potent antioxidants in HS that can reduce peroxidation of liver cells, reducing liver lesions and hepatocytes swallowing by increasing glutathione peroxidase and catalase activity and decreasing malondialdehyde levels in aged rats (Shi et al. 2006).

Regarding the effect of PO on the liver, the study of Sudhakar and Krishna (2010) has demonstrated the significant recovering level of hepatic damage induced by cisplatin administration to chicks through pretreatment with extract of This looks to be a return to normal values of biochemical parameters (Sudhakar and Krishna 2010). In addition, due to the antioxidant groups in PO, research on the effect of PO on rats injected with Alloxan indicated an improvement in vacuolation in the cytoplasm and inflammatory cells in the liver when treated with PO (Adel et al. 2018).

Eruca sativa extraction has a hepatoprotective effect against phosphoric acid and paracetamol, may be due to the suppression of the cytochrome P450 oxygenase enzyme system and the presence of glucoerucin (the major glucosinolate in a rocket) which has indirect and direct antioxidant actions, in addition to hydroperoxides and H2O2 decomposition properties (ELSadek 2014; Mashi 2017). Kamil et al. (2019) reported that biopotential stem cells in the liver may differentiate into either hepatocytes or cholangiocytes after treating experimental mice with *E. sativa* (Kamil et al. 2019). When comparing the results of the current study to previous studies of herbs treatment individually, it can be concluded that the synergistic effect of herbs extract combination on the skeletal muscle and liver of mice is evident throughout the regeneration activity of myocells and hepatocytes as a result of antioxidants component activity. The limitation of this study is lack of molecular study to determine active ingredients that responsible for regeneration processes. An absence of enough studies on the same composition to compare, and there aren't enough studies on herb combinations, in conclusion, it is recommended that more research be done employing herbal mixtures based on molecular studies to identify active substances responsible for physiological changes as well as to detect the mode of action to generate new possibilities for future health care derived from natural resources.

Conclusion

When comparing the results of the current study to previous studies of herbs treatment individually, it can be concluded that the synergistic effect of herbs extract combination on the skeletal muscle and liver of mice is evident throughout the regeneration activity of myocells and hepatocytes as a result of antioxidants component activity. The limitation of this study is the lack of molecular study to determine active ingredients that responsible for regeneration processes. An absence of enough studies on the same composition to compare, and there aren't enough studies on herb combinations. it recommends that more research be done employing herbal mixtures based on molecular studies to identify active substances responsible for physiological changes as well as to detect the mode of action to generate new possibilities for future health care derived from natural resources.

Author's Contribution

All the participants performed a significant contribution to the aim, design and conducting of the study. SJA provided and prepared the laboratory (workplace) with all the required tools and devices. AMK and LMM participated in the preparation of histological sections which were examined by LMM histologically. The writing, interpretation of the results, and the arrangement of the publishing paper and revision were the responsibility of AMK. All authors revised and accepted the conclusive manuscript, and contributed funding to the study.

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