The protective effect of non-thermal plasma against gamma irradiation in albino rats

Shaimaa M. Eldeighdy, Tarek M. Allam, and Walaa F. Hassan

Biological Applications Dept, Nuclear Research Center (NRC), Egyptian Atomic Energy Authority (AEA), Cairo, Egypt

ABSTRACT

Background, plasma-activated water (PAW) is generated by exposing water to the fourth state of matter (plasma). Plasma emits several kinds of reactive nitrogen and oxygen species (RNOS). This wealth of active species potentiates the PAW modulation of gamma radiation-induced oxidative stress, which is the most likely cause for DNA damaging effect and/or cell death following gamma irradiation. Aims, this study is an endeavor to investigate the role of oral administration PAW, against high dose of gamma irradiation. Methods, tap water was exposed to plasma jet under consideration delivering a plasma dose of 0.425 J/cm². Rats, were divided into seven groups, normal control 'G1,' group 'G2' including rats accessed to normal tap water and third group 'G3' including rats accessed to PAW. Each group drank the designated water for 60 consecutive days. After 60 days, the second and third groups were irradiated with a single dose of 7 gray gamma rays and anesthetized then sacrificed 24 following the irradiation. Blood samples were collected and assayed for DNA damage detection using comet assay. Biological functions including blood count, liver and kidney function as well as oxygen and glucose levels were also assayed in the blood of all rats in the three considered groups. Results, although exposure to this dose of gamma radiation showed an adverse effects on rats accessed to normal tap water at both cellular and organ level, PAW group remained highly protected as was clearly shown by their cellular and biochemical parameters. Conclusions, our findings suggest that, PAW is considered a potentially promising technique for combating the acute effects of high radiation doses that damage as well as impaired some biological functions. This could increase and support the hope in utilizing PAW as safer, economic, and efficient drinking water as radio-protective agent in expected wide scales.

1. Introduction

Ionizing radiation (IR), such as gamma radiation, can cause a number of potentially severe physiological and morphological alterations in mammals (Begum et al., 2012; Fajardo, 2005).

The ionization of biological molecules or the production of free radicals, such as superoxide, can cause the negative biological consequences of IR. Gamma radiation makes an array of biological consequences, such as inflammation, oxidative stress, carcinogenesis, that may be lead to death. The exposure of humans as well as animals to different types of radiation happens via radiotherapy, experimentation, work in nuclear stations, and nuclear battlefields as well as during nuclear accidents (Nair et al., 2001). Ionizing radiation induces highly oxidative stress as a result of potential production of free radicals that attack several components in different cells leading to biochemical changes and impaired in some biological functions via, lipid peroxidation, oxidation of protein, and DNA damages (Fuchs-Tarlovsky, 2013 & Shirazi et al., 2013). However, radiotherapy play important role in controlling cancer in human and animals, It may makes some harmful effects to different tissues such as bone marrow and liver. These damages lead to a necessity of limiting the therapeutic dose of ionizing radiation which is very important for controlling cancers (Abou-Zeid et al., 2018). Therefore, use age of radio-protective agents, is rendered very important to protect normal cells from the expected damages, which are stimulated by ionizing radiation as well as decreasing the adverse effects on organs functions. Protection of biological cells from different types of radiation is of great importance in planned and unplanned accidental events during exposures to ionizing radiation (Arora et al., 2005 & Jagetia & Jagetia, 2007). Through development of novel, as well as effective agents to prevent or at least to control radiation damages by using new and effective nontoxic radio-protectors.

Hence, many natural and synthetic agents have been evaluated in the recent past few years for their potential efficacy to reduce the harmful effects of ionizing radiation on living cells. Such as antioxidants, plant extracts, sulfhydril compounds, immune-modulators, and other potential agents (Raisin, 1998). Meanwhile, the ingained oxidative and harmful effects of the synthetic agents at the efficient radio-protective concentration guaranteed a lot of research for safer, economic, and best efficient radio-protector’s agents.
Plasma is the fourth state of matter, either partially or completely ionized gas, including different type of ions, electrons, free radicals as well as energetic photons which, is typically produced in specific conditions. Few years ago, several plasma jet devices, from which, cold atmospheric plasma (CAP) were generated. The attention to this field is drawn by potential economic properties from numerous cold plasma techniques (Ahmed, 2014). The CAP applications has been examined and a proof-of-principle demonstration is given, and these newly discovered properties make CAP particularly interesting for applications involving biomedical applications. The Egyptian Atomic Energy Authority atmospheric nonthermal plasma jet (EAEA-ANPJ) device under consideration has been designed, constructed and operated in the Plasma and Nuclear Fusion Department, Nuclear Research Center, Egyptian Atomic Energy Authority to conduct researches in several fields of application of CAP including biological fields (Ahmed et al., 2014). Generation of plasma-activated water (PAW) by plasma jet is considered one of the most important applications of the device (Eldeighdy et al., 2020). PAW- as an activated has a number of reactive species, including reactive oxygen and nitrogen species (RONs) produced by the interaction of plasma species with water surface (Tasaki et al., 2017).

PAW has antimicrobial potential effects (Kamang-Youbi et al., 2008), which is reported to occur due to the synergetic effects between RONS and/or pH of the water (M J et al., 2011). Plasma-activated solutions as well as media of cell culture have also been studied for therapeutically purposes (Takai et al., 2014), and its potency in tumors treatment has been shown (Utsumi et al., 2013). Besides, in agriculture field, PAW has potential role in both the plants growth and amelioration of seeds germination (Thirumdas et al., 2018).

According to a recent study (Eldeighdy et al., 2020), PAW has significantly increasing levels of dissolved oxygen, and NO₂ (6.15 and 7.87 mg/dl) compared with tap water (5.8 and 0.2 mg/dl), respectively, as well as an increase in pH (8) as compared to tap water of pH (7.73), which gives PAW unique properties. Moreover, PAW was ingest to rats for long time (60 consecutive days) and showed no adverse effects on biological functions as well as the behavior of rats. There is an ongoing search for more effective alternatives to conventional radioactive protecting agents, one of which is suggested in the present study. The potential properties of PAW are employed for this purpose by testing if it would meet the desired criteria. An investigation is given to the possible protective role of PAW against oxidative stress of high dose ionizing radiation, which impaired biological functions. This attempt, when proved feasible, would start to unlock the power of PAW against IR and bridge the gap between PAW and the convincing proof to its usage for oral therapy.

2. Materials and methods

2.1. Experimental animals

In the current study 30 male’s albino rats weighing approximately 120 ± 20 gm, were used. The rats were selected from the animal house of atomic energy authority, nuclear research center Inshas, Egypt, housed in special cages (10-rats/cage) and kept under standard laboratory conditions, with a 12-hr dark-light cycle. Each group had free access to standard commercial laboratory food as well as water, and were allowed to adapt to the laboratory conditions for 10 days before the beginning of the study. All experiments were performed in accordance with the rules of the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, as well as the Research Ethics Committee of the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, (the experimental protocol approval No. 5A/21) for the purpose of monitoring and supervising experimental animals.

2.2. Chemicals

All chemicals under current study with high analytical grade and were kept from different commercial sources includes, chemical for comet assay were obtained from Sigma-Aldrich, Inc., St Louis, MO, USA and diagnostic kit for liver and kidney functions obtained from (Biotech, Spinreact and Diamond) from their branches, Cairo, Egypt.

2.3. Atmospheric non-thermal plasma jet (ANPJ)

The ANPJ device was created by the ANPJ team (Plasma and Nuclear Fusion Department, Nuclear Research Center, (NRC), Egyptian Atomic Energy Authority (EAEA). The ANPJ device is based on a tiny hollow cathode structure with readily available and low-cost components. This device uses air as an input gas to generate a nonthermal plasma jet at atmospheric pressure. The plasma jet gadget can be held in one hand, and the plasma can be touched without injury. The electrode system and power supply weigh a total of 1 kg, and the gadget’s entire cost is under $200. The device is portable since the air compressor may be replaced with a much smaller one (Kamal et al., 2018).
2.4. Generation of plasma activated water (PAW)

In the current study, tap water obtained from the Plasma and Nuclear Fusion Department, Nuclear Research Center (NRC), Egyptian Atomic Energy Authority (EAEA) was exposed to plasma jet. Except for tap water samples, acidification was observed in all cases of discharges operated nonthermal plasma in contact with or in close proximity to water (Michael et al., 2019). Therefore, in the current study, we chose tap water for exposure to plasma jet to rule out any probability of acidification. Plasma activated water was obtained by exposure (100 ml) of tap water to direct nonthermal plasma jet for (10) minutes with corresponding exposure plasma dose (0.425 J/cm²), according to (shaimaa et al., 2020) as shown in fig.(1).

The PAW samples were generated daily for administration to rats under current study. The RONS in PAW usually include long-lived species such as nitrates (NO₃⁻), nitrites (NO₂⁻), hydrogen peroxide (H₂O₂), and ozone (O₃), with half-lives of years, days, 104 s, and several to dozens of minutes, respectively, as well as (relatively) short-lived species such as hydroxyl radicals (•OH), nitric oxide (NO), superoxide (O₂⁻), peroxynitrate (OONO²⁻), and peroxynitrates (ONOO⁻), with life times 1 ns, a few seconds, 1.5 s, and less than 1 s, respectively (Thirumdas et al., 2018) (Thirumdas et al., 2018). Meanwhile, it is known that NO₂ is a key breakdown product of NO (life time in milliseconds) in water (Yan et al., 2012), and that NO₂ is produced by the mechanisms described below (Popov, 2009). As a result, measuring NO₂ levels infers NO levels.

\[
\begin{align*}
N₂ + e & \rightarrow 2 \text{N} + e 1 \\
N + O₂ & \rightarrow NO + O 2 \\
4\text{NO} + O₂ + 2\text{H}_2\text{O} & \rightarrow 4\text{NO}_2 + 4 \text{H}^+ 3
\end{align*}
\]

On the other hand, dissolved oxygen (DO) concentration is also an important metric for characterizing natural and wastewaters, as well as assessing the overall status of water quality (Vidyasagar & Global Minute, 2007). Furthermore, one of the most significant operational water-quality criteria is pH (Marc & Chambon, 2013). As a result, the focus of our recent research was on detecting NO₂, DO, and pH levels in PAW. Overcoming this, the current study aims to see if considerable increases in (NO₃, DO, and pH) in PAW (Eldeighdy et al., 2020) allow it to be used as a radioprotector agent or not.

2.5. Irradiation procedure

Whole-body gamma-irradiation was done at the Egyptian Atomic Energy Authority, Cairo, using a Gamma Cell-40 Carlo irradiator, cesium 137 source. Rats were exposed to at an acute single dose level of 7 gray delivered at a dose rate 0.831 rad/s.

2.6. Experimental design

The current study was designed to evaluate the role of PAW against high dose (7 Gy) of gamma irradiation in albino rats. Via investigation of some biological functions including (liver, kidney, glucose level, complete blood count, oxygen value in blood) as well as level of DNA damages using comet assay technique for all rats. Therefore, albino rats were classified into three main groups:

- Group 1: Negative control group, healthy and untreated rats (10 rats drank tap water only).
- Group 2: Positive control group (10 rats drank tap water) for 60 days then irradiated with 7 gray gamma radiation.
- Group 3: Plasma treated group (10 rats drank PAW) for 60 days then irradiated with 7 gray gamma radiation.

![Figure 1. Generation of PAW and interaction of plasma active species with tap water, both ‘G2,’ rats drank tap water and ‘G3,’ rats drank PAW were exposed to one dose gamma irradiation (7Gy) then blood samples were obtained for both biochemical analysis and molecular study.](image-url)
Rats, drank water for 60 days, then groups (2 and 3) are irradiated with 7 gray. After 24 hours of irradiation, all rats were anesthetized then sacrificed, and blood samples were obtained for analysis of some biological functions as well as comet assay studies.

2.7. Samples collection (blood sampling)

At the end of experiment (after 61 days) rats were sacrificed and blood samples were collected for different analysis. About 4 ml into separated fresh vials (2 ml for every vial) containing anticoagulant (for comet assay and blood count) and the rest of blood left to coagulate, serum was separated by centrifugation at 2000 rpm for 4 min for assaying the other biochemical parameters.

2.8. Biochemical analysis

Blood glucose level was measured immediately using blood glucose meter, liver functions (total protein, albumin, alkaline phosphatase, total bil., ALT and AST) urea and creatinine by using diagnostic kit methods, blood (Oxygen) level by using arterial blood gases (ABG Roch analyzer) and CBC (WBC, RBCs, and platelets), by complete blood cell counter (Abacues 380 CBC counter).

2.9. Comet assay (single-cell gel electrophoresis) analysis

According to Singh et al., a comet assay was done (Singh et al., 1988) (1988). The comet experiment involves embedding cells in agarose, lysing them in alkaline buffer, and then exposing them to an electric current. Because the charged DNA is pulled away from the nucleus by the electric current, relaxed and fragmented DNA fragments travel further away from the nucleus than intact DNA. According to the protocol, the comet test was carried out in an alkali environment (Ostling & Johanson, 1994). Sigma provided all of the chemicals utilized in the comet assay. On totally frosted slides, two liters of whole blood were suspended in 0.5% low melting agarose and sandwiched between 0.6% normal melting agarose and a top layer of 0.5% low melting agarose. The slides were held on ice during the polymerization of each gel layer. The slides were immersed in lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris-HCl, 1% Triton X-100, and 10% DMSO) at 4°C after the 0.6% agarose layer had solidified. After 1 hour, the slides were immersed in electrophoresis buffer (0.3 M NaOH, 1 mM Na2EDTA, pH 10) at room temperature for 20 minutes to allow for the unringaling of DNA Electrophoresis was carried out in a horizontal electrophoresis platform for 20 min at 300 mA and 19 V in fresh, refrigerated electrophoresis buffer. The slides were neutralized three times with Tris-HCl solution (pH 7.5) for 5 min each time before being stained with 10% ethidium-bromide for ten minutes.

2.9.1. Comet capture and analysis

An epifluorescence microscope (Zeiss) was used to analyze 100 randomly collected comets from each slide at 400× magnification using a black and white camera coupled to an image analysis system (Comet Assay II; Perceptive Instruments Ltd, UK). We approximated the comet cell components using a computerized image analysis method that captures images and computes integrated intensity profiles for each cell, as Tail moment = Tail Length (px) × Tail DNA (%) according to (Boeck et al., 2000).

2.10. Statistical analysis

Using version 16 of SPSS software, data were statistically analyzed using one way ANOVA, followed by Duncan’s Multiple Range test for post hoc analysis. When the (P <0.01), the results were considered significant (Levesque & SpSS, 2007).

3. Results

The present study showed very high significant damaging effects of gamma radiation in DNA of leukocytes as well as malfunction in some biological functions of irradiated rats, which is discussed in details in the following sections.

3.1. Effect of gamma radiation on DNA fragmentation in irradiated rats compared with normal group

Gamma radiation with high dose (7 Gy) had very highly damaging effects on DNA, shown in this study, as it is increasing fragmentation level in ‘G2,’ which is shown by highly significant increasing (P < 0.01) in comet assay parameters (Comet %, Tail length, tail DNA% and Tail moment), as (77.6, 5.76, 6.14 and 0.35) compared with ‘G1’ (19.6, 1.99, 3.03 and 0.06), respectively. While pretreatment of rats with PAW showed protective effect against DNA damaging in ‘G3’ by (29, 2.59, 3.32, and 0.085), respectively, compared with ‘G2’ (see figures (2, and 3).

3.2. Effects of gamma radiation on complete blood count, kidney functions, and glucose level in all rats under study

The data of current study showed highly significant decrease (P < 0.01) in blood parameters (Hb, RBCs, WBC, and platelets) in irradiated group ‘G2’ by (12.44, 6.81, 1.98 and 191) compared with control group ‘G1’ which showed (14.08, 8.16, 6.95, and 641), respectively.
Figure 2. (A, B, C, and D) Damaging effect of gamma radiation is clearly showed by significant increase in comet assay parameters (comet %, tail length, percent of DNA in the tail, tail moment) recorded in white blood cells in irradiated group compared with control group, and protective effect of PAW against damaging effects of gamma irradiation.

Figure 3. (A, B, and C) Comet assay of blood leukocyte in all groups, (A 1, 2, 3) DNA of ‘G1’ which shows absence of DNA fragmentation as compared with (B 1, 2, 3) of irradiation group ‘G2’ which showed significant DNA fragmentation. Meanwhile (C 1, 2, 3) PAW + irradiated group ‘G3,’ showed protective effect against of DNA fragmentation by Gamma radiation when compared with irradiation group ‘G2.’
On the other hand, rats in ‘G3,’ which provided with PAW, were more protected against the hazards of gamma radiation, when compared with ‘G2’ (provided with regular tap water) by (14.18, 7.67, 5.28, and 504), respectively, as demonstrated by their blood components in table (1).

Moreover, there were also significant increase in levels of urea, creatinine as well as glucose in ‘G2’ by (45.6, 1.34, and 135), when compared with ‘G1’ that showed (20.2, 0.57, and 118.6). Meanwhile, ‘G3’ showed radio-protective effect against harmful effects of gamma radiation shown by significantly reduced urea, creatinine as well as glucose levels by (21.5, 0.588, and 119.7), respectively, when compared with ‘G2’ as shown in table (1).

### 3.3. Effect of gamma radiation on liver functions and level of blood oxygen

As is expected and confirmed in previous reports, the adverse effects of gamma radiation are well represented in liver functions of exposed rats according to our current study. Indeed, a significant increase in liver enzymes (alkaline phosphatase, AST, and ALT) as well as total bilirubin, in ‘G2’ by (92.6, 59.4, 58.6, and 0.56), as compared with ‘G1’ which showed (71.4, 34.6, 32.1, and 0.39). On the other hand, PAW play protective role against harmful effect of gamma irradiation as there are significant reduction in level of liver enzymes compared with ‘G2’ by (77, 35.2, 35.2, and 0.4), respectively. In addition, although, there were significant decrease in the level of total protein, albumin and blood oxygen in ‘G2’ by (6.58, 3.68, and 80) as compared with ‘G1’ that showed (7.36, 4.48, and 87.6). While, Rats in ‘G3’ had the radio-protective effect of PAW by (7.3, 4.32, and 87.4), respectively, as compared with ‘G2,’ as illustrated in table (2).

#### Table 1. Effect of gamma radiation on CBC, kidney functions, and glucose levels.

| Groups | Hb (%/g/dl) | RBCs 1/μl/cm³ | WBCCs 10⁹/μl/cm³ | PLTs 10³/μl/cm³ | Glucose (mg/dl) | Urea (mg/dl) | Creatinine (mg/dl) |
|--------|-------------|---------------|-------------------|-----------------|----------------|-------------|------------------|
| ‘G1’ Mean ±SD | 14.08 ± 0.37 | 8.16 ± 0.56 | 6.95 ± 0.82 | 641.2 ± 103.5 | 118.6 ± 2.3 | 20.2 ± 1.9 | 0.57 ± 0.01 |
| ‘G2’ Mean ±SD | 12.44 ± 0.38* | 6.81 ± 0.21** | 1.98 ± 0.41*** | 191.2 ± 46.6** | 135.8 ± 3.0* | 45.6 ± 1.5** | 1.34 ± 0.11** |
| ‘G3’ Mean ±SD | 14.18 ± 0.55** | 7.67 ± 0.33** | 5.28 ± 0.4** | 504 ± 65.4** | 119.7 ± 2.5** | 21.5 ± 2.2** | 0.588 ± 0.4** |

Numerical data were expressed as mean ± SD. Gamma-irradiated group ‘G2’ was compared to Control group ‘G1’ (*) and PAW+ irradiation group ‘G3’ compared with irradiation group ‘G2’ (**) P value <0.01 was considered significant.

#### Table 2. Effect of gamma radiation on liver functions and level of blood oxygen.

| Groups | Total protein (g/dl) | Albumin (g/dl) | Alkaline phosphatase (U/l) | Total bilirubin (mg/dl) | AST (U/l) | ALT (U/l) | Blood oxygen (mm/Hg) |
|--------|----------------------|---------------|---------------------------|------------------------|-----------|-----------|---------------------|
| ‘G1’ Mean ±SD | 7.36 ± 0.36 | 4.48 ± 0.13 | 71 ± 1.6 | 0.39 ± 0.01 | 34.6 ± 1.14 | 32 ± 2.6 | 87.6 ± 1.1 |
| ‘G2’ Mean ±SD | 6.58 ± 0.14** | 3.68 ± 0.2** | 92.6 ± 2.07** | 0.36 ± 0.02** | 59.4 ± 4.8** | 58.6 ± 5.4** | 80 ± 1.58** |
| ‘G3’ Mean ±SD | 7.30 ± 0.24** | 4.32 ± 0.12** | 77 ± 2.1** | 0.4 ± 0.01** | 35.2 ± 3.2** | 35.2 ± 2** | 87.4 ± 1.14** |

Numerical data is expressed as mean ± SD. Gamma-irradiated group ‘G2’ was compared to control group ‘G1’ (*) and PAW+ irradiation group ‘G3’ as compared with irradiation group ‘G2’ (**) P value <0.01 is considered significant.

### 4. DISCUSSION

It had a long history, that ionizing radiation (IR) exposure causes an assortment of hazardous changes to living cells depending on both exposed and absorbed dose as well as the postirradiation conditions, also, the susceptibility of tissues (Karbownik & Reiter, 2000). IR causes oxidative stress by producing reactive oxygen species, which causes an imbalance of prooxidants and antioxidants in various cells, leading to cell death (Reyners & De Ruyscher, 2015). Intracellular generation and accumulation of free radicals in the stressed cells overcome the natural antioxidant defense, causing impaired in biological macromolecules, including lipids, proteins as well as nucleic acids (Behn et al., 2007). The antioxidant agents neutralized the free radical species so they can inhibit the harmful effects induced by irradiation (Said & Hanafy, 2006). The present study is an attempt to investigate the protective role of PAW against gamma irradiation. PAW has unique properties as it contains high level of dissolved oxygen and NO₂ as well as alkaline pH (Eldeighdye et al., 2020). According to the data of present study, PAW possesses a protective effect against oxidative stress generated by gamma radiation in rats. For example, the highly significant DNA damaging effect, that is revealed by great increase in comet assay parameters and detected in the ‘G2’ irradiated animals, was much attenuated in the group of rats exposed to the same dose of irradiation but drinking PAW (G3). That is, undoubtedly, related to the cell damage occurred by ionizing radiation and it is fundamentally due to DNA damage. This DNA damage, may be single and/or double strand fragmentation, DNA-DNA and DNA-protein cross-links, and damages to nucleotide bases (Singh, 2000).

These harmful effects of irradiation was detectable, not only on the cellular level but, also, at the level of organ functions. For example, there are some biological parameters related to liver and kidney functions as
well as blood count that showed impairment. In addition, glucose and oxygen levels in blood of rats were also affected. As, there are very high significant decreasing in blood component (WBC, platelets, and RBCs). This was consistent with the previous reports on the effect of gamma irradiation. In previous study, exposure of rats to whole body gamma radiation-induced leukopenia (Mishima et al., 2004), and reduces lymphocytes, neutrophils, and monocytes count. The high radio sensitivity of hematopoietic tissue may be to blame for these declines (Chew & Park, 2004).

Furthermore, the significant decreases in erythrocytes in “G2 may be related to increase of permeability in the hemolytic process as well as the erythrocytic membrane stability, which was the main cause of the expressed decreases in RBCs count, after Gamma radiation (Sharma & Purohit, 2012). Moreover, current study showed, significant increases in the levels of serum ALT, AST and Alkaline phosphatase. These increments of blood transaminases is symbiotic of liver cells damage, leading to increase in cell membrane permeability which, facilitates the passage of cytoplasmic enzymes to blood (Abou-Zeid et al., 2018).

Also, impaired in kidney and liver functions after high dose gamma irradiation, may be due to the impaired of vital biological processes or attributed to a change in the permeability of kidney liver as well as other tissues (Mahmoud et al., 2019).

In current study, it is vital to examine PAW’s radioprotective properties. Given its ability to kill microorganism (Jun-seok et al., 2018), and to react with different living cells (Dehui et al., 2020), PAW showed a protective effect against fragmentation of DNA, as well as protecting liver, kidney, blood component from adverse effects of ionizing radiation. The radioprotective effect of PAW observed in this study could be attributed to dissolved oxygen and NO₂ of PAW as documented by recent researches, which, suggests that RONS play a considerably more intricate and subtle function in terms of health (Graves, 2012). In addition, due to the anti-oxidative effect of plasma active species, including (NO, NO₂, O₃, O₃, and H₂O₅), could also have a role according to (Schmidt et al., 2015) who showed that the wealth of plasma active species make an influence on the nuclear factor erythroid-related factor 2NRF2 pathway, that regulates the expression of antioxidant proteins and sets cells against exogenic noxae and increases their resilience against oxidative stress, via paracrine pathways, in which cell-cell communication benefits distant cells. In addition, Schmidt et al. (2015) found that cold plasma has a significant impact on gene and protein expression, highlighting the possible involvement of NRF2 in regulating cellular defenses against oxidative stress. These findings showed that cold plasma provides an interesting effective therapeutic tool to control redox-based processes, as cold plasma-based delivery of RONS stimulates or inhibits cellular processes.

Furthermore, it is thought that consuming oxygenated water increases oxygen availability, which may promote vitality and immune function (Bock et al., 2012). The antioxidant properties of oxygenated water help to reduce oxidative stress and return the cell to its natural state by increasing oxygenation levels and decreasing hypoxia (Al-Dosoki Mohamed et al., 2018).

Overcome, according to (Jon et al., 2008) it is now clear that nitrite (NO₂) is biologically recycled in blood and tissues to create NO and other beneficial nitrogen oxides, according to research conducted over the last decade (Cosby et al., 2003). As a result, they should now be seen as NO-like bioactivity storage pools, supplementing the NO synthase (NOS)-dependent system. Hemoglobin (Nagababu et al., 2003), xanthine oxidoreductase (Godber et al., 2000), and ascorbate are all involved in the further reduction of NO₂ to NO in the body (Carlsson et al., 2001), which occur through the following interactions according to, Lundberg et al., 2008.

(1) Deoxy-haemoglobin/myoglobin

\[ \text{NO}_2 + \text{Fe}^{3+} + \text{H}^+ \rightarrow \cdot \text{NO} + \text{Fe}^{2+} + \text{OH}^- \]  \hspace{0.5cm} (1)

2-Xanthine oxidoreductase

\[ \text{NO}_2^- + \text{Mo}^{4+} + \text{H}^+ \rightarrow \cdot \text{NO} + \text{Mo}^{5+} + \text{OH}^- \]  \hspace{0.5cm} (2)

3-Ascorbate

\[ \text{NO}_2^- + \text{H}^+ \rightarrow \text{HNO2}(3) \]

2 HNO₂ + Asc → 2 ·NO + dehydro Asc + 2 H₂O(4)

The ability of NO to reduce oxidative stress plays a significant role in both physiological and pathological mechanisms including properties at the chemical, cellular, and physiological levels, and this radical molecule serves to counterbalance oxidative stress. This equilibrium between NO and oxidative stress serves as a key regulating mechanism in a variety of physiological processes (Wink et al., 2001).

In addition, several reports at the genomic level postulated protective as well as anti-oxidative roles of nonthermal plasma in liquid media (Kurita et al., 2014). Plasma-activated media (PAM) types are better scavenging agents of active oxygen because they are predominantly made of water and have a high reduction potential (250 mV–300 mV) (Lotfy, 2016). Using nuclear magnetic resonance analysis, it was also discovered that tap and well water contain clusters of 10–13 H₂ O molecules. PAM electrolysis, on the other hand, turns tap and well water alkaline by reducing cluster size to about half of its normal size, i.e. five to six water molecules per cluster (Santos et al., 2013). Moreover, the lower surface tension as a result of exposure of
water to plasma species) makes PAW as an alkaline water easier, to absorb via cellular osmosis. Thus, PAW swiftly pervades the body and stops biological molecule oxidation by contributing its copious electrons to active oxygen, allowing biological molecules to spontaneously renew themselves without oxidative effects, causing potentially various damages (Kaushika et al., 2018).

In addition, few research efforts have, recently, indicated that alkaline natural water may have antioxidant action in animals, inhibit free radicals (Nassini et al., 2010), and protect DNA. (Sanetaka et al., 2012).

Through, mentioned above, the current study can reveal that the antioxidant properties of (DO, NO₃, and alkaline pH), made PAW play very important role as a radio-protector agent.

5. Conclusions and future work

The current study highlights the potential radioprotective effect and ambiguity of PAW that is attributed to (NO₂ and DO, and alkaline pH). In addition, the achievement of PAW will be a glimmer of hope in plasma medical applications as well as radio-protector agent. As, PAW, here, is considered the link between the living tissues and cold plasma biomedical field. PAW can, also, be delivered to different places for specific biological applications. Although there are considerable advances recently in the field of PAW applications, but several questions still remain without a clear answer. For example, evaluation of the unique properties of PAW solutions to explain the nature and time-dependent concentration profiles of the active species in different preservation conditions of liquid media. The life time or activity retention time of PAW may depend on the interaction of the reactive species and/or the composition of the media. It is also crucial to look into the impact of plasma species on other types of solutions besides water. In addition, a future study should elucidate the nature and main mechanism of nonthermal plasma active species to make PAW, potentially, more interesting and economic radioprotector agent.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research does not have funding.

Ethical approval

The care and use of animals were conducted in accordance with all applicable international, national, and/or institutional guidelines.

References

Abou-Zeid, S. M., El-bialy, B. E., EL-borai, N. B., AbuBakr, H. O., & Elhady, A. M. A. (2018). Radioprotective effect of date syrup on radiation-induced damage in rats. Scientific Reports, (8) 7423, 1–10. https://doi.org/10.1038/s41598-018-25586-3

Ahmed, K. (2014). Design and experimental investigations of electrical breakdown in a plasma jet device and applications (pp. 130–140). Faculty of Engineering at Shoubra Electrical Engineering Department. Benha University.

Ahmed, K., Allam, T., El-sayed, H., Soliman, H., Ward, S., & Saied, E. (2014). Design, construction and characterization of AC atmospheric pressure air non-thermal plasma jet. J Fusion Energ, 33(6), 627–633. https://doi.org/10.1007/s10894-014-9720-7

Al-Dosoki Mohamed, A., Mansour, A. M., & Ahmed, M. M. (2018). Effect of oxygenated water as a new treatment modality on experimentally induced hamster buccal pouch carcinogenesis. Al-Azhar Journal of Dental Science, 21(3), 261–273. Record 1110–2624 | the portal portal.org. https://doi.org/10.21608/ajds.2018.71567

Arora, R., Gupta, D., & Chawla, R. (2005). Radioprotection by plant products: Present status and future prospects. Phytotherapy Research, 19(1), 1–22. PMID: 15799007. https://doi.org/10.1002/tr.1605

Begum, N., Prasad, N., & Thayalan, K. (2012). Apigenin protects gamma-radiation induced oxidative stress, hematological changes and animal survival in whole body irradiated Swiss albino mice. International Journal of Nutrition, Pharmacology, Neurological Diseases, 2(1), 45–52. https://www.ijnpnd.com/text.asp?2012/2/1/45/93134. https://doi.org/10.4103/2231-0738.93134

Behn, C., Araneda, O. F., Llanos, A. J., Celedón, G., & González, G. (2007). Hypoxia-related lipid peroxidation: evidences, implications and approaches. Respiratory Physiology & Neurobiology, 158(2-3), 143–150. PMID: 17662674. https://doi.org/10.1016/j.resp.2007.06.001

Bock, J., Lee, A., & Bong, L. (2012). Oxygenated drinking water enhances immune activity in pigs and increases immune responses of pigs during salmonella typhimurium infection. J Vet Med Sci, 74(12), 1651–1655. https://doi.org/10.1292/jvms.12-0051

Boeck, M., De, Touil, N., Visscher, G., De, Vande, P. A., & Kirsch-Volders, M. (2000). Validation and implementation of an internal standard in comet assay. Mutation Research, 469(2), 181–197. PMID: 10984679. https://doi.org/10.1016/S1383-5718(00)00075-9

Carlsson, S., Iklund, N. P., Engstrand, L., Weitzberg, E., & Lundberg, J. O. (2001). Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. Nitric Oxide : Biology And Chemistry / official journal of the Nitric Oxide Society, 5(6), 580–586. PMID: 11730365. https://doi.org/10.1006/niox.2001.0371

Chew, B., & Park, J. (2004). Carotenoid action on the immune response. The Journal of Nutrition, 134(1), 2575–261S. PMID: 14704330. https://doi.org/10.1093/jn/134.1.2575

Cosby, K., Partovi, K. S., Crawford, J. H., Patel, R. P., Reiter, C. D., & Martyr, S. (2003). Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nature Med, 9(12), 1498–1505. PMID: 14595407. https://doi.org/10.1038/nm954

Dehui, X., shuai, W., Bing, L., & Maiao, Q. (2020). Effects of plasma-activated water on skin wound healing in mice. Microorganisms J, 1091(8), 1–14. https://doi.org/10.3390/microorganisms8071091
Eldeighdy, S. M., Allam, T. M., & Elsayed, H. A. (2020). Non-thermal plasma applications: impact of non-thermal plasma treated water on some biological functions in albino rats. World Journal of Pharmacy and Pharmaceutical Sciences, 9(10), 114–130. https://doi.org/10.20959/wjpps202010–17171

Fajardo, L. F. (2005). The pathology of ionizing radiation as defined by morphologic patterns. Acta Oncologica (Stockholm, Sweden), 44(1), 13–22. PMID: 15848902. https://doi.org/10.1080/02841860510007440

Fuchs-Tarlovsky, J. & Eldeighdy, S. (2000). Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. The Journal of Biological Chemistry, 275 (11), 7757–7763. PMID: 10713088. https://doi.org/10.1074/jbc.275.11.7757

Graves, D. B. (2012). The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. Journal of Physics D: Applied Physics, 26(45), 1–42.doi:10.1088/0022-3727/45/26/263001 IOP Publishing Ltd 263001.

Jagetia, G. C., & Jagetia. (2007). Radioprotective potential of plants and herbs against the effects of ionizing radiation. Journal of Clinical Biochemistry and Nutrition, 40(2), 74–81. PMCID: PMC2127223. https://doi.org/10.3164/jcbn.40.74

Jon, L. O., Weitzberg, E., & Gladwin, M. T. (2008). The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. Nature Reviews. Drug Discovery, 7, 156–167. https://doi.org/10.1038/nrd2466

Jun-seok, J., Sitzl, E. J., & Kotaro, Q. (2018). UV-vis spectroscopy study of plasma activated water:dependence of chemical composition on plasma exposure time and treatment distance. Japanese Journal of Applied Physics, 57 (1) 1–9. the journal society of applied physics, 010289. https://doi.org/10.7567/JJAP.57.010289

Kamal, A. M., Eldeighdy, S. M., Allam, T. M., & Hassanin, W. F. (2018). Power density measurements to optimize AC plasma jet operation in blood coagulation. Australasian Physical & Engineering Sciences in Medicine, 41(3), 621–632. https://doi.org/10.1007/s13246-018-0654-7

Kamgang-Youbi, G., Berry, J.-M., Brisset, J.-L., M.-n., B.-F., Doubia, A., & Naitali, M. (2008). Microbial inactivation using plasma activated water obtained by gliding electric discharges. Applied Microbiology and Biotechnology, 48(1), 13–18. PubMed. https://doi.org/10.1007/j.14372-765X.2008.02476.x

Karbowiak, M., & Reiter, R. J. (2000). Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Experimental Biology and Medicine, 225 (1), 9–22. https://doi.org/10.1046/j.1523-1730.2002.22502.x

Kaushika, N. K., Ghimirea, B., Lia, Y., & Adhikaria, M. (2018). Biological and medical applications of plasma activated media, water and solutions. Biological Chemistry J, 400(1), 39–62. PMID: 30044757. https://doi.org/10.1515/hsz-2018-0226

Kurita, M., Shimizu, M., Sano, K., Nakajima, T., Yasuda, H., Takashima, K., & Mizuno, A. (2014). Radical reaction in aqueous media injected by atmospheric-pressure plasma jet and protective effect of antioxidant reagents evaluated by single-molecule DNA reagents measurement. Jap. J. Appl. Phys, 53(S1) 1–4, the Japan Society of Applied Physics 05FR01. https://doi:10.7567/JJAP.53.05FR01.

Levesque, R., SpSS. (2007). Programming and Data Management: A Guide for SPSS and SAS Users (Fourth Edition).

Lofy, K. (2016). Cold atmospheric plasma and oxidative stress: Reactive oxygen species vs. antioxidant. Austin Biochem, 1(1), 2578–9481, 1001.googlesocular.

M. T., M. J., & Eldeighdy, S. (2011). Long-term antibacterial efficacy of air plasma-activated water. Journal of Physics D: Applied Physics, 44(47), 44472. Matthew J Taylor et al 2011 J. Phys. D: Appl. Phys. 44 472001.

Mahmoud, A. H., & El-Abd, K. M. T. (2019). Study of the effects of naturally occurring radioactive materials on blood indices in blood’s rats. Egypt J.Biophys. Biomed. Engng, 20(1), 1–7. https://doi.org/10.21608/ejbbe.2019.7930.1024

Marc, H., & Chambon, J. (2013). Physico-chemical, biological and therapeutic characteristics of electrolyzed reduced alkaline water (ERAW). Water,5(4) 2094–2115. https://doi.org/10.3390/w5042094

Michael, S., & Eldeighdy, S. (2013). Plasma-activation of larger liquid volumes by aninductively-limited discharge for antimicrobial purposes. Appl. Sci, 9(10), 1–12. https://doi.org/10.3390/app9102150

Mishima, S., Ito, K., Maruyama, H., Inoue, M., Yamashita, T., & Gu, Y. (2004). Antioxidant and immuno-enhancing effects of echinacea purpurea. Biological & Pharmaceutical Bulletin, 27(7), 1004–1009. PMID: 15256730. https://doi.org/10.1248/bpb.27.1004

Nagababu, E., A. Ramasamy, S., Abemethy, D. R., & Rifkind, J. M. (2003). Active nitric oxide produced in the red cell under hypoxic conditions by deoxymegoglobin mediated nitrite reduction. The Journal of Biological Chemistry, 278(47), 46349–46356. PMID: 12952953. https://doi.org/10.1074/jbc.M307572200

Nair, C. C., Parida, D. K., & Nomura, T. (2001). Radio protectors in radiotherapy. Journal of Radiation Research, 42(1), 21–37. PMID: 11393887. https://doi.org/10.1269/jrr.42.21

Nassini, R., & J.-L., Parida, D. K., & Nomura, T. (2001). Radio protectors in radiotherapy. Journal of Radiation Research, 42(1), 21–37. PMID: 11393887. https://doi.org/10.1269/jrr.42.21

Ostling, O., & Johanson, K. J. (1984). Microelectrophoretic study of radiation-induced DNA damages in individual cells. Biochem Biophys Res Commun, 123(1), 291–298. PMID: 6477583. https://doi.org/10.1016/0006-291X(84)90141-x

Popov, N. (2009). Associative ionization reactions involving excited atoms in nitrogen plasma. Plasma Physics Reports, 35(5), 436–449. https://doi.org/10.1134/S1063780X09050092(5)

Raisin, J. (1998). Baq andAlexander award lecture chemical radioprotection: Past, present and future prospects. International Journal of Radiation Biology, 73(4), 443–450. PMID: 9587083. https://doi.org/10.1080/0955300981422284

Reynders, K., & De Ruyscher, D. (2015). Radiotherapy and immunotherapy: improving cancer treatment through synergy. Progress in Tumor Research, 42, 67–78. PMID: 26383848. https://doi.org/10.1159/000437185

Said, U., & Eldeighdy, S. (2006). Effect of grape seed extract on hepatic function and antioxidant status of mouse bearing Ehrlich ascites carcinoma and exposed to gamma radiation. J. Radiat Res, 38(1), 225–240. Radiation Biology Department, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo (Egypt).ISSN 0021-1907
Sanetaka, S., Hamasaki, T., & Teruya, K. (2012). Advanced research on the health benefit of reduced water. Trends in Food Sciences & Technology, 23(2), 124–131. https://doi.org/10.1016/j.tifs.2011.10.009

Santos, D. M. F., Sequeira, C. A. C., & Figueiredo, J. L. (2013). Hydrogen production by alkaline water electrolysis. Quim, 36(8), 1176–1193. https://doi.org/10.1590/S0100-40422013000800017

Schmidt, A., Stephan, D., Anna, S., Klaus-Dieter, W., Thomas, W., & Kristian, W. (2015). Non-thermal plasma activates human keratinocytes by stimulation of antioxidant and phase II pathways. The Journal of Biological Chemistry, 290(11), 6731–6750. PMCID: PMC4358097PMID:25589789. https://doi.org/10.1074/jbc.M114.603555

Sharma, R., & Purohit, R. K. (2012). Protective role of liv.S2 against radiation and cadmium induced haematological changes in the Swiss albino mice. Int. J. Life Sci. Bt & Pharm. Res, 1(3), 114–123. http://www.ijlbpr.com/0413/20150413050204624.

Shirazi, A., Mihandoost, M., Gholbadi, G., Mohseni, M., & Ghazi-Khansari, M. (2013). Evaluation of radio-protective effect of melatonin on whole body irradiation induced liver tissue damage. Cell J, 14(4), 292–297. PMCID: PMC3593934, PMID: 23577309.

Singh, N. P. (2000). Microgels for estimation of DNA strand breaks, DNA protein crosslinks and apoptosis. Mutation Research, 455(1-2), 111–127. https://doi.org/10.1155/2013/953079

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantification of low levels of DNA damage in individual cells. Experimental Cell Research, 175(1), 184–191. PMID: 3345800. https://doi.org/10.1016/0014-4827(88)90265-0

Takai, E., Ohashi, G., Yoshida, T., K.M.S., Zako, T., Maeda, M., Kitano, K., & Shiraki, K. (2014). Chemical modifications of amino acids by atmospheric –pressure cold plasma in aqueous solutions. Applied Physics Letters, 47(28) https://doi.org/10.1088/0022-3727/47/28/285403.104 023701. IOP Publishing.

Tasaki, T., Ohshima, T., Usui, I., Ikawa, S., Kitano, K., Maeda, N., & Momoi, Y. (2017). Plasma-treated water eliminates streptococci mutants in infected dentin model. Dent. Mater. J, 36(4), 422–428. PMID: 28367914. https://doi.org/10.4012/dmj.2016-358

Thirumdas, R., Kothakota, A., Annapure, U., Siliveru, K., Blundell, R., Gatt, R., & Valdramidis, V. P. (2018). Plasma activated water(PAW):chemistry, physic-chemical properties, applications in food and agriculture trends. Food Sci. Technol,(27) 21–31. https://doi.org/10.1016/j.tifs.2018.05.007

Utsumi, F., Kajiya, H., Nakamura, K., Tanaka, H., Mizuno, M., Ishikawa, K., Kondo, H., Kano, H., Hori, M., & Kikkawa, F. (2013). Effect of indirect nonequilibrium atmospheric pressure plasma on anti-proliferative activity against chronic chemo-resistant ovarian cancer cells in vitro and in vivo. PLoS One, 12(8), 1–10. https://doi.org/10.1371/journal.pone.0081576

Vidyasagar, D., Global Minute. (2007). Water and health–walking for water and water wars. Journal Perinatol, 1(27),56–58. https://doi.org/10.1038/sj.jp.7211629

Wink, D. A., Miranda, K. M., Espey, M. G., Pluta, R. M., & Grisham, M. B. (2001). Mechanisms of the Antioxidant effects of Nitric Oxide. Antioxidants and Redox Signaling Journal, 3(2), 203–213. PubMed. https://doi.org/10.1089/152308601300185179

Yan, X., Xiong, Z., Zou, F., Zhao, S., Lu, X., Yang, G., He, G., & Ostrikov, K. K. (2012). Plasma-induced death of HepG2 cancer cells: intracellular effects of reactive species. Plasma Processes and Polymers, 9, 59–66. https://doi.org/10.1002/ppap.201100031