Original Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

THE REMARKABLE ROLE OF *ROSEMARY OFFICINALIS* & *TAMARIX APHYLLA* AGAINST RADIATION BASED THERAPY

SOHAIR ALY HASSAN*[1], EL SHERBINY M[1], AMANI N. HASHIM[2]

[1]Therapeutic chemistry Dept. / National Research Center, (NRC) Cairo/ Egypt
[2]Phytochemistry and plant Systematics Dept.(NRC), Cairo /Egypt

ABSTRACT

Rosmarinus officinalis L and *Tamarixaphylla* are among herbal plants known in the Mediterranean region, with a long history of medicinal and culinary use. These plants are rich source of antioxidants which mainly attributed to its components. The modifying influence of leaves extract of both *Rosmarinus officinalis* (rosemary) and *Tamarixaphylla* (Athel) against irradiation intoxication were investigated in experimental albino rats. Selected doses were chosen as radio protectors against Gamma [**γ**] radiation hazards. The experimental Westar rats were exposed to 5 Gry for 15 min before and after treatment with rosemary and tamarix extracts in a dose of 100 mg/kg/ b/ wt of each respectively. The treated animals were autopsied for collection of blood serum at days 1, and 15 post-irradiation. A decrease in the hematological parameters and Glutathione level was registered in the positive irradiated control group. A recovery pattern were recorded in the pre and after treated groups. The whole hematological parameters were ameliorated back to the normal by day 15; as a significant elevation was achieved with groups treated with rosemary and tamarix extracts compared to irradiated one. An increment in the level of lipid peroxidation above normal was noted in serum of irradiated rats. This increment was significantly reversed upon treatment by rosemary and tamarix extracts. Moreover interlukin 6 levels were highly modified in the treated groups. The whole results were confirmed Histologically by the improvement seen in the (PCNA) (proliferating cell nuclear antigen)level antigen. Considering these biochemical and histological results, the present study suggests the significant importance for both herbs with the superior role of rose marry as a magnificent radio modifier herb.

KEYWORDS: ***Rosemary** officinalis, **Tamarix aphylla, gamma radiation, antioxidative effect, PCNA (proliferating cell nuclear antigen).***

SOHAIR ALY HASSAN*

Therapeutic chemistry Dept. / National Research Center, (NRC) Cairo/ Egypt

Received on: 03.06.2017
Revised and Accepted on: 19.09.2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b292-301

INTRODUCTION

Scientifically radiation is the energy that travels as waves or in the form of particles [1]. Although radiation is became one of the double edged tools in the meantime, the steadily increasing use of radiation technologies in medicine, industry, agriculture and scientific research has been paralleled by increasing potential risk for overexposure[2-3]. The hazards of radiation exposure lie in the severe cellular damage and stress both directly, by energetic perturbation of DNA integrity, and indirectly, by the formation of intracellular free radicals [4-5]. It is believed that free radicals are considered the main venue leading to a complex different health conditions, including the ageing process, cancer, radiation damage, atherosclerosis, etc [6]. Thus, protecting living systems from the damaging impacts of radiation is very vital in radiation biology in general and in particular importance for those of cancer patients when they undergo to radiotherapy while their normal tissues are completely exposed to ionizing radiation[7]. Luckly, there are many plants derived natural antioxidants that interfere with free radicals resulted from ionizing radiation lead to dwindle the bad influence to the body [6]. Antioxidants have the ability to capture the free radicals and halt its damaging effect [8].The expansion of radioprotective modifiers has been the target of intense research in view of their impact for use within a radiated environment[9]. However, no standard safe chemically synthesized radio protectors are known up till now. Therefore, a necessary need to find new & safe sources including medicinal herbs and plants to be used as alternative treatment for radiation hazards, [10-12]. A mountain herb Rosemary (*Rosmarinus officinalis L*.) is one among herbal plants known in the Mediterranean region, with a long history of medicinal and culinary use [13]. This plant is a rich source of antioxidants which mainly attributed to its components of diterpenes carnosol , carnosic acid, , ursolic acid, rosmarinic acid, and caffeic acid [14-15]. Rosemary also was proved to be safe in toxicity studies, when added as an antioxidant to food in animal models [16-17]. Moreover Rosemary has been used for many medical purposes as a tonic and stimulant, diuretic, antireumatic ,analgesic, carminative, DNA repairer, anti-spasmodic in renal colic expectorant, anti- epileptic, human fertility and, dysmenorrhoea, relieving respiratory disorders [18-19]. On the other hand, *Tamarix aphylla* plant is a well identified species which belongs to the genus Tamarix, F. Tamaricaceae [Sadaf et al [20]. It has already mentioned in Quran taking the name of (Athel), and in Islamic literature for its medicinal use as a folk treatment for different ailments like jaundice, rhewnatism, wound and abscesses [21-22]. Alkaloids, flavonoids, tannins and terpenoids. and other polyphenolic compounds were identified among about 62 active constituents of *T. aphylla* [23-25]. *T aphylla* is a rich source of flavonoids that appear to have a protective effect for human health when extracted by Methanol , [26-27]. While a mixture of water-methanol was better than any other extracts as it has total phenolic content acts as potent free radical inhibitors. In this respect and in view of the wide diversity of chemical constituents and the pharmacological properties together with the anti-oxidative properties of both *Rosmarinus officinalis* and *Tamarixaphylla*; the present study has designed to focus and shed light to the radio modulatory effect of their extracts against the harms resulted from gamma irradiation exposure in rats.

MATERIALS AND METHODS

Animals

Animal care and handling were performed according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland and followed the Institutional Animal Ethics Committee applied at NRC (National Research Center), Cairo, Egypt Wistar rats, 6-8 weeks old, weighing 120-180 g., from an inbred colony were used for the present study. These animals were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). They were provided standard mice feed (procured from Arab chemicals co) and water ad libitum. Tetracycline water once a fortnight was given as preventive measures against infections.

Preparation of Rosmarinus officinalis &Tamarix aphylla extracts.

The identification of the commercial plant *Rosmarinus officinalis*& *Tamarix aphylla* were done by a competent botanist from the Herbarium, Department of Botany, NRC (Cairo). Both plant leaves were washed with distilled water; air-dried and chopped into fine homogenized powder in a grinder, passed through 0.5 mm mesh screen and were kept in clean polythene bags in the sterile environment of the laboratory. The dried leaves of both R. *officinalis* and *Tamarix aphylla* were extracted with double distilled water by refluxing for 36 hrs (12 hrs x 3) at 38 ± 5°C. Pellets of the drugs were obtained by evaporation of their liquid contents in an incubator, it is known that different solvents can separate certain compounds, eg normal N-hexane and diethyl ether can separate fatty acids, sterols and hydrocarbons. Chloroform can separate glycone compounds, while ethanols remove glycoside. So using of water was preferred in this study to ensure that all traces of the acid or base have been removed.

Determination of optimum dose.

A dose selection of *R. Officinalis* or *Tamarix aphylla* was carried out on the basis of drug tolerance study. For this purpose, various doses of plant extract (100, 50, 25, 12.5, 5, mg/kg body weight) were dissolved in bi distilled water and tested for their tolerance (once daily for 15 days) in rats. The most optimum and tolerable dose obtained of both extracts were (100 mg/ kg b. wt.) used for further detailed experimentation.

Irradiation

Cobalt teletherapy unit (Co-60) (The National Centre for Radiation Sciences), Radiotherapy Department, & Cobalt unit for irradiation. Un anaesthetized animals were restrained in well-ventilated Perspex boxes and exposed to gamma radiation at a distance (SSD) of 80 cm from the source at a dose rate of 5 Gry /min.

Experimental design
A total of 60 animals used for the experiment were assorted into 6 groups of 10 rats in each. Rats in group I served as negative control giving neither extract nor irradiation. Group II served as positive control exposed to whole body gamma irradiation [TBI] (total body irradiation) at a dose rate of 5 Gry /min. All the experimental animals were prepared for intraperitoneal (IP) (intraperitoneal) injection as follow: Group III and Group IV injected 24 hrs post irradiation with *R. officinalis* extract (0.5 ml/animal at a dose of 100 mg/ kg b/ w daily for 15 days) either before or after exposure to radiation; Group V and VI injected with *Tamarix aphylla* extract 24 hour post irradiation (0.5 ml/animal at a dose of 100 mg/ kg/ b/ w daily for 15 days) either before or after exposure to irradiation. The animals were observed daily for any sign of sickness, morbidity, behavioral toxicity and mortality. A minimum of 5 animals from each group were necropsied on days 1, 15 post-treatment intervals to evaluate hematological and biochemical parameters.

Hematological study.
24 hour before and after irradiation, blood was collected from the orbital sinus of animals from each group in a vial containing 0.5 M EDTA. Total number of erythrocytes (RBC), leucocytes (WBC), hematocrit (Hct) and hemoglobin (Hb) percentage were determined by adopting standard procedures.

Biochemical analysis.
Biochemical alterations were studied in blood serum of the all experimental animals of all groups at 24 hour pre and post-exposure to gamma radiation. The level of glutathione (GSH) was determined according to the methods of Moron et al.[28]. The lipid peroxidation (LPx) level in the serum was measured by the assay of thiobarbituric acid reactive substances (TBARS) following the method of Ohkhawa et al [29]. Serum IL-6 levels were measured by using a polyclonal ELISA kits (RapidBio Lab., Calabasas, California, USA).

Immuno-histochemistry for Detection of tissue proliferating cell nuclear antigen (PCNA) (proliferating cell nuclear antigen)
Immuno-histochemical reaction was performed using an avidin biotin complex (ABC) (avidin biotin complex) immune-peroxidase technique according to Hsu et al [30] using anti mouse PCNA on paraffin sections, which were de-waxed in xylene and hydrated in descending grades of ethanol. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide in 100% methanol for 20 min. Antigen retrieval was performed by microwaving the sections in citrate buffer (pH 6.0) for 15 min at 700 W. Sections were incubated overnight at 4°C with the anti-human primary antibodies against PCNA, (purchased from Dako, Demark), monoclonal antibody, diluted at 1:150 in PBS (Phosphate buffer solution). Next day, after thorough washing in PBS (Phosphate buffer solution) , the sections were incubated with streptavidin-biotin-peroxidase preformed complex and evidentiated using a peroxidase/DAB (diaminobenzidine) enzymatic reaction for PCNA & Caspase3. Staining is completed by 5-10 min incubation with 3, 3'-diaminobenzidine (DAB) (Diaminobenzidine) + substrate - chromogen which results in a brown-colored precipitate at the antigen site (nucleus). Slides were washed in PBS (Phosphate buffer solution) for 5 min. Slides were placed in 70%, 95% and then 100% alcohol each for 5 min. The cell nuclei were counterstained with Mayer's hematoxylin. The cover slips were mounted using DPX. Positive and negative control slides for each marker were included within each session. As a negative control, liver tissue section was processed in the above mentioned sequences but the omission of the primary antibody and PBS (Phosphate buffer solution) was replaced.

Immuno-histochemical scoring of proliferating cell nuclear antigen (PCNA)(proliferating cell nuclear antigen)
Sections were examined under Zeiss light microscopy at x40, the proliferation index (P.I) is the number of positive nuclei stained by PCNA in 1000 hepatocytes then the mean of them calculated, and the mean for each group is taken according to [31]. Zero% was given to unstained sections.

STATISTICAL ANALYSIS

The results were expressed as Mean ± SD. The data were subjected to one-way ANOVA student t-test using graph Pad Prism 5 software $p<0.001$was considered as significant.

RESULTS

The hematological parameters for the different groups are tested and the results are represented in (Table1).A significant attenuation in all hematological parameters, RBCs, WBCs counts, HB &HCT were seen in positive irradiated group compared to healthy control ($P<0.05$). While, a gradual improvement in these parameters were observed when treated with rosemary and tamarix extracts before [prophylactic group] and after radiation exposure [treated group].The most improvement was seen at the group treated with rosemary extract at the dose of 100 mg/kg b/ wt) Post irradiation exposure ($P<0.01$). On the other hand the effect of the treatment by both extracts either prophylactic or therapeutic on the level of some antioxidant and inflammatory markers (Glutathione, Lipid peroxidation, Interleukin 6) were studied (Table 2). The results revealed a significant decrement in glutathione level in the irradiated group compared to healthy control one. Consistently MDA was extremely increased ($P<0.01$). Level of Interleukin 6 was markedly increased after irradiation compared to control. All these parameters got a noteworthy improvement ($P<0.01$) in the groups received both extracts of T. Aphyla and R. officinal's. Glutathione level was restored to near normal values in both treated groups. Moreover hydropic degeneration &sinusoid dilatation is seen clearly and

significantly ($p<0.05$) in irradiated group compared to healthy one.(Table3 &Fig 1).which rectified significantly ($p<0.01$) after treatment with both extracts. A significant increase in apoptosis percentage as well as PCNA (proliferating cell nuclear antigen) protein were observed ($p < 0.05$) in irradiated group in comparison to healthy control(Table 4,Fig 2), suggesting that the expression pattern of both apoptosis and PCNA (proliferating cell nuclear antigen) increases during the course of time of radiation. Treatment with both extracts displayed a moderate, significant pattern of PCNA (proliferating cell nuclear antigen) expression ($P < 0.01$) as seen in Fig 2

Table 1
Effect of both extracts on hematological parameters before and after radiation.

	Mean ± S.D			
Groups	**RBC ($10^6 mm^3$)**	**WBC ($\times 10^3/mm^3$)**	**HB(mg/dl)**	**HCT (%)**
Healthy Control	5.86 ± 0.27	6.42 ± 0.47	15.00 ± 0.58	43.50 ± 1.50
Irradiated Control	4.04 ± 0.3*	4.00 ± 0.44	9.80 ± 0.56**	32.80 ± 1.87**
Prophylactic Tam	4.45±0.33** ^	4.54 ± 0.50**	10.39±0.44**^	35.00 ± 1.45**^
Post Irradiated Tam	4.72±0.24** ^	4.90±0.48**^	10.90±0.65**	37.10 ± 1.79**^
Prophylactic Rose	4.88±0.27**^	5.42±0.42**^	12.01±0.83**^^	37.10 ± 1.59**^
Post Irradiated Rose	5.53 ± 0.14*^^	6.60±0.31* ^^	13.71± 0.38*^^	41.50 ± 1.17*^^

p<0.05 compared to healthy control. ** p<0.01 compared to healthy control
^ p<0.05 compared to irradiated control. ^^ p<0.01 compared to irradiated control

Table 2
Effect of both extracts on redox state and Il6 before and after irradiation.

	Mean ± S.D.		
Groups	**Glutathione**	**Lipid peroxidation**	**Interleukin 6**
Healthy Control	1.11 ± 0.31	1.75 ± 0.50	74.16 ± 10.10
Irradiated Control	0.30 ± 0.13**	2.17± 0.57* ^	101.68 ± 12.06**
Prophylactic Tam	0.55 ± 0.10** ^	1.78± 0.55	61.93 ± 4.75*^^
Post irradiated Tam	0.83 ± 0.15* ^^	1.57 0.51^	65.90 ± 3.97^^
Prophylactic Rose	0.69 ± 0.12** ^	1.56 ± 0.41^^	85.78 ± 9.62*^
Post irradiated Rose	0.88 ± 0.12* ^^	0.99 ± 0.41**^^	68.6 ± 7.96^^

p<0.05 compared to healthy control. ** p<0.01 compared to healthy control
^ p<0.05 compared to irradiated control. ^^ p<0.01 compared to irradiated control

Table 3
Effect of both extracts on liver architecture before and after irradiation.

Groups (n=10)	Hepatocytes				Sinusoids			
	Normal		Hydropic degeneration %		Normal		Dilated	
Healthy Control (n=10)	10	100%	0	0%	10	100%	0	0%
Irradiated Control	4	40%**	6	60%	2	20%**	8	80%
Prophylactic Tam	5	50%**^	5	50%	5	50%**^^	5	50%
Post Irradiated Tam	8	80%*^^	2	20%	7	70%*^^	3	30%
Prophylactic Rose	5	50%**^	5	50%	3	30%**^	7	70%
Post Irradiated	8	80%*^^	2	20%	7	70%*^^	3	30%

Cross table, chi square
p<0.05 compared to healthy control. ** p<0.01 compared to healthy control
^p<0.05 compared to irradiated control. ^^p<0.01 compared to irradiated control

Table 4

Effect of both extracts on the Apoptosis Percentage and PCNA (proliferating cell nuclear antigen) scoring before and after irradiation.

Groups	Apoptosis				PCNA-Proliferation index
	Absent		Present		Mean±S.D
Healthy Control	10	100%	0	0%	2.4 ± 1.35
IrradiatedControl	6	60%	4	40%	29.30 ± 3.34**
Prophylactic Tam	6	60%	4	40%	11.50 ± 4.06**^
Post Irradiated Tam	8	80%	2	20%	12.70 ± 3.37**^
Prophylactic Rose	7	70%	3	30%	7.70 ± 3.2* ^^
Post Irradiated Rose	8	80%	2	20%	8.70 ± 1.50*^^

Cross table, chi square

****p<0.05 compared to healthy control. ** p<0.01 compared to healthy control.***

^ p<0.05 compared to irradiated control. ^^ p<0.01 compared to irradiated control.

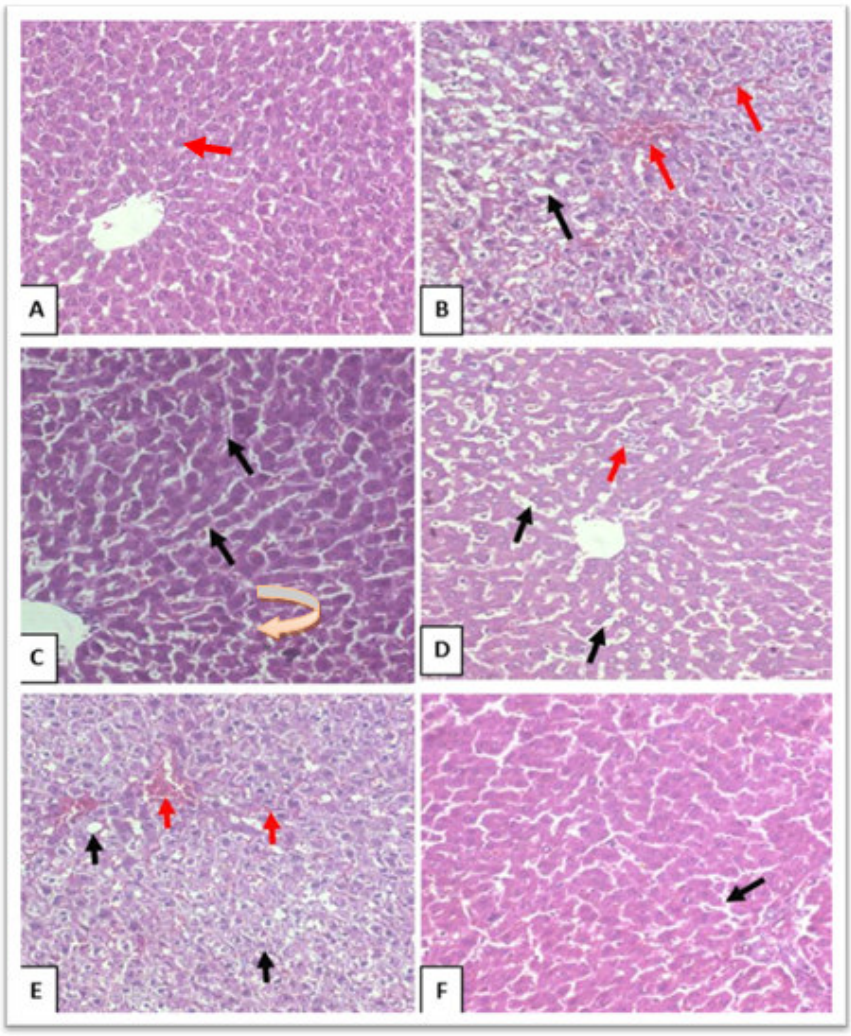

Figure 1(A)

The normal control preserved hepatic lobular architecture with normal hepatocytes arranged in thin plates (red arrows) in comparison with liver sections from irradiated control [B]which showed partial loss of hepatic architecture with moderate hydropic degeneration of hepatocytes, congested central vein and sinusoids with p <0,05. liver section from Prophylactic Tam [C] showed preserved hepatic architecture with normal hepatocytes arranged in thin plates, congested, dilated sinusoids (arrows) . (H&E,x200), [D] liver section from Post irradiated Tam showed preserved hepatic architecture with almost normal hepatocytes arranged in thin plates, congested, dilated sinusoids black arrows), collection of lymphocytes(red arrow) . (H&E,x200)[E] liver section from prophylactic Rose showed partial loss of hepatic architecture with moderate hydropic degeneration of hepatocytes (black arrow), congested central vein and sinusoids (red arrow) . (H&E,x200). [F] liver section from Post irradiatedRose treatment showed preserved hepatic Architecture with normal hepatocytes arranged in thin plates, congested, dilated sinusoids (arrows) (H&E,x200)

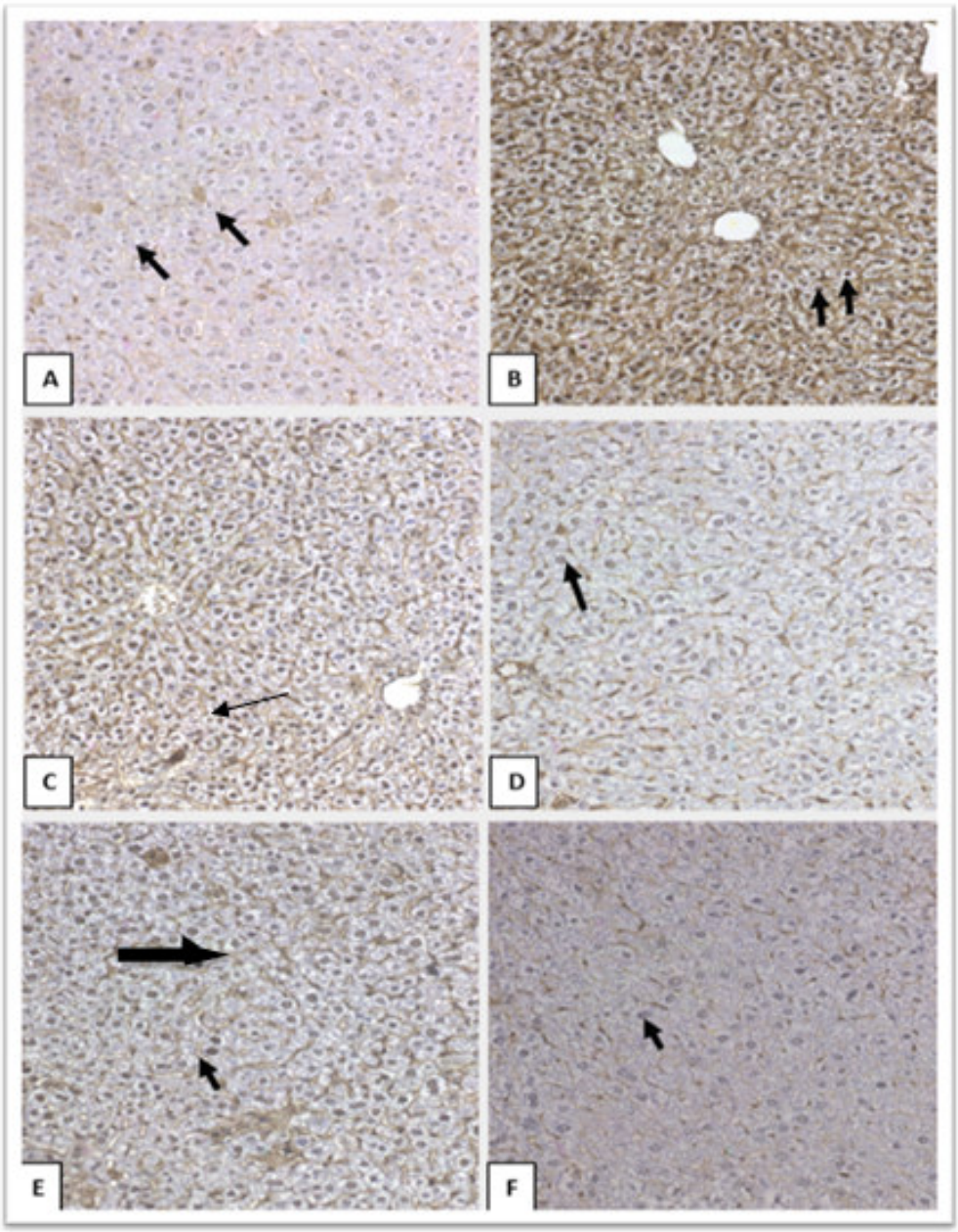

Figure 2(A)
liver section from normal control negative expression of PCNA(proliferating cell nuclear antigen). (IHC, DAB,x200),[B) : liver section from irradiated control showing positive brown nuclei expression of PCNA(proliferating cell nuclear antigen)(arrow). (IHC, DAB,x200) [C] : liver section from prophylactic Tam showing positive brown nuclei expression of PCNA(proliferating cell nuclear antigen)(arrow). (IHC, DAB,x200),[D) liver section from post irradiated Tam showing positive brown nuclei expression of PCNA(proliferating cell nuclear antigen)(arrow). (IHC, DAB,x200), [E] liver section from prophylactic Rose showing positive brown nuclei expression of PCNA(proliferating cell nuclear antigen)(arrow). (IHC, DAB,x200), [F] : liver section from post irradiated Rose showing positive brown nuclei expression of PCNA (proliferating cell nuclear antigen)(arrow). (IHC, DAB,x200

DISCUSSION

Although radiation therapy has been extensively employed as a curative or palliative intervention against cancer along the last century, it still have unpleasant side effect. Therefore, the needs to avoid extensive damage to normal tissues caused by the ionizing irradiation become a must [32]. A reasonable number of studies revealed the radio protective efficacy for some medicinal plants against the damaging effect of IR [33 -37]. According to Geraci and Mariano, [38] and El-Shanshoury [39], who reported that a single dose of <15 Gry can damage both types of liver cells, nonparenchymal and parenchymal cells, leading to other hypoxic, partial hepatic dysfunction. Therefore, in our study a rat total body irradiation by 5Gry gamma was designed to investigate the potential power of each of Rosemary and Tamarix herbal extracts to reverse the possible damage effects of irradiation on the hematological parameters, redox state as well as

the liver architecture as the most susceptible organ to irradiation from one side and for having the characteristic of self regeneration from the other side . The results profile of this study showed a fall in erythrocyte count post exposure to 5 Gry gamma irradiation, which may be attributed to radiation-induced injury, inhibition of new cells entering into the blood or loss of cells through hemorrhage [40] or due to irradiation damage of lymphocyte DNA since there is no lethal damage to RBCs because they lack nuclei. However, other damages to erythrocytes may influence the quality of RBC concentrates. These results are in full agreement with [41-42,39] who reported that whole blood irradiation damaged lymphocyte DNA, but there is no lethal damage to RBCs. Hemoglobin level showed a similar depression as in RBC. That level of hemoglobin was modulated upon treatment by both extracts back to near normal level especially with Rosemary treatment. This effect may be due to the potent antioxidant component like, carnosic acid, rosemarinic acid alkaloids, flavonoids,tannins and terpenoids. and other polyphenolic compounds present in both extracts with specific attention to rosemary [7], [44].These components trigger an early responding component of tissues (epithelial and blood cells) which accelerate proliferation of the relevant stem cells, in order to balance the cellular loss [43,39].Same pattern was seen in hematocrite percentage. Similar findings were reported earlier by Daga et al. [44] and Nunia et al. [45] as they used a sub-lethal dose of gamma rays. They attributed the decrement in the hematocrit to the depletion in the peripheral blood due to disturbances in steady state mechanisms in blood forming organs as well as an increase in plasma volume after irradiation. In addition a marked change in the redox state was recorded. A decrease in GSH concentration was in parallel mode with an elevation in MDA levels. The decrement in GSH level was due to the enhanced utilization of the antioxidant system in order to detoxify the free radicals generated by radiation, [46]. Surprisingly the results revealed an improvement in each of glutathione and lipid peroxide levels upon pre and post treatment by both extracts. The improvement was greater with rosemary rather than tamarix .This improvement can be interpreted as one of the mechanisms that belong to rosemary extract [RE] protection against radiation through an elevation in GSH level mediated through the modulation of cellular antioxidants level. The increase in GSH level in turn leads to a reduction in the lipid propagation [16].The results of the present study demonstrated by evidence that the effect of *R. officinalis* extract (RE) is comparatively more active than *T.aphylla* extract (TE) to restore the original values of hematological and antioxidant parameters to near normal. However, previous investigation of the cellular mechanisms of radiation-induced augmentation of the immune response may contribute to the improvement of both low-dose TBI (total body irradiation) and conventional radiotherapy. It has been reported previously that initiation of a pro-inflammatory reaction post-irradiation exposure in the surrounding tissue leads to an activation and production of a lot of cytokines and chemokines. These cytokines include interleukin-1, interleukin-6, [47-48]. These can initiate a long term of inflammatory responses that ends up to chronic inflammation and tissue injury [49-50].The present study confirmed the previous mentioned facts as a prominent elevation of serum IL6 level upon irradiation by 5Gry was recorded as a type of irradiation resistance concurrent with a partial loss of hepatic architecture to be in coherence with previous studies, [49], [51, 47and 52] , Depending on the fact that Cytokine production peaking is lemmatized by a time-, often ranging from 4–24 hrs after irradiation with exponential decrease to baseline levels may extend up to few days. [50] The study results have drowned our attention as the level of IL6 was kept around the normal level when treated by both, rosemary and tamarix extracts prior and post irradiation exposure. Further Il6 level was stabilized within 15 days. These can be interpreted by the potential role of both rosemary and tamarix potent antioxidant constituents that have the ability to block the over release of cytokines, overcome the inflammatory reactions and at the same time down-regulate the expression of IL-6 [52], The present results also may predict the cytokine production to be dose dependent, in a contradiction with Ha et al., 2016 [53]. In the present study we suggest the great action of the anti-oxidative and anti-inflammatory components of both extracts with conjunction to low toxicity, which successfully stabilized it within 15 days and improved the liver architecture Fig 2. Such finding suggested that both plants extracts inhibited the inflammatory response induced by gamma radiation-intoxication. Furthermore an important central role in DNA replication and repair processes has been proven by (PCNA The proliferating cell nuclear antigen) [54-56]. In the current study the level of PCNA protein significantly elevated post irradiation compared with normal control group, which reflect the high susceptibility of the liver cells to radiation damage .Accordingly treatment with both extracts either before and after radiation were proved to be potent enough to encounter the uncontrolled increment of PCNA (proliferating cell nuclear antigen) reversing it back to lower levels [Table 4, Fig 2]. These results seem to be inconsistent with previous studies employed melatonin[as an antioxidant] pre-treatment to enhance the expression of PCNA protein in response to irradiation-and/or enhance loading of PCNA on to DNA in response to radiation in cells with low p21 levels, although this increased loading appears to reduce repair fidelity.

CONCLUSION

An obvious alteration of hematological parameters, Redox state, cytokines and PCNA (proliferating cell nuclear antigen) markers resulted due to an oxidative stress induced by γ-radiation intoxication. The selected doses of rosemary and tamarix extracts were successfully able to ameliorate these damaging effects. To the best of our knowledge, no study has reported the radio protector/modifier role of both extracts against the possible injuries resulted from γ-radiation intoxication even in low doses. Both plants extracts conferred a reasonable therapeutic protection due to their active constituents, with an Indication to the superior role of rosemary to be with an advantageous benefits over *Tamarix Aphylla*, intense studies are demanded to verify their therapeutic efficacy

ACKNOWLEDGMENTS

Authors thank Prof Eman, staff of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt, for her help in animal irradiation.Prof dr Olfat hammam ,Dept of Pathology, Theodor Bilharz

,Cairo, Egypt for her great assistance in the pathology part ,Dr Mona mentawy ,Dr Mahmoud sanad, Medicinal Chemistry Dep,National Research Center for their effort and assistance.

FUNDING ACKNOWLEDGMENT

This work was self-supported and did not receive any specific grant from funding agencies in the public or commercial.

Abbreviations

TBI : total body irradiation
(IP) : intraperitoneal
(ABC) : avidin biotin complex
(PCNA) : proliferating cell nuclear antigen
(DAB) : Diaminobenzidine
(PBS) : Phosphate buffer solution
(DBX) : dinbutyl phthalate

CONFLICTS OF INTEREST

Conflict of interest declared none.

REFERENCES

1 Arora R. Herbal radiomodulators: applications in medicine, homeland defence and space. CABI; 2008.
2 Naoki T, *et al*., Blockade of TLR3 protects mice from lethal radiation-induced gastrointestinal syndrome. *Nat Commun,* (2014). 18(5): 3492-506.
3 Hassan FA, Bazaid S, Ali EF. Effect of deficit irrigation on growth, yield and volatile oil content on Rosmarinusofficinalis.L. plant. J. Med. Plant. Stud. 2013;1(3):12-21.
4 Hall S, Rudrawar S, Zunk M, Bernaitis N, Arora D, McDermott CM, Anoopkumar-Dukie S. Protection against radiotherapy-induced toxicity. Antioxidants. 2016 Jul 5;5(3):22.
5 Simone NL, Soule BP, Ly D, Saleh AD, Savage JE, DeGraff W, Cook J, Harris CC, Gius D, Mitchell JB. Ionizing radiation-induced oxidative stress alters miRNA expression. PloS one. 2009 Jul 27;4(7):6377.
6 Nwachukwu KC, Asagba SO, Nwose C, Okoh MP. Radiation protection and anti-oxidative effects of garlic, onion and ginger extracts, x-ray exposed albino rats as model for biochemical studies. Afr. J. Biochem. Res. 2014 Nov 30;8(9):166-73
7 Hassan HA, Hafez HS, Isa AM. Neuro-protective effect of Mentha extract against oxidative stress in pre-treated gamma irradiated Swiss albino mice . J. Med. Plants Res 2012 Apr 23;6(15):2995-3002.
8 Basri DF, Heng KY, Meng CK, Ghazali AR. Screening of antioxidant phytoextracts of Canariumodontophyllum (Miq.) leaves in vitro. J. Pharm.. 2014;4(12):1-6.
9 Aprotosoaie, A.C.,Trifan, A., Gille, E., Petreus, T., Bordeianu, G. and Miron, A., 2015. Can phytochemicals be a bridge to develop new radioprotective agents?. Phytochem Rev , 14(4), pp.555-66.
10 Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnopharmacol. 2000 Jul 31;71(1):23-43.
11 Arora R, Gupta D, Chawla R, Sagar R, Sharma A, Kumar R, Prasad J, Singh S, Samanta N, Sharma RK. Radioprotection by plant products: present status and future prospects. Phytother Res 2005 Jan 1;19 (1):1-22.
12 Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol . 2014 Jan 10;4:177.
13 Tsai TH, Chuang LT, Lien TJ, Liing YR, Chen WY, Tsai PJ. Rosmarinus officinalis extract suppresses Propionibacterium acnes–induced inflammatory responses. J Med Food . 2013 Apr 1;16(4):324-33.
14 Kontogianni VG, Tomic G, Nikolic I,Nerantzaki Alexandra AA, Sayyad N, -Grujicicb S S, Stojanovic I, . Gerothanassis P I , Tzakos G A. Phytochemical profile of Rosmarinus officinalis and Salvia officinalis extracts and correlation to their antioxidant and anti-proliferative activity, , Food chem. , 2013 Vol 136, Issue 1 1 January 2013, Pages 120–129.15-
15 Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M. Antioxidant activity of rosemary (Rosmarinusofficinalis L.) essential oil and its hepatoprotective potential. BMC Complement Altern Med 2014 Jul 7;14(1):225.
16 Sancheti G, and Goyal PK,): Modulatory influence of Rosmarinus officinalis on DMBA-induced mouse skin tumorigenesis. Asian Pac. J. Cancer Prev. (2006) 7: 299-302
17 Gutiérrez R, Alvarado JL, Presno M, Pérez-Veyna O, Serrano CJ, Yahuaca P. Oxidative stress modulation by Rosmarinusofficinalis in CCl4 induced liver cirrhosis. Phytother Res. 2010 Apr 1;24(4):595-601
18 Hassan SA, Lam KL, Haikal T, Mantawy M, Siddiqui MJ, Al-Jassabi S. Comparative Study for the Egyptian and Malaysian Rosemary Water Extracts For their Active, Antioxidant and Anti-proliferative Contents. IJPSL 2012.;1(2):37-43.
19 ZainabE,Hanafy and Sohair A, Hassan(2010): Rosemarry protection against γ-irradiation Induced clastogenecity.Globalvetrinaria 4 (3): 230-6.

20 Sadaf B, Muhammad A, Nazia A, Burhan U, Muhammad B, Anum K, Hezba K. Antifungal Activity of Tamarixaphylla (L.) Karst. (2015): American-Eurasian J. Agric. & Environ. Sci., 15 (4): 541-545.

21 Marwat SK, Khan MA, Khan MA, Ahmad M, Zafar M, Rehman F*et al*.,. Fruit Plant Species Mentioned in the Hol Marwat SK, Khan MA, Khan MA, Ahmad M, Zafar M, Rehman F, Sultana S. Fruit Plant Species Mentioned in the Holy Qura'n and Ahadith and Their Ethno medicinal Importance. Am. Eurasian J. Agric. Environ. Sci. 2009;5(2):284-95.

22 Yusufoglu S H, and Aqasoumi Si .. Anti inflammatory and wound healing activities of herbal Gel containing antioxidant Tamarixaphylla leaf extract. Int J Pharmacol (2011)7,(8):829-835.

23 Abbas B, Al-Qarawi AA, Al-Hawas A. The ethnoveterinary knowledge and practice of traditional healers in Qassim Region, Saudi Arabia.Journal of Arid Environments. 2002 Mar 1;50(3):367-79.

24 Nawwar MA, Hussein SA, Ayoub NA, Hofmann K, Linscheid M, Harms M, Wende K, Lindequist U. Aphyllin, the first isoferulic acid glycoside and other phenolics from Tamarixaphylla flowers. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2009 May 1;64(5):342-7.

25 Merfort I , Buddrus J, Nawwar MA, Lambert J. A triterpene from the bark of Tamarixaphylla.Phytochemistry. 1992 Jan 1;31(11):4031-2.

26 Shafaghat A, phytochemical investigations of quranic fruits and plants.J.Med.Plants,9,61-66 (2010)

27 Mohammedi Z, Atik F. Impact of Solvent Extraction type on total polyphenols content and biological activity from Tamarix Aphylla (L.) karst. I. J. Pharma& Bio Sci, (2011); 2 (1): 609-617.

28 Moron MS, Depiere JW, Mannervik B. Levels of GSH, GR and GST activities in rat lung and liver. Biochemica Biophysica. ,(1979); 582: 67-78.

29 Okhawa H, Ohishi N, Yogi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Analyt.Biochem. , (1979); 95: 351.

30 Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) (avidin biotin complex) in immunoperoxidase techniques: a comparison between ABC (avidin biotin complex) and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. (1981): 29:577-80

31 Akyol UM, Dursun A, Akyol G, Edaliota N. Proliferating cell nuclear antigen immunoreactivity and the presence of P53 mutation in basaloid squamous cell carcinoma of the larynx. Oncology 1998;55:382 – 383

32 Delanian S, Porcher R, Rudant J, Lefaix JL.Kinetics of response to long-term treatment combining pentoxifylline and tocopherol in patients with superficial radiation-induced fibrosis.J Clin Oncol. (2005);1,23(34):8570-9.

33 Arora R, Goel HC. Herbal radioprotectors. InProceedings of the International Conference on Radiation Biology, Radiobiology 2000 Feb.

34 Arora R, Gupta D, Sharma AK, Sagar RK, Prasad J, Singh S, Samanta N, Kumar R, Goel HC, Sinha AK, Chawla R. Modification of radiation-induced damage in mammalian model systems by natural plant products: implications for radiation protection. InProceedings of the IUPAC International Conference on Biodiversity and Natural Products, Delhi University, Delhi, India 2004 (Vol. 33).

35 Jagetia GC, Baliga MS. Influence of the leaf extract of Mentha arvensis Linn.(mint) on the survival of mice exposed to different doses of gamma radiation. Strahlentherapie und Onkologie. 2002 Feb 1;178(2):91-8.

36 Kamat JP, Boloor KK, Devasagayam TPA, Kesavan PC, Protection of superoxide dismutase by caffeine in rat liver mitochondria against gamma irradiation. Curr.Sci, (1999);. 77: 286-289

37 Maharwal J, Samarth R, Saini M. Radiomodulatory influence of Rajgira (Amaranthuspaniculatus) leaf extract in Swiss albino mice. Phytother. Res. 2003 Dec 1;17(10):1150-4.

38 Geraci JP, Mariano MS. Radiation hepatology of the rat: parenchymal and nonparenchymal cell injury. Radiat Res. 1993 Nov 1;136(2):205-13.

39 El-Shanshoury H, El-Shanshoury G, Abaza A. Evaluation of low dose ionizing radiation effect on some blood components in animal model. J Radiat Res Appl Sci. . 2016 Jul 31;9(3):282-93.

40 Sanzari JK, Wan XS, Krigsfeld GS, Wroe AJ, Gridley DS, Kennedy AR. The effects of gamma and proton radiation exposure on hematopoietic cell counts in the ferret model. Gravitational and space research: publication of the American Society for Gravitational and Space Research. 2013 Oct;1(1):79.

41 Maks CJ, Wan XS, Ware JH, Romero-Weaver AL, Sanzari JK, Wilson JM, *et al*.,. Analysis of white blood cell counts in mice after gamma-or proton-radiation exposure. Rad res. 2011 Apr 8;176(2):170-6.

42 de Oliveira GC, Maia GA, Cortes VF, de Lima Santos H, Moreira LM, Barbosa LA. The effect of γ-radiation on the hemoglobin of stored red blood cells: the involvement of oxidative stress in hemoglobin conformation. ANN HEMATOL. 2013 Jul 1; 92(7):899-906.

43 Koukourakis MI. Radiation damage and radioprotectants: new concepts in the era of molecular medicine. . Br J Radiol 2014 Mar 5

44 Daga SS, Jain VK, Goyal PK,: Radioprotective role of Liv-52 on circulating erythrocytes against sub-lethal doses of gamma radiations. Proceedings of International Symposium on Radiomodifiers in Human Health.Manipal, India. (1995).

45 Nunia V, Sancheti G, Goyal PK. Protection of Swiss albino mice against whole-body gamma irradiation by diltiazem. Br. J. Radiol. (2007); 80: 77-84.

46 Uma Devi P, Ganasoundari A, Rao BSS, Srinivasan KK.In vivo radioprotection by Ocimum flavonoids, survival of mice.Radiat Res (1999); 151: 74-78.

47 Kim JH, Jenrow KA, Brown SL. Mechanisms of radiation-induced normal tissue toxicity and implications for future clinical trials. Radiat Oncol J. 2014 Sep 30;32(3):103-15.

48 Giuseppina D, Francesco P, Salvatore P, Giuseppe C, Filippo R, Salvatore P, et al,. Concomitant cetuximab and radiation therapy: A possible promising strategy for locally advanced inoperable non-melanoma skin carcinomas. Mol Clin Oncol. (2016).; 4(4): 467-471.

49 Graves PR, Siddiqui F, Anscher MS, Movsas B. Radiation pulmonary toxicity: from mechanisms to management. In Seminars in radiation oncology 2010 Jul 31 (Vol. 20, No. 3, pp. 201-207). WB Saunders.

50 Di Maggio FM, Minafra L, Forte GI, Cammarata FP, Lio D, Messa C*et al*. Portrait of inflammatory response to ionizing radiation treatment. J Inflamm 2015 Feb 18;12(1):14.

51 Wu CT, Chen MF, Chen WC, Hsieh CC. The role of IL-6 in the radiation response of prostate cancer. Rad oncol. 2013 Jun 27;8(1):159.

52 Liu J, Zhang QY, Yu LM, Liu B, Li MY, Zhu RZ. Phycocyanobilin accelerates liver regeneration and reduces mortality rate in carbon tetrachloride-induced liver injury mice. World J Gastroenterol.:. 2015 May 14;21(18):5465

53 Ha CT, Li XH, Dadin Fu, Moroni M, Fisher C, Arnott R, *et al*.,. Circulating Interleukin-18 as a Biomarker of Total-Body Radiation Exposure in Mice, Minipigs, and NonhumanPrimates (NHP) PLOS ONE 1 October 2014 | Vol 9 | Issue 10 | P,1-12

54 Feng W, Guo Y, Huang J, Deng Y, Zang J, Huen MS. TRAIP regulates replication fork recovery and progression via PCNA (proliferating cell nuclear antigen). Cell discovery. 2016;2:16016.

55 Strzalka W, Ziemienowicz A. Proliferating cell nuclear antigen (PCNA): a key factor in DNA replication and cell cycle regulation. Annals of botany. 2011 May 1;107(7):1127-40.

56 Wang M, Shi Q, Zhang R, Qiu H, Mao D, and Long F . Herbal Compound “Jiedu Huayu” Reduces Liver Injury in Rats via Regulation of IL-2, TLR4, and PCNA Expression Levels Evid Based Complement Alternat Med . . (2017).Vol, 2017 ,1-9.

Reviewers of this article

Prof. Afaf K. El-Ansary

Biochemistry Department, Science College, and Central Laboratory, King Saud University

Prof.Dr.Prapurna Chandra Rao

Assistant Proffessor, KLE University, Belgaum, Karnataka, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript