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PHYTOCHEMICAL POTENTIAL OF β-SITOSTEROL, β-AMYRIN AND EPIAFZELECHIN IN NICKEL INTOXICATED MULTIPLE ORGAN DAMAGE IN RAT

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ABSTRACT

Background: Chronic inflammation increases the risk of DNA damage and promotes tumor progression and metastasis. Persistent microbial infections induced by bacteria, viruses and parasites and physical or chemical stimuli can cause inflammation. **Aims**: The present study was planned to elucidate the crucial role of phytocompounds β-sitosterol, β-amyrin and epiafzelechin in different dose regimen to ameliorate the damaging effect of pro inflammatory mediators produced as a result of inflammation driven carcinogenesis by heavy metal nickel. **Methods**: Hundred albino rats were included, divided into ten groups and each group contains ten rats of weight 160-200g. Group A was control while group B, C, D, E, F, G, H, I, J were injected with 1ml/kg nickel intraperitoneally per week for three months. Further groups C, D and E were treated with β-sitosterol (100mg/kg b.w), β-amyrin (100mg/kg b.w) and epiafzelechin (200mg/kg b.w) respectively for one month. Later groups F, G, H and I were fed with combinatorial treatments of the three phyto compounds for one month. The group J was administered with commercial drug Nalgin (500mg/kg b.w). The biochemical parameters ALT, CRT, hs-CRP, 8-

OHdG, TNF-α, IsoP-2α, MDA and COX-2 were estimated by ELISA kits. **Results**: All the parameters were significantly raised in group B indicating the significant inflammation while the levels of the foresaid biomarkers were decreased in the serum in groups C, D, E, F, G, H, I, J with the treatment of the three phytocompounds in different dose pattern. However, the best recovery was observed in group I in which the three active compounds were administered concomitantly. The correlation matrix indicated significant and positive correlation of COX-2 (r=0.65**, p=0.01) with TNF-α (r=0.759**, p= 0.01), IsoP-2α (r=0.661**, p=0.01), 8OHdG (r=0.842**, p=0.01), MDA (r=0.945**, p=0.01). **Conclusion:** The study concluded that the three phytocompounds have potent defensive ability against inflammation more prominently in comparison with synthetic drug Nalgin and can be used in the near future to treat inflammatory diseases.

Keywords: COX-2, β-sitosterol, β-amyrin, Inflammation, Malondialdehyde (MDA), Nalgin, Nickel, tumor necrosis factor-alpha (TNF-α), Isoprostanes, C-reactive protein

INTRODUCTION

Human exposure to heavy metals boosts the mutagenicity and carcinogenicity of cells by directly inhibiting the ability to repair the damaged DNA. Nickel is such a genotoxic agent which has the highest intake of its compounds through dietary exposure and drinking water. Binding of nickel with DNA interferes with the process of nucleotide and base-excision repair creating cytotoxicity [1]. This contributes to the alterations in gene expression, stimulated proto-oncogenes and activation of cellular proliferation and inhibition of tumor suppressor genes resulting in the down regulation of cell growth [2]. Substantial in vivo data has been provided to prove that oxidative stress due to ROS generation is one of the important mechanisms of cytotoxicity in nickel induced carcinogenesis. Overuse of synthetic drugs for the treatment of oxidative damage results in the increased rate of destructive side effects which has motivated man to turn to nature based complementary and alternative medicine [3].

Beta-sitosterol is one of the most potent phytosterols present in *Parkinsonia acculenta* which is known for its properties to combat inflammatory diseases, viral damage, ulcer and cancer at different levels including inhibition of tumor invasion and metastasis as well as in the enhancement of immune system [4]. Beta sitosterol is structurally related to cholesterol but is more slowly absorbed in the intestinal tract

blocking the cholesterol absorption in the blood and preventing its rise in the serum. In addition to this, beta sitosterol is demonstrated to modulate the immune system and pain levels by interference of the production of inflammatory cytokines [5]. The environmental toxins, heavy metals and radiations causes the increase in Th-2 activity and lowering of Th-1 activity and this modulating response of the body is critical for managing autoimmune disorders and inflammation. β-sitosterol is also applied as an ROS scavenger as it is reported to increase the non-enzymatic antioxidants glutathione (GSH) and also excites enzymatic antioxidants through the stimulation of estrogen receptor in PI3 kinase dependent pathway [6]. β-amyrin is a pentacyclic triterpene which is largely derived from vegetable oils, vegetarian foods, medicinal herbs, cereals and fruits and regarded as natural component of human diet. Estimated daily human consumption of β-amyrin was approximated to 250mg per day in developed countries [6]. It is an important plant triterpene which is of immense medicinal importance obtained from the leaf and bark incision of *Albizia lebbeck* through the extraction with dichloromethane, chloroform, n-hexane and methanol and identified through thin layer chromatography, further, quantified through high performance liquid chromatography (HPLC) [6].

The clinical data suggests that β-amyrin ameliorates the production of excessive nitric oxide by reducing the nitrosative stress inside the body and lowers the graph of oxidative stress created by reactive oxygen species causing pancreatic cell death in insulin dependent diabetes mellitus patients. The insulin sensitivity is improved due to the property of β- amyrin to decrease the glucose absorption from gut and reduced rate of glucose synthesis in liver [7]. β- amyrin is reported to function as good antioxidant and anti-inflammatory agent and improves the lipid profile of the diabetic patients by vanishing hyperglycemia and dyslipidemia by reducing the atherogenesis and possibly improves the glucose tolerance through the altered intracellular Ca^{+2} regulation [8]. Modulation of anti-inflammatory action of this active constituent is related to the systemic inhibition of local cytokine and down regulation of NF-κB, cyclooxygenase-2 (COX-2) and mitogen activated protein kinases (MAPK) underlying the therapeutic effects of β-amyrin possibly through the reticence of both NF-κB and CREB signalling cascades. The transformation of arachidonic acid into prostaglandins (PGs) is

carried out by two inflammatory enzymes COX-1 and COX-2 which are exclusively responsible for the induction of inflammation, stimulation of cytokine expression and infiltration of neutrophils [9]. However, stimulation of both COX-1 and COX-2 is not necessarily induced I response to growth factors, cytokines and tissue injury. Colon cancer induced by nickel induced rats leads to macroscopic damage that was associated to PGE_2 synthesis, COX-2 protein expression and leukocyte infiltration in the stroma of inflammatory colon suggesting that arachidonic acid metabolites derived from COX-2 plays an important role in this model of nickel induced inflammation in rats [10]. β-amyrin is indeed related to its ability to ameliorate the inflammatory pattern of cytokine expression observed in colitic rats [11].

Epiafzelechin (EFZ) has been identified as a bioactive phenolic compound in *Acacia chundra,* a tree that is widely distributed in central, southern and western regions of India and used as an important traditional therapy for the treatment of inflammation and pathologies related to heart, liver and other organs [12]. It is a widely studied phenolic compound in cancer research and the potential underlying mechanisms are important to understand. The oral administration of 40mg/kg body weight of EFZ in rats infected with acute myeloid leukemia for 22 consecutive days were observed with reduced DNA damage, p65 dephosphorylation and enhanced apoptosis. The antiproliferative activity of epiafzelechin beats the simple inhibition of tumor formation into prevention of cancer cell proliferation, promotion and development [13]. In 7, 12 dimethylbenz (a) anthracene induced papilloma in albino mice model and indorsed by using croton oil, the topical application of 100 mg epiafzelechin/kg body weight showed 90% inhibition of the formation of papilloma. A number of scientific reports suggest that epiafzelechin defends the normal and non-tumorigenic tissues from exposure by radiation and chemotherapy [14].

For instance, a group of rats were pre-treated with epiafzelechin and then exposed to doxorubicin which induced neurotoxicity. The neurotoxicity was reduced which was evident by the decreased expression of TNF-α, iNOS and NF-κB including the reduced nitrite levels in the rat brain. Epiafzelechin also impede the mitochondrial and renal injury in vitro using cultured mice proximal tubular cells and in vivo in mouse pre-treated with epiafzelechin preceding intraperitoneal administration of cisplatin [15]. Recent

report demonstrated a powerful stimulatory effect of epiafzelechin on the oxidative phosphorylation mechanism of mitochondrial enzymes in vitro. The mitochondrial respiration was activated in *in vitro* pancreatic cancer cells and in *in vivo* rat muscle tissues in which the epiafzelechin up regulated the expression of oxidative phosphorylatory protein complexes. In context of protective effect of this phenolic compound, the epiafzelechin pre-treated pancreatic cancer cells Panc-11, U87 and MIA PaCa-2 when exposed to radiation show significant reduction in the cell survival; however, it had small protective effect in normal pancreatic cells. This property of epiafzelechin is an ideal characteristic as an adjunct therapy of cancer cells with chemo-rehabilitation or radiation [16]. In this connection, the present work was designed to evaluate the anti-inflammatory role of β-sitosterol, β-amyrin and epiafzelechin bioactive compounds alone and in different therapeutic combinations to assess the key processes which are responsible to ameliorate the damaging effect of nickel.

MATERIALS AND METHODS

Adult Wister male albino rats were taken from the Institute of Molecular Biology and Biotechnology, Lahore, Pakistan with body weight ranging from 160 to 200g. The study was approved by the Ethics Committee for the Scientific Research at the University of Lahore.

PLANT EXTRACTS

The standardized bioactive compounds of B-sitosterol, β-amyrin and epiafzelechin were purchased from the Sigma-Aldrich Corporation (St. Louis, MO, USA).

EXPERIMENTAL DESIGN

Hundred (100) albino rats divided into ten groups. Every group had ten rats. For ninety days every group was administered intraperitoneally with nickel (1ml/kg b.w.) to induce toxicity. To check the multiple organ protective effects, B-sitosterol, β-amyrin and epiafzelechin were fed to group C, D, E, F, G, H and I for one month in different doses pattern. Group J treated with synthetic drug Nalgin 1tab/kg that contains several extracts such as Silybum marianum (200mg), Picrorhiza kurroa (50mg), Glycyrrhiza glabra (50mg) and Cichorium intybus (75mg).

BLOOD AND SERUM SEPARATION

During the dissection 5mL blood was collected from the rat heart and serum was separated by centrifugation at 1500 rpm for 10 min. After the separation of serum it was stored at -60ºC until further biochemical analysis.

BIOCHEMICAL ASSAYS

The biochemical markers Alanine transaminase (ALT), creatinine, High sensitive C-reactive proteins (hs-CRP), 8-hydroxydeoxyguanosine (8-OHdG), tumor necrosis factor alpha (TNF-α), Isoprostanes (Iso)-2α, Malondialdehyde (MDA) and cyclooxygenase-2 (COX-2) were measured spectrophotometrically through commercially available ELISA kits purchased from the Sigma-Aldrich Corporation (St. Louis, MO, USA).

Table 1: Experimental Design

GROUPS	TREATMENTS
A	Control
B	Ni
C	Ni+ β-sitosterol (100mg kg b. w.)
D	Ni+β-amyrin (100mgkg b. w.)
E	Ni+ epiafzelechin (200mgkg b.w.)
F	Ni+ β-sitosterol (100mg kg b. w.), β-amyrin (100mg kg b. w.)
G	Ni+ β-amyrin (100mg kg b. w.) and epiafzelechin (200mg kg b. w.)
H	Ni+ β-sitosterol (100mg kg b. w.) and epiafzelechin (200mg kg b. w.)
I	Ni+ β-sitosterol (100mg kg b. w.), β-amyrin (100mg kg b. w.) and epiafzelechin (200mg kg b. w.)
J	Ni+ NALGIN©

Ni@ (1 ml/kg B.w. per week for three months)
E1+E2+E3@ (2.5mg/ Kg b.w. / for one month)
NALGIN© @ (500mg/Kg b.w/day) for one month

RESULTS

Inflammation associated environment requires the presence and activation of inflammatory cells like macrophages and granulocytes at the site of inflammation in addition to the production of inflammatory mediators and angiogenesis, tissue necrosis activation biomarkers which are responsible for the expression of growth factors like IL-6 and VEGF, inflammatory cytokines like cyclooxygenase-2, tumor necrosis factor alpha, isoprostanes, 8-hydroxydeguanosine, malondialdehyde and heat shock proteins. The data presented in table 2 clearly illustrates that nickel had a significant effect on all the biochemical markers' levels Alanine transaminase (ALT), creatinine, High sensitive C-reactive proteins (hs-CRP), 8-hydroxydeoxyguanosine (8-OHdG), tumor necrosis factor alpha (TNF-α), Isoprostanes (Iso)-2α, Maliondialdehyde (MDA) and cyclooxygenase-2 (COX-2) with the rise of 24%, 10%, 21%, 2.3%, 29%, 7.5%, 8.8% and 12% respectively as compared to the healthy group of rats (Group A).

The sole treatments of the three plant active constituents β-sitosterol (100mg/kg b. w), β-amyrin (100mg/kg b. w) and epiafzelechin (200mg/kg b. w) in group C, D and E showed significant reduction in the levels of the foresaid biomarkers as compared to the group of rats receiving nickel with maximum recovery in group of rats receiving β-sitosterol @100mg/kg b. wt (77%, 80%, 90%, 76%, 95% in ALT, Creatinine, Hs-CRP, 8OHdG, TNF-α respectively). Different combination treatments (Group F, G, H, I) showed significant reduction in the levels of the forediscussed biomarkers. However, maximum restoration was obtained in the group of rats receiving combinatorial treatment of β-sitosterol (100mg/kg) + β-amyrin (100mg/kg) + epiafzelechin (100mg/kg) with 44%, 87%, 23%, 14%, 28%, 12%, 11% and 16% respectively in all the biomarkers as compared to the group of rats receiving nickel. A standard drug Nalgin which is commercially available for the treatment of inflammation was used to compare the recovery rate of our under observation treatment combinations. Highly significant results were obtained as Nalgin recovered ALT (37%), creatinine (16%), Hs-CRP (22%), 8-OHdG (18%), TNF-α (22%), IsoP-2α (7.9%), MDA (10%) and COX-2 (17%) as compared to the rats induced with nickel toxicity which is quite below the rate of recovery in group of rats receiving combination of β-sitosterol (100mg/kg) + β-amyrin (100mg/kg) + epiafzelechin (100mg/kg) (Group I). These observations concluded that the combination of β-sitosterol (100mg/kg) + β-amyrin (100mg/kg) + epiafzelechin (100mg/kg) is the best treatment regimen for the recovery of inflammation which is even better than the commercially used anti-inflammatory Nalgin with quite less side effects. The least significant deviation (LSD) value predicted that the difference of the recovery rate between the groups of rats receiving sole treatments of the active constituents was non-significant as it is below the LSD value (4.55). However, the difference of the recovery rate between any group of rats receiving sole treatment and group of rats receiving combinatorial treatment of all the three standardized active constituents was significant (greater than LSD 4.55). This also confirms that amalgamation of the three standardised active constituents is the best regimen for the management of inflammation and restoration of inflammatory mediators.

Table 2: Biochemical Response Of B-Sitosterol, B-Amyrin And Epiafzelechin In Rat Model Following Nickel Induced Toxicity

GROUPS		MEAN±SD (n=10)						
	ALT IU/L	CREATININE mg/L	Hs-CRP mg/L	8-OHdG pg/ml	TNF-α pg/ml	IsoP-2α pg/ml	MDA nmol/ml	COX-2 ng/ml
A	16.35±3.06	0.56±0.014	0.95±0.018	1.06±0.054	21.25±4.09	17.25±1.09	0.85±0.056	0.66±0.0023
B	66.56±2.05	5.26±1.25	4.35±1.22	44.26±1.88	71.26±1.99	229.36±8.56	9.65±1.08	5.15±1.08
C	51.26±5.11	4.26±1.08	3.95±0.96	33.65±2.08	68.06±7.55	189.58±4.88	8.26±2.02	4.19±1.04
D	48.26±1.07	4.03±1.11	3.01±0.88	30.26±5.66	55.26±6.66	202.19±12.25	7.69±1.81	3.28±0.29
E	42.32±4.11	3.89±0.91	2.09±0.58	28.26±3.08	31.26±5.18	181.25±6.65	8.29±2.11	3.59±0.85
F	33.26±1.08	3.26±1.08	2.06±0.44	23.25±1.24	54.26±7.16	96.66±3.88	6.33±1.18	2.19±0.41
G	40.16±2.00	2.88±0.73	1.86±0.18	23.29±4.19	35.26±2.08	75.26±4.55	5.18±1.55	1.29±0.27
H	33.19±4.01	1.08±0.88	1.45±0.23	19.65±2.10	29.06±1.08	56.36±1.56	3.28±0.95	1.33±0.12
I	29.35±3.11	0.46±0.16	1.03±0.17	6.55±3.08	20.19±2.99	28.29±3.29	1.09±0.16	0.87±0.055
J	25.26±1.08	0.86±0.24	0.99±0.06	8.19±3.33	23.18±2.07	18.29±1.25	1.02±0.36	0.89±0.011
LSD (0.05)	4.55	1.33	2.53	1.08	4.16	0.956	2.33	3.08
p-VALUE	0.003	0.014	0.008	0.004	0.031	0.022	0.000	0.008

DISCUSSION

Human body is enriched with natural antioxidants which are responsible for the onset as well as for the treatment of diseases caused due to the free radical mediated oxidative stress. Antioxidants are naturally taken thorough diet but human body can also produce antioxidants within the body [17, 18]. Voluminous literature suggests that antioxidants of plant origin with anti-inflammatory property and free radical scavenging activity have gained much importance as therapeutic agents in several pathologies [19]. The phytoconstituents inhibit lipid peroxidation and prevent DNA damage thus, lessen the oxidative damage through several biochemical and metabolic pathways. B-sitosterol, β-amyrin and epiafzelechin used in the present study have been known to be potent anti-inflammatory induced anti-carcinogenic compounds which increase the recovery rate of the organ damage by normalising the inflammatory biomarkers' levels in the serum [20].

COX-2 metabolites have been implicated in the functional and structural alterations in glomerular and tubulointerstitial inflammatory damage and thus, are prominent biomarker of inflammation [21]. Current researches have suggested that prostanoids mediate changes in the renal function and glomerular prostaglandin levels in the kidney of mouse, rat, rabbit and dog and this COX-2 localization is same in human kidney [22]. A recent study demonstrated the localization of COX-2 in the macula densa and medullary interstitial cells of a 60 years old man kidney and a preliminary report detected the increased levels of COX-2 in the macula densa with congestive heart failure and Bartter's syndrome. No gene abnormality of COX-2 in human cancer has been reported but several oncogenes and growth factors can induce the expression of

COX-2 [23]. For instance, epidermal growth factor (EGF) enhances the COX-2 production. Tumor growth factor (TGF) induced the up regulation of COX-2 stimulated by EGF in rat intestinal epithelial cells. Inhibition of EGF receptor tyrosine kinase activity with AG1478 completely diminished the expression of COX-2 [24]. The standardised active phytoconstituents B-sitosterol, β-amyrin and epiafzelechin targets COX-2 by inhibiting the over expression of COX-2, angiogenesis and VEGF expression which results in the tumor necrosis and tumor regression.

The correlation study depicted highly significant and direct interrelationship of the COX-2 with TNF-α (r=0.525**, p=0.01) and 8-OHdG (r=0.525*, p= 0.01) showing that both TNF-α and 8-OHdG were raised in serum of rats induced with inflammation. Since, TNF-α is a pleiotropic cytokine with associated inflammatory cascade leading to renal injury and its immunosuppressive role is evident in experimental models of lupus nephritis and the anti- TNF-α treatments have shown promising results in the experimental animal models of glomerulonephritis [25]. However, 8-OHdG is reported as ubiquitous oxidative product of DNA damage and was first analysed in the urine of experimental animals and humans in 1989. It is a widely used biomarker of oxidative stress and carcinogenesis and is a known risk factor for many diseases [26]. Therefore, the table 4 shows direct correlation of TNF-α and 8-OHdG with COX-2 as all were showing increased levels in the serum of toxicity induced rats (71.26pg/ml, 44.26pg/ml and 5.15ng/ml respectively) (Table 3). Similarly, the correlation of creatinine (CRT) with COX-2 (r=0.526, p=0.01) and with isoprostanes (r=0.661**, p=0.01) was highly significant because CRT is a gold standard for renal injury biomarker. Often CRT becomes raised in the serum when the injury is well established and acute kidney diseases are mainly diagnosed by an increase in serum creatinine [27]. Several researches have provided the evidence that the concentration of isoprostanes 2-alpha (IsoP-2α) is quantitatively increased during oxidative stress condition which proves it to be significant biomarker of prooxidative status in response to different pathologies and induction of toxins in the biological system [28]. A significant elevation in IsoP-2α in the rat hepatocytes was observed after the toxic dose of carbon tetrachloride (CCl_4). The intensification in this metabolite showed incremental inclination for the next 4 hour dose of CCl_4. The phenobarbital compounds that induce microsomal enzyme damage enhance the metabolic rate of CCl_4 and decrease the glutathione (antioxidant) accumulation which induces the synthesis of IsoP-2α in the cell [29].

Our experimental model also exhibited the same results with increased levels of IsoP-2α (2299.6pg/ml) in nickel intoxicated rats which were further metabolised and normalised up to 85% during the treatments with standardised active phytoconstituents using different combination regimens (Table 3). A report on oxidative stress clearly depicted the role of isoP-2α in lipid peroxidation in the case of smokers with continuous intake of tobacco. The free and lipid ester bound forms of IsoP-2α were measured in smokers' blood plasma and urine [30]. The levels of IsoP-2α in blood plasma were prominently increased and they were correlated with concentrations of metabolites in urine. The levels of these free and lipid ester bound forms of IsoP-2α in blood plasma of smokers after two weeks of complete parting of smoking. The decrease in smokers' blood serum levels of IsoP-2α after intake of vitamin C was also observed. These studies proved that estimation of IsoP-2α in blood plasma, serum and urine can be utilised as a gold biomarker of oxidative stress and lipid peroxidation in cells [31]. Following the diagnosis of inflammation, malondialdehyde was also studied which is the main oxidation product of omega-3 and omega-6 fatty acids because it reacts readily with thiobarbituric acid (TBA). The table 4 showed positive and direct interrelationship of MDA with 8-OHdG (r=0.945**, p= 0.01), TNF-α (r=0.845**, p=0.01) and high sensitivity C-reactive proteins (hs-CRP) (r=0.745*, p=0.01) as all were showing elevated serum levels in inflamed group of rats. The increased levels of MDA in cells indicated that the continued oxidation of fatty acid side chains due to nickel administration in the body released polyunsaturated fatty acids (PUFA) and the fragmentation of peroxides producing aldehydes eventually leads to the loss of membrane integrity by changing its fluidity which in result inactivates the membrane bound proteins [32]. The aldehyde produced as a result of lipid peroxidation can easily cross the membranes and can modify any protein in the cytoplasm and nucleus, thus causing DNA damage or inflammation. Hs-CRP is produced by the liver in response to inflammation and thus is a known non-specific biomarker of inflammation in a wide variety of conditions from infection to cancer [33]. The increased and positive correlation of hs-CRP with other inflammatory biomarkers means that the rats with high levels of CRP are more likely to have heart disease or high liver profile or chronic obstructive pulmonary disease (COPD). CRP test is usually recommended to diagnose inflammatory autoimmune diseases including liver inflammation, inflammatory bowel disease, lupus or arthritis. Therefore, elevated levels of CRP in serum or plasma may be a good prognostic biomarker of inflammatory complications [34, 35].

CONCLUSION

The current study concluded that nickel induction caused biochemical alterations in the body and the standardised phytoconstituents β-sitosterol, β-amyrin and epiafzelechin have potent anti-inflammatory induced anti-carcinogenic activity which recovered the multiple organ damage up to 90% than the commercial drug Nalgin. Therefore, these active phyto compounds would be useful as an antioxidant and free radical scavenging agent and help in the treatment of various pathologies including cancer.

Table 4: Pearson S' Correlation Coeeficients Of Different Variables In Liver Tissues Of Rats Under Nickel Stress Receiving E1, E2. E3

VARIABLES	ALT	CRT	hs-CRP	8-OHdG	TNF-α	IsoP-2α	MDA	COX-2
ALT	1.00	0.526*	0.745*	0.842*	0.664	0.596	0.674	0.685
CRT		1.00	0.645	0.432	0.759**	0.661**	0.641	0.745**
hs-CRP			1.00	0.329	0.648	0.415	0.329	0.156
8-OHdG				1.00	0.426	0.548	0.945**	0.761**
TNF-α					1.00	0.648	0.845**	0.525*
IsoP-2α						1.00	0.348	0.245
MDA							1.00	0.684
COX-2								1.00

** Correlation is significant at the 0.01 level (Two-tailed)

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

REFERENCES

[1] Cortijo J, Milara J, Mata M, Donet E, Gavara N, Peel SE, Nickel induces intracellular calcium mobilization and pathophysiological responses in human cultured airway epithelial cells, Chem Biol Interact, 183(1), 2010, 25–33.

[2] Kanwal R, Gupta S, Epigenetics and Cancer, J Appl Physiol, 84, 2010, 83-118.

[3] Bell JT, A twin approach to unraveling epigenetics, Trends Genet, 27, 2011, 16–125.

[4] Hou L, Environmental chemical exposures and human epigenetics, Int. J. Epidemiol, 41, 2012, 79–105.

[5] Barouki R, Developmental origins of non-communicable disease: implications for research and public health, Environ. Health, 11, 2012, 42.

[6] Xia D, Prostaglandin E2 promotes intestinal tumor growth via DNA

methylation, Nat. Med, 18, 2012, 224–226.

[7] Huang SK, Prostaglandin E(2) increases fibroblast genespecific and global DNA methylation via increased DNA methyltransferase expression, FASEB J, 26, 2012, 3703–3714.

[8] Peveling-Oberhag J, Dysregulation of global microRNA expression in splenic marginal zone lymphoma and influence of chronic hepatitis C virus infection, Leukemia, 26, 2012, 1654–1662.

[9] Abdalla M.A, Promising candidate urinary microRNA biomarkers for the early detection of hepatocellular carcinoma among high risk hepatitis C virus Egyptian patients, J. Cancer, 3, 2012, 19–31

[10] Arzumanyan A, Epigenetic repression of E-cadherin expression by hepatitis B virus x antigen in liver cancer, Oncogene, 31, 2012, 563–572

[11] Lim JS, Hepatitis C virus core protein overcomes stress induced premature senescence by down-regulating p16 expression via DNA methylation, Cancer Lett, 321, 2012, 154–161

[12] Jiang J, Hypomethylated CpG around the transcription start site enables TERT expression and HPV16 E6 regulates TERT methylation in cervical cancer cells, Gynecol Oncol, 124, 2012, 534–541.

[13] Hyland PL, Evidence for alteration of EZH2, BMI1, and KDM6A and epigenetic reprogramming in human papillomavirus type 16 E6/E7-expressing keratinocytes, J. Virol, 85, 2012, 10999–11006.

[14] McLaughlin-Drubin ME, Human papillomavirus E7 oncoprotein induces KDM6A and KDM6B histone demethylase expression and causes epigenetic reprogramming, Proc. Natl Acad. Sci. USA, 108, 2011, 2130–2135.

[15] Bodily JM, Human papillomavirus E7 enhances hypoxiainducible factor 1-mediated transcription by inhibiting binding of histone deacetylases, Cancer Res, 71, 2011, 1187–1195.

[16] Hsu CH, The HPV E6 oncoprotein targets histone methyltransferases for modulating specific gene transcription, Oncogene, 31, 2012, 2335–2349.

[17] Yu H, Epstein-Barr virus downregulates microRNA 203 through the oncoprotein latent membrane protein 1: a contribution to increased tumor incidence in epithelial cells, J. Virol, 86, 2012, 3088–3099.

[18] Angrisano T, Helicobacter pylori regulates iNOS promoter by histone

modifications in human gastric epithelial cells. Med. Microbiol, Immunol, 201, 2012, 249–257.

[19] Manayi A, Saeidnia S, Ostad SN, Hadjiakhoondi A, Shams Ardekani MR, Vazirian M,Akhtar Y, Khanavi M, Chemical constituents and cytotoxic effect of the main compounds of Lythrum salicaria L, Z Naturforsch, 68, 2013, 367-75.

[20] Shi C, Wu F, Zhu X, Xu J, Incorporation of β-sitosterol into the membrane increases resistance to oxidative stress and lipid peroxidation via estrogen receptor-mediated PI3K/GSK3β signalling, Biochim Biophysic Acta, 1830, 2013, 2538-44.

[21] Radika MK, Viswanathan P, Anuradha CV, Nitric oxide mediates the insulin sensitizing effects of β-sitosterol in high fat diet-fed rats, Nitric Oxide, 32, 2013, 43-53.

[22] Saeidnia S, Barari E, Shakeri A, Gohari AR, Isolation and identification of main compounds of Lagochilus cabulicus, Asian J Chem, 25, 2013, 1509-11.

[23] Naseri M, Monsef-Esfehani HR, Saeidnia S, Dastan D, Gohari AR, Antioxidative coumarins from the roots of Ferulago subvelutina, Asian J Chem, 25, 2013, 1875-8.

[24] Ruggiero A, Vitalini S, Burlini N, Bernasconi S, Iriti M, Phytosterols in grapes and wine and effects of agrochemicals on their levels, Food Chem, 141, 2013, 3473-79.

[25] Anyanwu GO, Ur-Rehman N, Onyeneke CE, Rauf K, Medicinal plants of the genus Anthocleista-A review of their ethnobotany, phytochemistry and pharmacology, J Etnopharmacol, 175, 2015, 648-667.

[26] Dwevedi A, Sharma K, Sharma YK, Cadamba: A miraculus tree having enormous pharmacological implications, Pharmacogn Rev, 9, 2015, 107-113

[27] García-Martínez O, De Luna Bertos E, Ramos-Torrecillas J, Manzano-Moreno FJ, Ruiz C, Repercussions of NSAIDS drugs on bone tissue: the osteoblast, Life Sci, 123, 2015, 72-77

[28] Goldstein JL, Cryer B, Gastrointestinal injury associated with NSAID use: a case study and review of risk factors and preventative strategies, Drug Healthc Patient Saf, 7, 2015, 31-41.

[29] Tovey FI, Role of dietary phospholipids and phytosterols in protection against peptic ulceration as shown by experiments on rats, World J Gastroenterol, 21, 2015, 1377-1384

[30] Sheeja MD, Beema SR, Karutha PS, Pandima DK, Cholinesterase inhibitory, anti-amyloidogenic and neuroprotective effect of the medicinal plant Grewia tiliaefolia – An in vitro and in silico study, Pharm. Biol, 55, 2017, 381–393

[31] Otto T, Sicinski P, Cell cycle proteins as promising targets in cancer therapy, Nat. Rev. Cancer, 17, 2017, 93–115

[32] Liu G, Kuang S, Wu S, Jin W, Sun C, A novel polysaccharide from Sargassum integerrimum induces apoptosis in A549 cells and prevents angiogensis in vitro and in vivo, Sci. Rep 6, 2016, 26722.

[33] Shin WS, Programmed activation of cancer cell apoptosis: A tumor-targeted phototherapeutic topoisomerase I inhibitor, Sci. Rep, 6, 2016, 29018.

[34] Pradhan M, Suri C, Choudhary S, Naik PK, Lopus M, 2016, Elucidation of the anticancer potential and tubulin isotype-specific interactions of β-sitosterol, J. Biomol. Struct. Dyn, 1102, 2016, 1–14.

[35] Nadeem T, Khan MA, Ijaz B, Ahmed N, ur Rahman Z, Latif MS, Ali Q, Rana MA, 2018. Glycosylation of Recombinant Anticancer Therapeutics in Different Expression Systems with Emerging Technologies. Cancer research, 78(11): 2787-2798.