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Original article

Optical and FTIR Spectroscopic Evaluation of Curcumin's Therapeutic Potential in Experimental Rats Liver Cirrhosis

Gehad Zakarai [1], Sahar M. Awad [1*], Amira M. Ali [1], Hebishy, M abdallah [2]

[1] *Physics department, Faculty of science, Al-Azhar University (Cairo-Egypt).*
[2] *National Research centre- physiology department (El-Dokki- Cairo- Egypt).*

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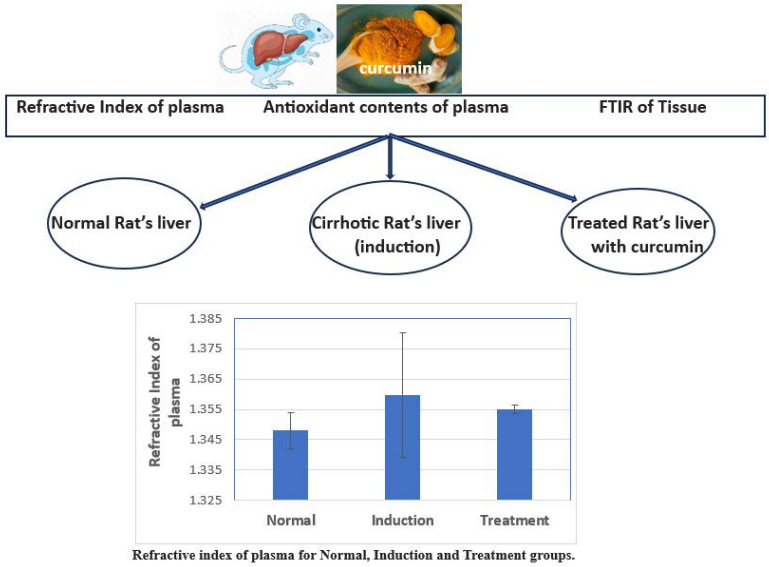
ABSTRACT

The aim of our work is a regression of liver fibrosis and cirrhosis by natural products. Cirrhosis is already a late stage of liver disorders. Our work gives more attention about natural herbal remedies as a therapy for liver cirrhosis, due to their plentiful source of antifibrogenic agents as well as their lesser side effects, lower costs, and higher acceptability. Curcumin is a natural compound extractable from turmeric and is widely used in middle eastern diets. Curcumin has an important role in the modulation of many biological mechanisms especially in liver injury. Our work investigates the effect of curcumin on curing liver damage in its late stages. Refractive index of plasma was measured in cirrhotic liver, indicating a high value due to the presence of high albumin level compared to normal cases, while after treatment with curcumin, the refractive index retained its normal value. Liver's tissue was also investigated spectroscopically using FTIR, which showed shifts of different bands in diseased tissues that were restored due to curcumin by stabilizing the phosphate group and restoring the normal pattern of hydrogen bonding. Results also showed an improvement in antioxidant behavior due to curcumin injection (which can be considered as a therapeutic agent). There is an improvement in the refraction of plasma because of the reduction of oxidative stress, which improves protein conformation, and decreased protein aggregation.

Graphical abstract



Refractive index of plasma for Normal, Induction and Treatment groups.

1. Introduction

Cirrhosis of the liver is a dangerous condition appears in the latest stage of disease, involving scarred and permanent damage of liver tissue. It is a significant factor for hepatocellular carcinoma. Symptoms of cirrhosis have increased one and a half to two-folds during the

* *Corresponding author*
E-mail address: saharwd@yahoo.com

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past two decades, reporting two million deaths worldwide every year by chronic liver disease and cirrhosis [1]. There are four stages of liver cirrhosis: First encloses certain scarring of the liver, but few symptoms and no complications. The second involves portal hypertension and varices. Third development of swelling in the abdomen and liver scarring with increasing complications. And last stage threatening, and people have end-stage disease, which is fetal without a transplant [2].

Researches give more attention to natural herbal remedies for treating liver cirrhosis, due to their plentiful source of antifibrogenic agents, beside their low cost and side effects [1, 3]. Curcumin is the active and important ingredient from turmeric; beside its role as an ancient Chinese medicine, it has been identified for its antioxidant and anti-inflammatory effect. It also has a cytotoxic effect on many cancer cells. Many types of carcinogenesis occurred due to oxidative stress and curcumin (curcuminoid compounds) can act as a scavenger of free radicals that reduces peroxidation of lipid [4]. Studies have shown that it improves systemic markers of oxidative stress, modulating enzymes activity as Glutathione (GSH), catalase, and Superoxide dismutase (SOD) by neutralization of free radicals [5]. Many reports revealed that curcumin enhances hepatic detoxification by increasing glutathione to glutathione disulfide ratio that reduces oxidative stress and inhibits the activation and nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells [6]. Recent studies postulated that curcumin protects against hepatic fibrosis by inactivating hematopoietic stem cells (HSCs) through activation of peroxisome proliferator-activated γ-receptor, which interrupts platelet-derived growth factor and epidermal growth factor signaling in activated HSCs [7].

Biophysical properties of blood can be assessed to reflect the physiological state and function of the liver, such as the refractive index of plasma and FTIR. The refractive index of blood is an easy and a widely used diagnostic tool for clinical applications. It is considered an important factor which the pathologists are newly interested is the refractive index profile of human blood and urine [8].
Fourier transformer infrared spectroscopy (FTIR) provides a high-speed, reliable, and powerful modality to investigate the structure of molecules. FTIR spectrum shows various absorption bands that arise from different modes of vibration of molecules as that of the functional and finger print groups present in biological macromolecules. FTIR can be used as a powerful noninvasive diagnostic tool for many diseases recently [9-11].

2. Material and methods

2.1. In vivo experiment

Experiments were made on three groups of male albino rats of average weight 250-500 g (7 rats for each group). First group is the control group: which received a normal diet without any additives or injections (Normal). Second is the cirrhosis group: At which rats were injected by carbon tetrachloride ccl4 of 1 mg/kg (Induction). The third group is the treated group: in which rats were injected with curcumin at 5 ml/kg prior injection of carbon tetrachloride, and three times after induction at the second, sixth, and twelfth week (Treatment).

A Fresh blood sample was taken from the rats by the end of the twelfth week (for all groups) and placed in anticoagulation tubes. Blood plasma was prepared by anticoagulating whole blood, which was centrifuged at a speed of 3000 rpm for 10 min [12]. Plasma was then investigated optically and spectroscopically. All animal experiments were performed in accordance with relevant ethical guidelines and regulations for the care and use of laboratory animals.

2.2. Characterization

1- Refractive index measurement of plasma

The instrument used to measure the refractive index of the samples is (Abbe) refractometer through which refractive index can be measured directly. The sample is placed as a thin layer between an illuminating prism and a refracting prism. The refracting prism has a high refractive index of 1.75; the refractive index of distilled water is used for calibration. Refractive index of all plasma groups was measured at room temperature (26 ± 0.5 ^{o}C) [13].

2- Oxidative Stress Biomarkers (MDA – TAC)

Lipid peroxide, a harmful oxidative product formed when lipids undergo peroxidation. This process compromises cell membrane integrity and contributes to oxidative stress, causing many implications and various diseases. Lipid peroxide content in plasma was measured by the colorimetric method by measuring malondialdehyde (MDA), which is a byproduct of lipid peroxidation [14, 15].

Antioxidants are molecules that inhibit or neutralize the formation of lipid peroxides by scavenging free radicals and enhancing enzymatic defenses like glutathione peroxidase, which reduces lipid hydroperoxides to nontoxic alcohols, superoxide dismutase, which converts superoxide radicals into hydrogen peroxide, and catalase, which breaks down hydrogen peroxide into water and oxygen. Total antioxidant capacity (TAC) in plasma was measured by the Koracevich colorimetric method [16].

Statistical analysis of data was analyzed using Microsoft Excel (mean ± SD) for each group. T-test was used to compare both induction and treatment group to control group, *p≤ 0.05 is considered statistically significant.

3- FTIR spectroscopy of liver tissue

Animals were scarified for further investigations. Biopsy from the liver of 2 g was taken from all groups, rinsed tissue with cold saline to wash them, and put drops of phosphate-buffered solution (1:5) was added. Use a homogenizer at a cold temperature and centrifuge at 3000 rpm for 10 min. Use the supernatant as liver emulsion [17]. FTIR spectra of freeze-dried liver samples from all groups were recorded using a spectrophotometer in the spectral range 400–4000 cm^{-1}. Spectrum of each group was presented as an average value of recorded data and further processed by smoothing to calculate the respective spectral band areas [11, 18]. Various spectral band areas, band area ratios, as well as bandwidth of specific absorption bands were calculated us-

ing Origin 2018. All experimental procedures on animals followed the ethical standards.

3. Results and discussion:

1- Refractive index of plasma:

The refractive index of normal plasma recorded an average of 1.348±0.006. After induction of liver cirrhosis, value of refractive index increased to 1.359±0.02, the wide variation of collected data of the induced disease indicates disturbance, either by a pronounced increase or decrease with respect to normal values, these changes can be contributed to disturbances caused in plasma compositions; either in protein content, or oxygen saturation levels [19-21]. The treatment group that takes curcumin after induction of disease has shown an obvious return to normal values with a slight deviation of 1.355± 0.0015, indicating curcumin still has a good effect in preventing the accumulation of byproducts in plasma, as shown in Fig. 1.

2- Antioxidant content of plasma:

MDA, that is a marker for oxidative stress, has shown a highly pronounced elevation in values in the induction group that exceeded ninefolds its normal content value as present in Table 1. By using curcumin as a treatment, lipid peroxides (MDA) level decreased effectively, revealing curcumin's antioxidant power for eliminating reactive oxygen species byproducts [22]. Lipid peroxides are markers of oxidative damage, and their accumulation signals a need for antioxidant intervention to restore cellular balance.

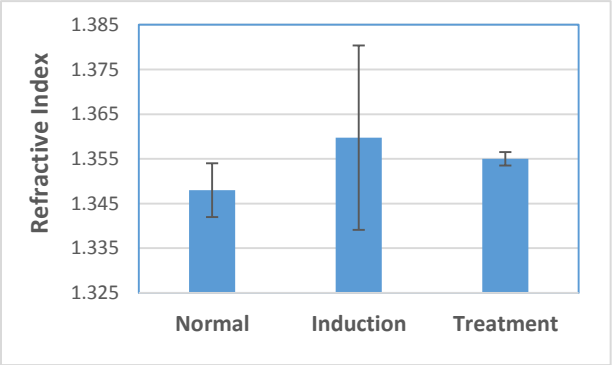

Fig. 1. Refractive index of plasma for Normal, Induction and Treatment groups

Table 1. Antioxidant content in plasma for different groups.

Antioxidants	Normal	Induction	Treatment
MDA (nmol/ml)	57.3±4.58	446.18*±86.05	192.91*±32.5
TAC mM/l	15.84±1.39	6.5*±3.29	9.826±2.34

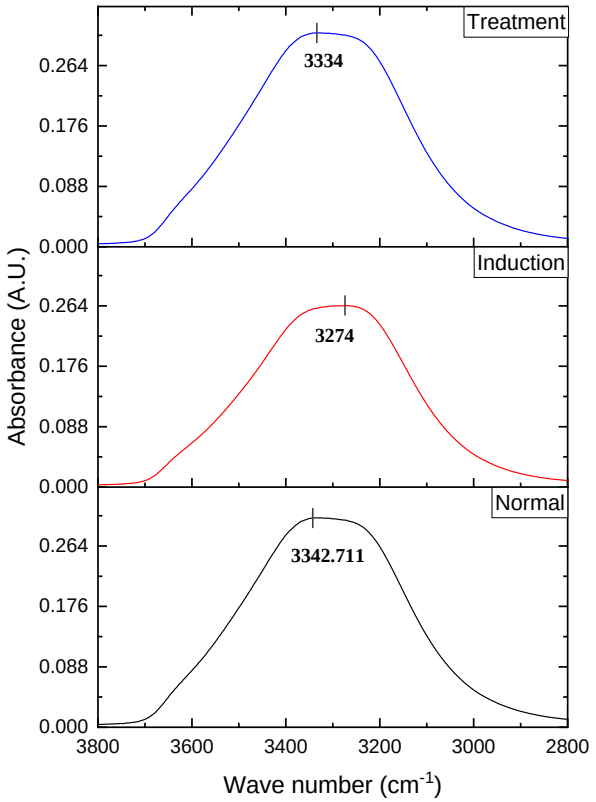

Fig. 2. Average FTIR Spectra of normal (Black), induction (Red), and treatment (Blue) groups of liver tissue in 3800 – 2800 cm^{-1}

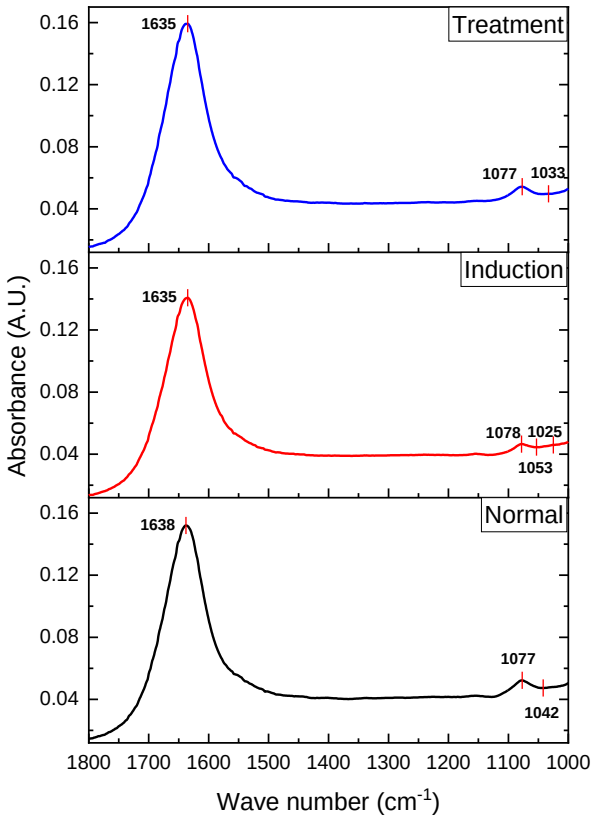

Fig. 3. Average FTIR Spectra of normal (Black), induction (Red), and treatment (Blue) groups of liver tissue in 1800 – 1000 cm^{-1}

The total antioxidant capacity measured after induction of disease was reduced comparable to its normal values, while using curcumin enhanced the level of TAC concentration in plasma, indicating its role in defending the oxidative stress caused by liver cirrhosis.

3 - FTIR Spectrum:

Figure 2 represents the spectrum belonging to the different groups in the functional group region. It shows that there is a shift in NH- stretching to lower wave number in the induction group (3272 cm^{-1}) compared to the control group of (3342 cm^{-1}), this shift due to liver cirrhosis indicates an increase in intermolecular interactions forming more hydrogen bonds yet weaker ones, while the treatment group reached (3334 cm^{-1}). In Fig. 3 which represents the fingerprint region, there is a shift in the amide I band in the induction group (1635 cm^{-1}) with respect to the control group of 1038 cm-1 and this is due to more beta sheet contents and aggregation of protein due to liver cirrhosis [23]. The slight change of the PO2 asymmetric band in the induction group (1078 cm^{-1}) is due to metabolic disorders in phosphate protein interactions. The presence of symmetric stretching of C-O (1042 cm^{-1}) indicates the adequacy of glycogen content. The splitting of C-O stretching to two bands, lower at 1053 cm^{-1} and higher at 1025 cm^{-1} in the induction group indicates the increase in glycogen content of cirrhotic liver cells due the decrease in glycogenolysis [24]. N-H, Amide I, PO2, and C-O bands in the treatment group with curcumin showed that there is a tendency to approach their normal values, as shown in Table 2 and accordingly the normal bond behavior [25].

Table 2. Different bands in FTIR Spectra of normal, induction and treatment groups of liver tissue in 700 -1 to 4000 cm^{-1}

	NH-Stretching	**Amide I**	**PO_2 Asymmetric**	**C-O stretching**
Normal	3342 cm^{-1}	1638 cm^{-1}	1077 cm^{-1}	1042 cm^{-1}
Induction	3274 cm^{-1}	1635 cm^{-1}	1078 cm^{-1}	1025, 1053 cm^{-1}
Treatment	3334 cm^{-1}	1635 cm^{-1}	1077 cm^{-1}	1033 cm^{-1}

4. Conclusion

Liver cirrhosis is a life-threatening disease that can be treated with natural products as curcumin, to prevent and may reverse its progression. This conclusion is stated due to the recorded results that have shown an improvement in the refraction of plasma because of reduction of oxidative stress, improve protein conformation, and decreased protein aggregation. Curcumin showed the capability of restoring normal behavior of molecules as indicated by bands in FTIR (functional and fingerprint regions) by stabilizing the phosphate group and restore normal hydrogen bonding, or approaching closer to normal values as indicated by refractive index and oxidative stress analysis.

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