RESEARCH ARTICLE

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Curcumin, Piperine and Taurine Combination Enhances the Efficacy of Transarterial Chemoembolization Therapy in patients with Intermediate Stage Hepatocellular Carcinoma: A Pilot Study

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Abstract

Introduction: Diagnosis of the majority of hepatocellular carcinoma (HCC) patients occurs at intermediate to advanced stages, with a few curative therapeutic options being available. It is therefore strongly urgent to discover additional adjuvant therapy for this lethal malignancy. This study aimed to assess the effectiveness of curcumin (C), piperine (P) and taurine (T) combination as adjuvant agents on serum levels of IFN-γ, immunophenotypic and molecular characterization of mononuclear leukocytes (MNLs) in HCC patients treated with Transarterial chemoembolization (TACE). **Patients and methods:** Serum and MNLs were collected from 20 TACE-treated HCC patients before (baseline-control samples) and after treatment with 5 g curcumin capsules , 10 mg piperine and 0.5 mg taurine taken daily for three consecutive months. Immunophenotypic and molecular characterization of MNLs were determined by flow cytometry and quantitative real time PCR, respectively. In addition, serum IFN-γ level was quantified by ELISA. **Results:** After receiving treatment with CPT combination, there was a highly significant increase in IFN- γ levels in the sera of patients when compared to basal line control samples. Additionally, the group receiving combined therapy demonstrated a downregulation in the expression levels of PD-1, in MNLs as compared to controls. MNLs' immunophenotyping revealed a significant decline in CD4+CD25+cells (regulatory T lymphocytes). Furthermore, clinicopathological characteristics revealed a highly significant impact of CPT combination on aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alpha feto protein (AFP) levels. **Conclusion:** This study introduces a promising adjuvant CPT combined treatment as natural agents to enhance the management of HCC patients who are candidates to TACE treatment.

Keywords: Hepatocellular carcinoma- TACE- curcumin- piperine- taurine

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Introduction

With escalating rates of morbidity and mortality, hepatocellular carcinoma (HCC), also known as primary hepatic carcinoma, is the most common kind of liver cancer and the second largest cause of cancer death [1]. The main causes of HCC, which is characterized by a high incidence, high degree of malignancy, frequent metastasis, and recurrence, are hepatitis B, C, and liver fibrosis [2]. Only 12.1% of liver cancer patients, especially those who are in the advanced and intermediate stages, survive for five years [3]. Patients with HCC in the intermediate stage are advised to undergo trans-arterial chemoembolization (TACE). Unfortunately, one of the main reasons for its failure is the emergence of multidrug resistance (MDR) [4].

In ancient China and Egypt, herbal medicines have been used for healing purposes for more than 4,000 years. An expanding range of herbal medications, including medicinal herbs and phytochemicals, have been used for treating chronic liver disorders globally in recent years due to their low cost, higher safety margins, long-lasting therapeutic effects, and lack of adverse effects [5].

The active component of the Curcuma longa plant, curcumin, is a small-molecule polyphenol compound derived from the rhizomes of Zingiberaceae, Araceae,

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and other plants. It has anti-inflammatory, antioxidant, anti-fibrosis, anti-tumor, and other pharmacological actions with low toxicity in normal cells [6]. It triggers cancer cell apoptosis, limits cancer cell growth and metastasis, reverses cancer cell multidrug resistance, increases cancer cell sensitivity to radiation and chemotherapy, and lessens related adverse side effects through a variety of methods [7].

Piperine is the most common plant alkaloid found in the Piperaceae species which recently gained a lot of attention due to a variety of positive biological and pharmacological properties, including chemopreventive, antioxidant, anticarcinogenic, immunomodulatory, and hepatoprotective effects [8]. Additionally, it is enhancing bioavailability of curcumin [9, 10].

Taurine (2-aminoethylsulphonic acid) is a semi-essential amino acid derivative that is abundant in mammalian cells and plasma. According to Lak and his team, it manifests in lymphocytes, monocytes, and neutrophilic granulocytes [11]. Under both acute and chronic oxidative and inflammatory stress, taurine helps cells maintain their homeostasis and cytoprotection [12, 13]. It stops tumor growth by inhibiting tumor markers, boosting antioxidant activity, and inducing tumor cell apoptosis [14].

Finding new treatments is crucial to lowering drug resistance, enhancing drug responsiveness, and lowering the cytotoxic side effects of traditional chemotherapies. Based on the existing evidence, our objective was to evaluate the effect of curcumin, piperine and taurine (CPT) combination treatment on serum levels of IFN-γ cytokine, and the expression levels of PD-1, CTLA-4 and FOXP3 genes as well as the immunophenotypes of MNLs in TACE-treated HCC patients.

Materials and Methods

Patients'samples collection

Twenty HCC patients (13 male and 7 females) were enrolled in this study. Patients admitted to HCC unit of National Hepatology and Tropical Medicine Research Institute (NHTMRI) in the period from 2021 to 2022. Study was approved by ethical committee 2021- NHTMRI- Cairo- Egypt (serial no.:17-2021) Eligibility criteria included patients with intermediate stage HCC, adequate organ function, and patients who can follow up for three months. One cycle TACE-treated HCC patients received 0.5 g of taurine (NOW FOODS, 395 S. Glen Ellyn Rd. Bloomingale, IL 60108, USA) and 5 g of curcumin containing 10 mg piperine (PURITAN'S PRIDE, INC. Ronkonjoma, NY. 11779, USA) orally once a day. Venous blood samples from HCC patients were collected in a tube without coagulant for serum separation and a tube containing EDTA for isolation of MNLs from patients prior (base-line) and after each treatment cycle with CPT combination. Serum was separated by centrifugation and stored immediately at -80°C for quantification of IFN-γ level. PD-1, CTLA-4 and FOXP3 gene expression levels as well as CD4+, CD8+ and CD4+25+ MNL phenotype in were evaluated. Further, a complete history, physical examination, radiological imaging and blood tests including a complete blood count (CBC), renal and liver function tests, alpha-fetoprotein (AFP) and lactate dehydrogenase (LDH) were reported for all patients at baseline and every month following each CPT treatment cycle.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted and purified from patients' MNLs using RNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentration was quantified by a NanoDrop-2000 spectrophotometer (ThermoFisher Scientific, USA). Complementary DNA (cDNA) was synthesized from 1 µg RNA using QuantiTect Reverse Transcription Kit (source) following the manufacturer's instructions. Relative expression of PD-1, CTLA-4 and FOXP3 mRNA levels were measured using SYBR Green reagent kit (Qiagen, Hilden, Germany) Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal house keeping control. Primers were obtained from Qiagen. All the qRT-PCR reactions were performed in triplicate in ViiA7 PCR system (Applied Biosystems, CA, USA). Relative gene expression levels were determined and expressed as fold change applying the2–ΔΔCt method as we previously described [15].

ELISA for IFN- γ

Amount of serum IFN-γ levels was assessed in triplicates using enzyme-linked immunosorbent assay (ELISA) kit provided by (Diaclone Research, Besancon, France), according to the manufacturer's instructions.

Flow cytometry

Peripheral blood samples were used to immunophenotype using antihuman CD4 FITC, CD8-PE, and CD25-APC antibodies (Beckman Coulter, California, USA). Applying the whole blood lysis staining method. Briefly, 100 µL blood samples were stained with the antibodies in the dark for 20 minutes. Afterwards, RBCs lysis reagent was added and left for 5 minutes at room temperature. A total of 3.5 mL PBS was added, and the whole samples were centrifuged at 300 xg for 3. Labeled cells were resuspended in PBS-and vortexed before being acquired and analyzed in FACS Canto Flow Cytometer (BD Bioscience, New York, United States). Data were analyzed with BD FACS Diva software .

Statistical analysis

Data were statistically analyzed using statistical software package (IBM-SPSS) version 23. Kolmogorov-Smirnov test was applied to check the data distribution. Most variables were normally distributed. Thus, one-way ANOVA was utilized to study the effect of sampling at different time intervals. Least significant difference (LSD) was used to check for significant differences among the studied groups. Pearson's correlation coefficient was applied to correlate the time with the studied variables. For non-normally distributed data. chi-squared test was applied to estimate the effect of sampling time on the non-parametric data. Wilcoxon test was used to illustrate statistical differences between males and females. Mann-

Whitney test was applied to show statistical differences for non-parametric data between different time intervals.

Results

Patients' characteristics

HCC patients enrolled in the study had ages ranging 35-65 years old. The male to female ratio was 2: 1. 65% was male (n=13), while 35 % were female (n=7). Approximately 100% of patients were HCV- positive and 100% of patients were child B. In addition, 95% of patients had no ascites and 100% had no encephalopathy as shown in Table 1.

Curcumin, piperine and taurine combination effect on some blood picture parameters in TACE-treated HCC patients. Hemoglobin (Hb) content, white blood cell (WBC) count and percentage of lymphocytes (LYM) of HCC-cases are depicted in Figure 1. Prior treatment, males had significantly higher a WBC count than. Interestingly, females' WBC counts significantly increased two and three months post treatment. No significant differences were observed for The LYM percentages and Hb content in HCC patients except for a marked reduction of Hb content in females 2 months post treatment, and that reduction was significantly increased 3 months post-treatment.

Effect of curcumin, piperine and taurine combination treatment on liver functions

Notably, serum activities of aspartate aminotransferase (AST) in both males and females were decreased post-treatment. Three months after treatment, the activity of AST in men was significantly lower than in women. The levels of Bilirubin, albumin, and ALT activity did not significantly alter over the course of the combined treatment (Table 2). Effect of the combined treatment on serum levels of lactate dehydrogenase (LDH) and the

Table 1. Characteristics of HCC Patients

Ages (years) (Mean ± SD)	(35-65) males (61.61 ± 1.47) females (63.00 ± 1.69)
Sex, No. (%) male/female	13 (65%)/7(35%)
Etiology, No. (%)	
HCV [a]	20 (100%)
HBV [b]	0
Other	0
Ascites, No. (%)	
None	19 (95%)
Mild/moderate	1(5%)
Severe	------
Hepatic encephalopathy	
None	20 (100%)
AFP[c]	
>200	9 (45%)
<200	11(55%)
Barcelona Clinic Liver Cancer (BCLC) staging system	
Child B	20 (100%)

a, Hepatitis C virus; b, Hepatitis B virus; c, Alpha fetoprotein

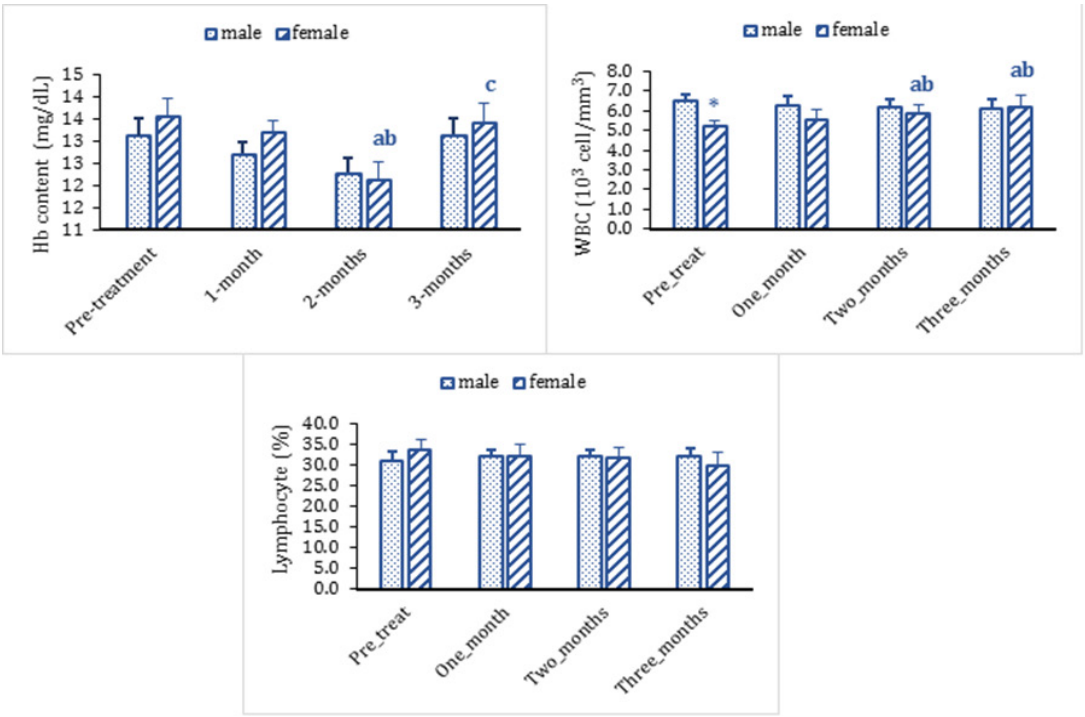

Figure 1. Hemoglobin (Hb) Content, White Blood Cell (WBC) Count and Percentage of Lymphocytes (LYM) of HCC-Patients Treated with TACE alone, or after 1-, 2- and 3-months following treatment with TACE together with a mixture of curcumin and piperine. Data is displayed as mean ± standard error. a: represents a significant difference, as compared to the corresponding pre-treatment value. b: represents a significant difference, as compared to the corresponding value after 1-month. c: represents a significant difference, as compared to the corresponding value after 2-months. *: significant difference ($p<0.05$), as compared to the female values.

Table 2. The Effect of Treatment Cycles on Serum Activity of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) as Well as the Levels of Bilirubin (Bil) and Albumin (Alb) of HCC-Patients

Parameter	Sex	Pre-treatment	Post-treatment			Effect of time
			One month	Two months	Three months	
AST (U/L)	Female (n=7)	33.71 ± 2.97	26.57 ± 1.95a	26.71 ± 1.60a	29.00 ± 1.86a	$F_{3,18}$=3.296, P=0.044, r=-0.26
	Male (n=13)	33.00 ± 2.34	25.55 ± 0.85a	25.39 ± 0.49a	25.46 ± 0.42a*	$F_{3,36}$=10.052, P=0.000, r=-0.50
ALT (U/L)	Female (n=7)	25.57 ± 2.86	23.86 ± 2.65	24.57 ± 2.72	25.43 ± 2.75	$F_{3,18}$=2.234, P=0.119, r=+0.00
	Male (n=13)	27.15 ± 2.78	25.39 ± 1.82	26.38 ± 1.66	25.15 ± 1.49	$F_{3,36}$=0.658, P=0.583, r=-0.08
BIL (mg/dL)	Female (n=7)	0.85 ± 0.11	0.80 ± 0.10	0.86 ± 0.09	0.79 ± 0.11	$F_{3,18}$=0.185, P=0.905, r=-0.06
	Male (n=13)	0.84 ± 0.09	0.79 ± 0.09	0.76 ± 0.08	0.74 ± 0.06	$F_{3,36}$=1.95, P=0.139,r=0.13
Alb (g/dL)	Female (n=7)	3.91 ± 0.09	4.01 ± 0.12	3.83 ± 0.10	3.83 ± 0.09	$F_{3,18}$=1.53, P=0.241, r=-0.19
	Male (n=13)	3.90 ± 0.12	3.95 ± 0.14	3.91 ± 0.10	3.69 ± 0.11	$F_{3,36}$=1.927, P=0.143, r=-0.18

Data is displayed as mean ± standard error. r, Pearson's correlation coefficient of the time with the studied variables; P<0.05: represent significant effect; [a], represents a significant difference, as compared to the corresponding pre-treatment value; [b], represents a significant difference, as compared to the corresponding value after 1-month; [c], represents a significant difference, as compared to the corresponding value after 2-months; *, significant difference, as compared to the female values.

coagulation factors prothrombin time and international normalized ratio on TACE-treated HCC patients.

In all HCC patients, prothrombin times (PT), the international normalized ratio (INR) and lactate dehydrogenase (LDH) levels are depicted in Figure 2. No marked variations were detected in the PT and INR during different time intervals or between males and females. After 1-month of the treatment, LDH activity in males was significantly elevated compared its basal level before treatment. However, LDH activity was significantly

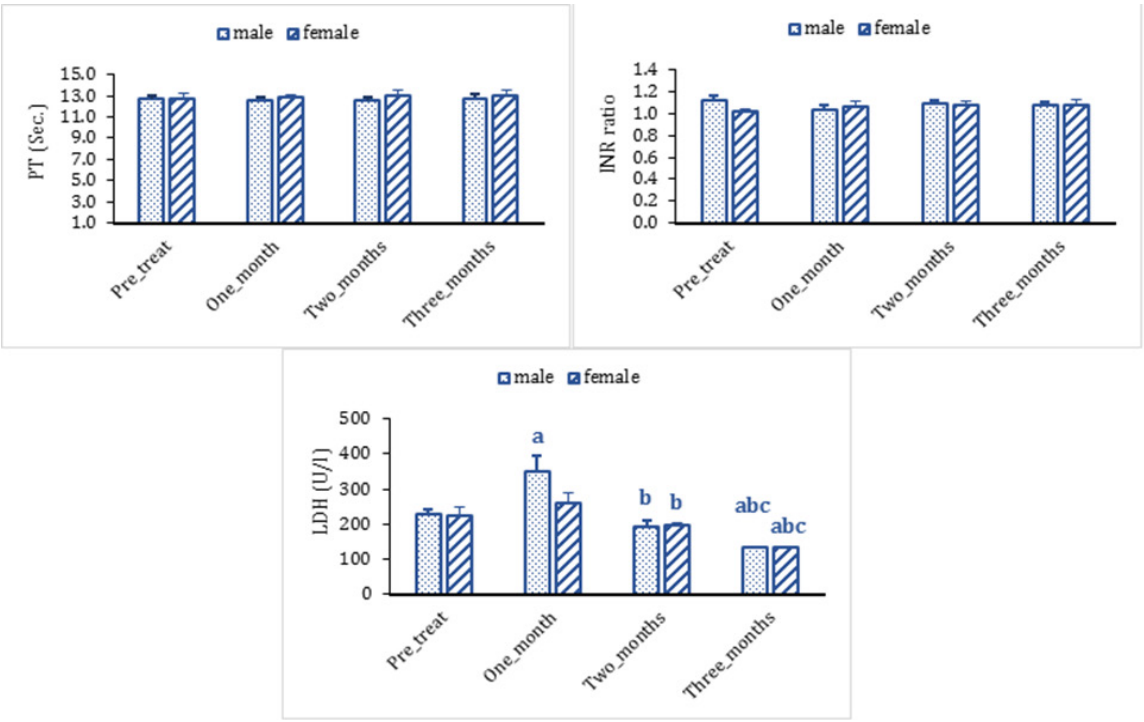

Figure 2. Prothrombin time (PT), the International Normalised Ratio (INR) and Lactate Dehydrogenase (LDH) of HCC-patients treated with TACE alone, or after 1-, 2- and 3-months following treatment with TACE together with a mixture of curcumin and piperine. Data is displayed as mean ± standard error. a: represents a significant difference, as compared to the corresponding pre-treatment value. b: represents a significant difference, as compared to the corresponding value after 1-month. c: represents a significant difference, as compared to the corresponding value after 2-months. *: significant difference (p<0.05), as compared to the female values.

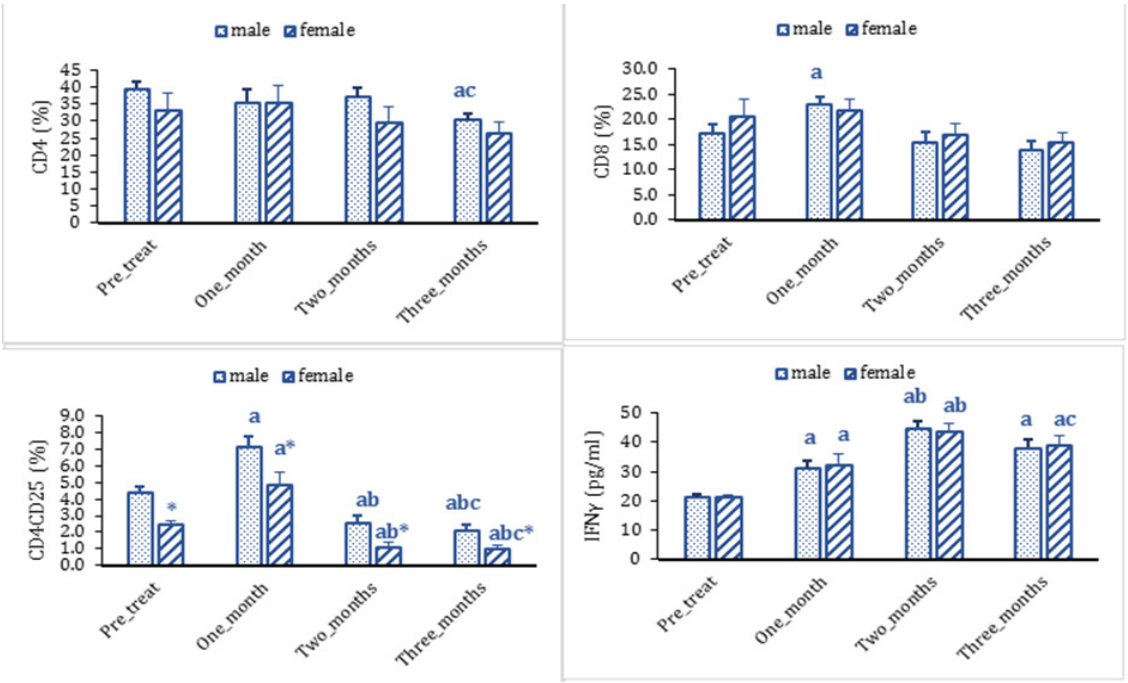

Figure 3. Percentages of CD4, CD8 and CD25 cells and INF- γ level in HCC-patients treated with TACE alone, or after 1-, 2- and 3-months of treatment with a mixture of curcumin, piperine, and taurine.Data is displayed as mean ± standard error. P<0.05: represent significant effect a: represents a significant difference, as compared to the corresponding pre-treatment value. b: represents a significant difference, as compared to the corresponding value after 1-month. c: represents a significant difference, as compared to the corresponding value after 2-months. *: significant difference , as compared to the female values.

decreased in both males and females after 2 and 3 months of the treatment.

Effect of treatment with curcumin, piperine, and taurine combination on level of alfa-fetoprotein in TACE-treated HCC patients

The levels of the HCC tumor marker alfa-fetoprotein (AFP) were assessed during the course of the study (Table 3). The levels of AFP were notably higher in females than in males. Interestingly, in males and females, after 3-months of treatment, the levels of AFP were significantly declined, in comparison to the pre-treatment and after 2 months treatment Effect of curcumin, piperine, and taurine combination on immunophenotyping profile of (MNLs)

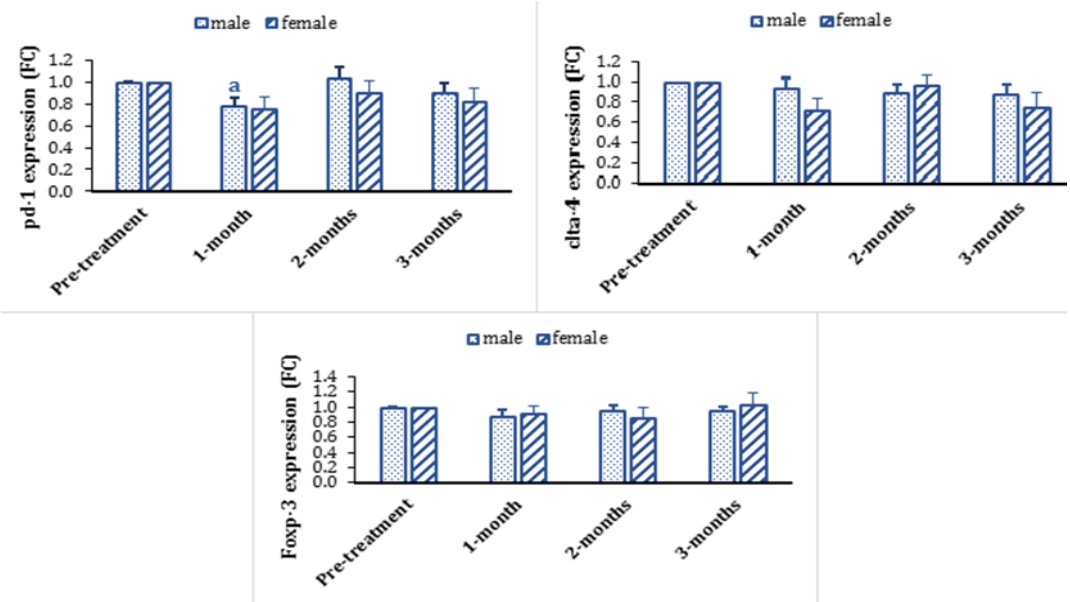

Figure 4. The Expression Level of PD-1, CTLA-4 and FOXP-3 mRNA Levels of TACE-treated HCC-patients before, after treatment with curcumin, piperine and taurine combination. Data is displayed as mean ± standard error. P<0.05: represent significant effect a: represents a significant difference, as compared to the corresponding pre-treatment value. b: represents a significant difference, as compared to the corresponding value after 1-month. c: represents a significant difference, as compared to the corresponding value after 2-months. *: significant difference as compared to the female values.

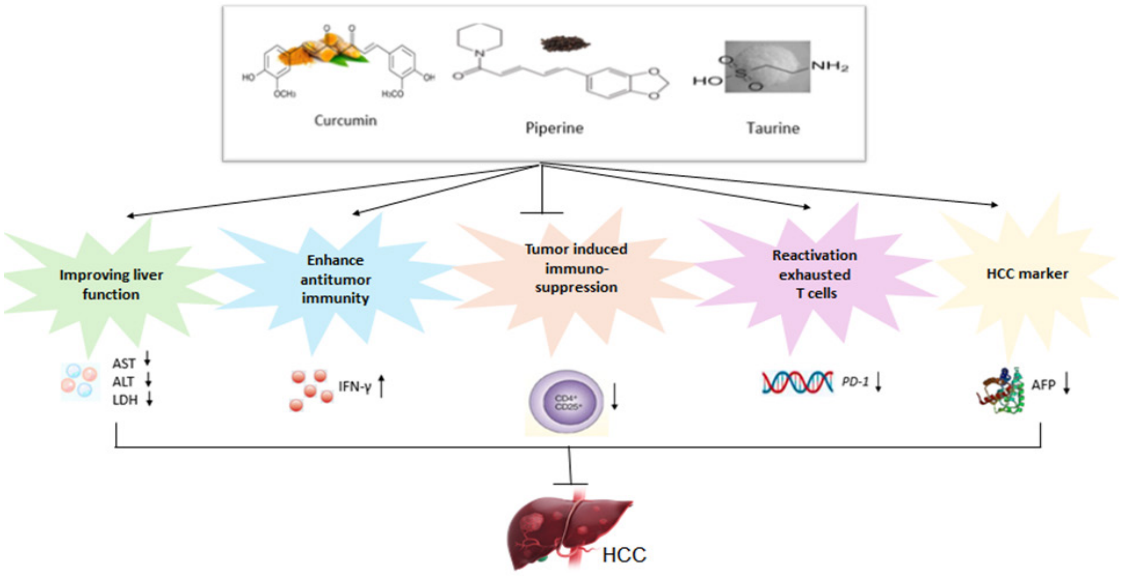

Figure 5. Mechanism by which HCC is Combated by CPT Treatment

Table 3. Levels of Alfa-Fetoprotein (AFP) of HCC Patients Treated with TACE (base Line Controls), after 1-, 2- and 3-Months Following Treatment with a Mixture of CPT. Data is displayed as median and interquartile range.

Parameter	Sex	Pre-treatment	Post-treatment			Chi-squared, df, P-value
			One month	two months	Three months	
AFP (ng/mL)	Female (n=7)	200.0 (5.28-312.3)	210.0 (3.77-333.20)	100.0 (3.80-210.00)	19.00 (3.70-20.00)[ac]	11.77, 3.0, P=0.008
	Male (n=13)	3.00 (2.83-14.30)*	3.36 (2.90-32.50)*	3.80 (3.80-33.00)*	3.20 (2.50-14.00)[ac]*	22.03, 3.0, P=0.000

P<0.05: represent significant effect; [a], represents a significant difference, as compared to the corresponding pre-treatment value; [b], represents a significant difference , as compared to the corresponding value after 1-month; [c], represents a significant difference , as compared to the corresponding value after 2-months; *, significant difference, as compared to the female values.

and level of interferon gamma (INF- γ) on TACE-treated HCC patients.

The percentages of CD4+, CD8+ and CD4+CD25+ T cells of HCC patients are shown in Figure 3. After one-month of treatment, males showed a significant elevation in the percentage of CD8+ cells, as compared to their percentage at basal level before the combined treatment Unexpectedly, CD4+ cells was significantly declined in males after three months of treatment, as compared to their frequency pre-treatment and after 2-months. By the 2nd month, both males and females showed a slightly decline in the CD8+ expression till the end of experiment. In males, the CD4+ CD25+ cells were remarkably higher by approximately two-fold than those in females either pre- or post-treatment. After 1-month of treatment, CD4+CD25+ cells were markedly elevated in both males and females, as compared to their levels before treatment. After 2 and 3 months of treatment, the CD4+CD25+ cells were significantly reduced in both sexes, as compared to their frequency at pre- and one-month treatment.

Effect of curcumin, piperine, and taurine combination on serum IFN-γ level

The combination therapy gradually increased IFN- γ levels in males and females with HCC patients after one- and two-month of treatment of note, a marked decline of IFN- γ levels in males was observed after 3 months of treatment, however, it don't reach the basal levels before the treatment (Figure 2).

Changes of PD-1, CTLA-4 and FOXP-3 expression levels in response to the combined treatment

The gene expression levels of PD1, CTLA-4 and FOXP3 of all cases were evaluated.We did not find any significant changes in the mRNA expression levels of PD-1, CTLA-4 and FOXP3 except for a significant downregulation of PD-1 after one month treatment in males (Figure 4).

Discussion

The outcomes of this study have shed light into the effects of daily CPT administration for three months on the management of TACE, the therapeutic stronghold in patients with intermediate stage HCC. Our findings suggest that the tested combination mediates a number of events including reduction of T-reg cells, suppression of T cell apoptosis, and a rise in INF-γ levels. Increased activities of transaminases the hepatotoxicity indicators were reversed. Moreover AFP and LDH levels were decreased during the time of investigation. All of these processes support liver functions and the reactivation of immune activity against malignancy, leading to tumor regression.

Our results show that in both males and females, after one month of receiving the adjuvant treatment of CPT

combination in HCC patients, serum levels of IFN- γ significantly increased compared to basal line values with a p value = 0.000 between different treatment cycles. Our results are in agreement with previous studies that showed a significant increase in IFN-γ level after administration of curcumin as well as CPT combination which lead to an enhanced antitumor immunity in liver cancer [16, 17]. A study done by Kotb and his team reported that after the first, second, and third cycles of CPT treatment, patients receiving CPT had significantly increased plasma levels of IFN- γ compared to baseline levels [18]. Another research by Elhouseini et al. reported that taurine combined with curcumin inhibited experimental hepatocarcinogenesis and the levels of IFN- γ in sera of mice were highly elevated after treatment [19]. In contrast, a study done by De Veer et al showed that increased IFN- γ levels were correlated with tumor progression and a worse of disease [20]. This can be attributed to the IFN-γ pleiotropic functions, as its action is mediated by cell-specific functions via regulation of genes, including inflammatory signaling molecules, apoptosis and cell cycle regulators, and transcriptional activators Based on its well documented cytostatic, pro-apoptotic anti-proliferative and antitumor functions, IFN-γ is considered potentially useful as adjuvant immunotherapy for different types of cancer [21]. Moreover, it was known that high IFN-γ levels positively associated with better survival in patients with different cancer entities [22]. Perforin/granzyme-induced apoptosis is the main pathway used by cytotoxic lymphocytes to eliminate virus-infected or transformed cells [23]. IFN- γ was known to operate as a cytotoxic cytokine in tumor cells, triggering apoptosis along with granzyme B and perforin [24, 25]. IFN- γ initiates the immune response in an inflammatory setting, promotes the destruction of pathogens and altered cells, and inhibits the over-activation of the immune system that causes tissue damage [26]. Additionally, it facilitates the production of indoleamine-2,3-dioxygenase (IDO) and immunological checkpoint inhibitory molecules, activating additional immune-suppressive pathways [27, 28, 29].

Regarding the immunophenotyping profile, males had a substantial increase in CD8+ T cells after receiving treatment for one month as compared to pre-treatment levels. Contrarily, our findings indicated that CD4+ T cells in TACE-treated HCC patients were marginally decreased in both males and females after 3 months of adjuvant CPT combination as compared to pre-treatment values. These ideas are consistent with prior research showing that CD4+ and CD8+ T lymphocytes proliferated in the early stages of the disease and exhausted in the middle and advanced stages in HCC patients treated with TACE and adjuvant combination of CPT [30]. According to earlier research [31] , curcumin is a useful drug for restoring CD4+ and CD8+ T cells in the tumor microenvironment, promoting a Th1 (CD4+) type response once more. Unexpectedly, our findings showed that CD4+ and CD8+ were somewhat decreased. Given that is a high level of lymphocyte exhaustion in cancer patients generally and particularly in HCC patients treated with TACE as they are frequently have leukopenia or neutropenia at the time of TACE due to underlying cirrhosis and portal hypertension [32], we suppose that this combination tolerated the greater suppression and exhaustion in lymphocytes that could occur in the absence of receiving the advised adjuvant therapy so the decrease in CD4+ and CD8+ was non-significant.

It has been proposed that a rise in T-reg cells is associated with the suppression of the immune system's anti-tumor response and, consequently, with the growth of tumor cells. The production of immunosuppressive cytokines by T-reg cells, a major strategy utilized by the developing tumor to evade immune monitoring, may help to explain this connection [33, 34]. Anti-CD25 antibody reduction of T-reg cells has been demonstrated to improve anticancer activity, which is pertinent to antitumor immunotherapy [35]. Our results showed that after two months of therapy, CD4+CD25+ T cells started to noticeably decrease in both sexes with a p value < 0.05, and they continued to decrease until the end of the experiment with a p value = 0.000 comparing different treatment cycles. Evidence that regulatory T cells (CD4+CD25+) suppress stimulated CD4+ and CD8+ T cell proliferation and activation and thereby prevent tumor-specific T cell responses as well as the immune system's capacity to recognize liver cancer cells in HCC patients supported the positive results for CD4+CD25+ T cells% [36, 37]. This outcome supports the claims made by other researchers who suggested that curcumin could considerably lower the T-reg population (CD4+ CD25+) [38, 16]. According to a different study, giving curcumin to tumor-bearing mice reduced the number of CD4+CD25+FOXP3+ Treg cells in the lymph nodes, tumor locations, and peripheral circulation [31]. Recent study by kotb et al showed that patients administered CPT had a significant decrease in the plasma level of CD25 after the first, second and third cycle of treatment compared to the baseline level [18].

By producing PD-L1, which interacts with PD-1 on T cells to negatively modulate immunological responses, tumors can evade host immune surveillance [39]. Accumulating evidence indicates that the inhibition of PD-1 promotes an effective immune response against cancer cells [40]. Our results showed a significant down regulation of PD-1 mRNA expression in males after the first month treatment and between different treatment cycles with P<0.05 and value=0.037 respectively. Many studies indicated that blocking PD-1 signaling might improve clinical outcomes for patients with various malignancies [41]. A research by Hamanishi and his group revealed that the number of intra-epithelial infiltrating CD8+ T cells is inversely linked with PD-L1 expression in ovarian cancer, indicating that PD-L1 expression on tumor cells inhibits CD8+ T cell infiltration at tumor locations [42]. Our findings are consistent with a study by Liu et al. 2021 that assessed the immune-modulating effects of curcumin and found that curcumin therapy reduced the expression of PD-1 in head and neck squamous cell cancer cell lines [43].

Additionally, the current study demonstrated that CPT treatment could effectively enhance hepatic functions and suppress a hepatocarcinoma marker, as demonstrated by

the considerable drop in AFP serum levels after the third cycle of CPT compared to the baseline level. Following several therapy cycles, the biochemical parameters of AST and ALT activities as well as LDH levels as indicators of liver functions all considerably decreased in the sera of HCC patients. LDH levels have been shown to constitute an indirect indicator of tumor hypoxia, neo-angiogenesis, and a worse prognosis in a variety of tumor forms. It has also been previously shown that LDH levels are a significant predictor marker in HCC patients undergoing TACE. The association of CPT administration LDH as a hypoxia marker with TACE, which is primarily focused against the angiogenetic pathway, could be a crucial tool in patients' care [44]. Our findings demonstrate a highly significant drop in LDH level over the duration of the experiment, with p values for females and males of 0.003 and 0.000, respectively. These results back up the hypothesis put forth by Faloppi et al. that curcumin may prevent HCC by reducing the concentration of AFP in the tumor tissue of the experimental animals. Our findings concurred with those of Salehi et al., who found that oral daily curcumin administration for 8 weeks was well tolerated and caused healthy females' levels of CRP and LDH to significantly decline [45].

In conclusion, all these evidences pointed to the fact that CPT enhanced the anticancer effects of TACE and slowed the growth of the tumor and protected against liver injury .The results of the present work support the notion that in a population of patients with intermediate stage HCC, the administration of CPT combined treatment as a potential immune-adjuvant for three consecutive months was able to increase the circulating level of the anti-inflammatory cytokine IFN-γ, and suppresses the expression levels of PD-1, and that in turn enhances liver functions and other clinicopathological features of TACE-treated HCC patients. This reflects the immunomodulation of CPT treatment that may offer new avenue to manage patients with intermediate stage HCC patients and give better outcome. The suggested mechanism by which HCC is combated by CPT treatment is illustrated in Figure 5.

Study limitation

There was a limitation to our study, as it considered a preliminary study performed on a small sample size. Larger numbers of participants might yield more robust statistical analysis. Even with this drawback, the study has unique benefits of its own because it could lead to the development of new and promising therapeutic strategies.

Author Contribution Statement

Conception and design :Emad M. Elzayat, Motawa E. El Houseini and Sherif Abdelaziz Ibrahim. provision the samples and data of the clinical part of this work. : Amr Abdelraouf. Performance the practical work :Reham A. Abd El Rahiem. Sharing in the practical part of the work: Randa A. Osman and Heba Effat. Data interpretation: Heba Effat. Writing the first draft of the manuscript: Heba Effat. All authors reviewed the manuscript. All authors approved the final manuscript.

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Scientific approval

This study is a part of PHD thesis of faculty of science, Cairo University, Cairo ,Egypt.

Ethics approval and consent to participate

Informed consent was obtained from all participants in this study. This study was approved by ethical committee 2021- NHTMRI- Cairo- Egypt (serial no. : 17-2021)

Data availability

All data is available in this manuscript

Competing interests

The authors declare that they have no relevant financial or non-financial conflicts of interests to disclose.

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