Effect of Conocarpus Erectus Extraction on Some Physiological and Biochemical Parameters on Male Rats

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Abstract

The present study was conducted to evaluated some of physiological and biochemical parameters ,we used eighteen of males rats divided randomly to three group (six in each), group one considered as control ,group two the animal treated with 250 mg /kg and group three treated with 500 mg /kg .The result show non-significant appeared in RBC , Hb, PCV ,MCH , MCV and MCHC ,while the WBC , lymphocytes and granular show increased significantly . The biochemical test show increased significantly in liver enzyme concentration ALT ,AST and ALP also the result of lipid profile appeared decreased in al parameters exception the HDL which appeared increased significantly .

Keyworlds ; *Conocarpus Erectus ; Liver enzyme ;lipid profile.*

Introduction

From ancient time till now and tomorrow, the use of natural resources especially plants increases day by day for the discovery of new therapeutic agents, natural products from some of these natural resources continue to be used in pharmaceutical preparations either as crude extracts, fractions, pure compounds or analogous compounds from highly active isolated compounds[1]. Plant-derived substances have recently become of great interest owing to their versatile applications medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[2,3] Conocarpus erectus, a member of combretaceae family, is widespread in tropical and subtropical areas, which is found in the form of a shrub,1.5 to 4 m tall [4], however it sometimes can grow up to 20 m. Conocarpus erectus L. (Combretaceae), known in English as buttonwood or button mangrove, is one of two species in the genus Conocarpus. The plant is spreading crown, grey or brown bark, glaucous medium-green leaves and greenish flowers in dense cone-like heads in terminal panicles[5]. It is a folk remedy for anemia, catarrh, conjunctivitis, diabetes, diarrhea, and fever ,the buttonwood tree, Conocarpus erectus, has not been investigated previously for its biological activity or for its constitutive phenolics ,due to the interest in the biological activity as well as the diverse plant bioactive metabolites[6,7,8] an investigation of the phenolics of the leaves of C. erectus was undertaken. Thirteen phenolics (1-13), including the new natural product 3,3′,4′-tri-O-methylellagic acid 4-O-β-glucupyranuronide have been isolated and purified from the aqueous alcohol extract of the leaves of this plant. all structures were confirmed by NMR analysis , in addition, the antioxidant potential based upon the principle of xanthine/ hypoxanthine oxidase assay of the plant leaf extract, the new compound together with the glucuronides, quercetin 3-O-glucupyranuronide, myricetin 3-O-β-glucupyranuronide 3′,5′-dimethoxymyricetin3-O-β-glucupyranuronide, syringetin 3-O-β-glucupyranuronide and gallic acid

Aim of Study

For lack of adequate study of the plant Conocarpus erectus, the study design to evaluate the efficacy of Conocarpus erectus extract on some physiological and biochemical parameters in male rats.

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Material and Method

1-Collection and Preparation of the Plant

The leaves of the plant under investigation were collected from some farms in basra city at Iraq. The leaves was collected and shade dried then finely powdered by electrical grinder , and kept in Dark glass containers and become ready for extraction process.

2- Preparation of Conocarpus erectus extract

Conocarpus erectus were cleaned, washed and dried at room temperature. Leaf were grounded for 2 minutes by electrical grinder. The leaf powder were refluxed with 250 ml (ethanol 70%) for 12 hours by Soxhlete, and then filtered by using Buchner funnel and filter paper . The solvent was dried and concentrated by using rotary evaporator at 50ºC. The final dryness was done by leaving residue in room temperature .

Experiments design

Eighteen of male rats about 3 months age and 200 – 250 weight were used in this experiment. The animals were divided randomly into three groups (6 rats for each group) as following :

Group one (control): In which rats were given 0.5 ml of normal saline by gavage tube daily for three weeks . .

Group two :- The rats were given 250 mg /Kg /Bw of Conocarpus erectus extract by gavage tube daily for three weeks .

Group three : The rats were given 500 mg /Kg /Bw of Conocarpus erectus extract by gavage tube daily for three weeks .

Collection of blood samples.

Blood sample (5 ml) were collected from heart puncher , after anaesthetised the rats with chlorophorme . The(2 ml) of blood collected from each animal were stored in plastic sample test tube containing ethylene diamine tetra acetic acid (EDTA) anticoagulant for hematological studies which done directly after collection , while another portion (3 ml) of blood was deposited in to tube without anticoagulant and allowed to clot at room temperature . Then the blood samples were centrifuged at (5000 rpm) for 30 minutes and serum sample were stored in polyethylene tubes at (– 20c) until used for biochemical analysis .

Hematological Study

The hematological tests were done in the laboratory by using Hematology auto analyzer (Huma Counts 5) made in Germany company serial no.160247 . the instrument can measures and calculates 22 different parameters .The Hematology auto analyzer containing four solution(HC5 – BASOLYSE containing cyanide free lyse reagent ,HC-LYSECF containing cyanide free lyse reagent ,HC5 – EOLYSE containing cyanide free lyse reagent and HC- Cleaner cleaning solution used to clean fluidics system) and the instrument have a printer mechanic inside with thermal paper . The hematological parameter estimated by this instrument were (RBC, WBC. DWBC ,Hb , PCV ,MCV ,MCH and MCHC)

Biochemical test

The biochemical tests were done in the laboratory by using chemistry auto analyzer made in Germany by human star company serial no.20628 ,the machine has 54 wells which numbered from 1 to 54 , The serum samples deposited in each specific wells . The reagent was put in a special container beside the wells. The serum biochemical parameters estimated by this instrument were lipid profile TG , TC ,HDL , LDL , AST, ALT, and ALP .

Results

1-Blood parameters

The results in table (1) revealed that conocarpus erectus extract administration to male rats in two doses 250 mg/kg/Bw and 500 mg/kg/Bw caused non significant in RBCs counts , hemoglobin concentration, hematocric ratio, MCV, MCH and MCHC when compared with control group.

Table (1): Effects of Conocarpus erectus extract on RBCs , Hb , PCV, MCV, MCH and MCHC . n=6

	RBC (1x106 mm)	Hb g/L	PCV %	MCV fL	MCH pg	MCHC g/L
Control group	7.55 ± 0.23 N.S	12.42± 0.90 N.S	37.1± 0. 25 N.S	57.17 ±3.35 N.S	18.42 ±0.96 N.S	30.8 ±2.18 N.S
250 mg/kg/Bw	6.31 ± 0.18 N.S	12.2 ± 0.22 N.S	37.5 ± 0.34 N.S	59.6 ± 2.14 N.S	19.7 ± 0.23 N.S	33.3 ± 1.25 N.S
500 mg/kg/Bw	6.62 ± 0.44 N.S	12.4 ± 0.51 N.S	38.4 ± 0.12 N.S	57.4 ± 2.89 N.S	18.3 ± 1.39 N.S	32.3 ± 2.10 N.S

The results in the present study table (2) showed that total leucocytes count and lymphocytes percentage increased significantly (P<0.05), when the animal treated with Conocarpus erectus extract compared with control group . On the other hand, neutrophil , eosinophil and basophil percentages were increased significantly (P<0.05),in all treated group .

Table (2): Effects of Conocarpus erectus extract on WBC , Lymphocytes and Granulocytes . n=6

	WBC(1x103 mm)	Lym	Gra.
Control group	5.84 ± 0.71 c	51.82 ± 3.23 c	31.80 ± 2.90 b
250 mg/kg	16.40 ± 3.62 a	64.30 ± 1.72 a	32.82 ± 2.24 b
500 mg/kg	12.60 ± 2.20 b	56.52 ± 3.41 b	40.11 ± 2.43 a

Different letters indicate significant difference at (P≤0.05)

Clinical chemistry data

The baseline data for clinical chemistry parameters collected from three group are shown in Table (3) . The result showed increased significantly (P<0.05),in AST enzyme especially in low dose 250 mg /kg compared with control ,also the same table appeared increased significantly (P<0.05),in ALT and ALP in two doses of conocarpus erectus extract compared with control group.

Table (4) showed decreased significantly (P<0.05) in total cholesterol , triglyceride and LDL in all treated group especially in low dose 250 mg /kg compared with high doses 500 mg /kg and control group ,while the HDL concentration in contrast appeared increased significantly (P<0.05) compared with control group .

Table (3): Effects of Conocarpus erectus extract on AST , ALT and ALP enzyme concentration . n=6

	AST(U/L)	ALT(U/L)	ALP (U/L)
Control group	45.64 ± 1.77 c	28.13 ± 1.26 b	55.7 ± 6.24 c
250 mg/kg	120.5 ± 10.20 a	48.6 ± 6.75 a	212.6 ± 13.62 b
500 mg/kg	96.7 ± 9.54 b	51.9 ± 4.83 a	244.6 ± 15.91 a

Different letters indicate significant difference at (P≤0.05)

Table (4): Effects of Conocarpus erectus extract on TC , TG, LDL and HDL concentration . n=6

	TC(mg/dl)	TG(mg/dl)	LDL (mg/dl)	HDL(g/ml)
Control group	119.1±6.13 a	18.7±9.6 a	46.15±8.57 a	37.3±1.90 c
250 mg/kg	63.6 ± 3.05 b	7.3 ± 1.10 b	28.3 ± 2.16 c	41.9 ± 1.10 b
500 mg/kg	59.3 ± 3.16 b	5.6 ± 0.25 c	35.6 ± 2.10 b	52.7 ± 1.20 a

Different letters indicate significant difference at (P≤0.05)

Discussion

This study was designed for examining toxicity of methanolic extracts of Conocarpus erectus herb , due to lack of resources found, we decided to study the chronic toxicity by giving daily for three week of two dose of extracts 250 and 500 mg /kg and observe the physiological and biochemical changes that appear on the laboratory animal during the administration.The results observed in the present study as represented in the Table (1) show no significant appeared in RBC count , Hb concentration , pcv percent , MCV, MCH and MCHC on male rats treated 250 and 500 mg/kg /BW of ethanol conocarpus erectus extract ,while the result of total WBCs count and differential leukocytic count as represented in table (2) has been shown significant increase in mean total WBCs count and lymphocyte following administration of Conocarpus erectus extract for three week that maybe due to the extract caused increasing stress may have resulted from the oxidative damage caused by ROS generated in response to stress which lead to increase in WBC count .

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified[9]. Serum liver enzyme significantly increased in blood serum AST ,ALT and ALP table (3) that maybe due to Plant possessed significant phytotoxic activities ,these properties may be due to the presence of flavonoids and phenols[10] . Also there was hepatotoxicity seen as great damage in interstitial component of cell like mitochondria caused by conocarpus erectus extract itself or reactive metabolites which can trigger oxidative stress lead to severe ATP depletion and finally cells necrosis and there are many signs pointed to the amount of Alkaloids which have major bioactive compounds in Conocarpus erectus herb may have slightly accountable for the defect present. In this study, the mainly defect or tissue changes have

emerged in high doses, there is clear sign there was a relationship between a number of toxic substances found in the plant and the extent of the existing poisoning in main organ like liver[11]. Also maybe the phenolic components of these plant play a weak role in cytotoxic activity and there is another natural product classes may be saponin compound play a part in cytotoxic activity [12].

The result indicated that Conocarpus erectus treated rats caused many change in lipid profiles like significant decreased in the level of cholesterol ,triglyceride ,Low density lipoprotein in two doses especially in low dose while the high density lipoprotein appeared increased significantly in two doses ,That maybe due to the Conocarpus erectus containing phenolic compounds are only responsible for the antioxidant activity and not only responsible for anticancer properties but it can be play indirect part as prevention by protects cells from the damage caused by free radicals. Many studies have revealed that intake of natural antioxidants is correlated with low incidence of cancer, heart disease, diabetes, and other diseases[12] .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

1. El-Sayed S, Salih AB, Mohamed M, Mortada M, Eman A. Phytochemical Studies and Evaluation of Antioxidant, Anticancer and Antimicrobial Properties of Conocarpus erectusL. Growing in Taif. J. Saudi Arabia European of M. Plants. 2012; 2(2): 93-112.
2. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afri. J. Biotechnol. 2008; 7, 1797-1806.
3. Nirmala MJ, Samundeeswari A, Sankar PD. Natural plant resources in anti-cancer therapy-a review. Research in Plant Biology. 2012;1, 184-185.
4. Bashir M, Uzair M, Bashir AC. A review of phytochemical and biological studies on Conocarpus erectus (Combretaceae). Pak J Pharm Res. 2015;1(1), 1-8.
5. Bailey LH. Hortus Third: A Concise Dictionary of Plants Cultivated in the United States and Canada. New York, MacMillan Collier MacMillan. 1976, p.306
6. Ayoub N, Hussein S, Reiss C, Mentel R and Lindequist U. .Antiviral and antibacterial phenolics from Rubus sanctusSchreb. Bull Fac Pharm Cairo Univ. 224;42: 223–231.
7. Hussein S, Ayoub N, and Nawwar M. Caffeoyl sugar esters and an ellagitannin from Rubus sanctus. Phytochemistry. 2003; 63: 905–911.
8. Ayoub N. Unique phenolic carboxylic acids from Sanguisorba minor. Phytochemistry. 2003; 63: 433–436.
9. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS and Seeff LB. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem. 2000 ;46(12):2050-68.
10. Haraguchi H, Yoshida N, Ishikawa H, Tamura Y, Mizutani K, Kinoshita T. Protection of mitochondrial functions against oxidative stresses by isoflavans from Glycyrrhiza glabra. J. Ethnopharmacol. 2000; 52: 219-223
11. Abdulrahman FI, Onyeyili PA, Sanni S, Ogugbuaja VO. "Toxic effect of aqueous root-bark extract of Vitex doniana on liver and kidney functions". International Journal of Biological chemistry (I). 2007.
12. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 Chinese medicinal plants associated with anticancer. Life Sci. 2004;74, 2157–2184.