Chapter

Lipid Peroxidation and the Redox Effects of Polyherbal

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Abstract

The use of more than one herb in a medicinal preparation also known as polyherbal has increased geometrically in recent times. Over a hundred thousand scientists have cited "herbal" to strengthen its ethnopharmacological relevance in literature. Polyherbal (PH) is effective potential therapeutic compound used globally to treat oxidative stress-induced injuries which give credence for their traditional applications. However, some issues related to safety and adverse reactions due to PH have raised important public health debates. Lipid peroxidation (LPO) assay is widely used to assess the toxic endpoint of PH. This paper discusses some important roles that PH plays during oxidation–reduction processes.

Keywords: Polyherbal, Lipid Peroxidation, Oxidative Stress, Antioxidant, Ethnopharmacology

1. Introduction

Lipid peroxidation (LPO) is one of the oldest risk factors for oxidative stress and its mechanistic processes in disease modulation were first observed during the oxidative deterioration of edible materials [1]. The independent works of scientists in the identification of membrane shedding formed the earliest breakthrough for the study of lipid peroxidation [1, 2]. The biochemistry of oxygenase moiety was a light to the further understanding of the peroxidation of lipids [2]. The central roles play by small lipid molecules, free radicals and cytochrome p450 have been well established [3, 4]. The small lipid molecules are transformed into low-density lipoprotein (LDL). LDL modulations have contributed immensely to the studies on cellular biology. Both enzymatic and non-enzymatic approaches to scavenging LPO have been reported [5]. LPO has its root in several diseases including neurodegeneration, cardiovascular, respiratory, and cancers [2]. Consequently, the chemopreventive and protective roles of several antioxidants with proven efficacies have been documented in the literature. Although the different compounds of antioxidants have been identified, researchers have shown that it is by coordinated efforts that they can quench oxidative damage [6]. Evidence abounds of the involvement of LPO in several diseases and the protective roles of medicinal plant antioxidants [7, 8]. Often time in traditional medicine practices, medicinal compounds that perform a similar function are combined to obtain synergy [9, 10]. Thus, potential antioxidants are capable of preventing, protecting, and eliminating any form of identifiable oxidants [11]. The medicinal effects of functional food have been known for a long time. This observation was the result of preservative, anti-oxidative and antimicrobial actions found in ginger, nutmeg, turmeric, etc. These help arrest, repair,

and restore potential injuries [12]. The scavenging efforts by antioxidants provide auto-inhibition or sometimes intervention to the activity of LPO metabolites [13]. Interestingly, in the case of progressive disease, the presence of combined efforts of antioxidants can offer a cascade of reactions to stop the process involved. Despite the tremendous roles of natural product medicines, some issues related to safety and adverse reactions due to PH have raised important public health debates [14]. Lipid peroxidation assay is one of the simplest methods widely used to assess the toxic endpoint of PH. Thus, this paper discusses some important roles that PH plays during oxidation–reduction processes.

2. Polyherbal (PH)

The use of herbal medicinal products has become popular for several reasons since they have always been the last order of resort when conventional therapy failed [15, 16]. Majorly, Polyherbal (PH, also known as herbal or traditional medicines) are used as complementary and alternative medicines [17]. The direct and indirect effects of polyherbal (PH) involvement in modulating LPO product formation have attracted scientific debates in recent times [15, 18]. Among the many reasons for use are that they are natural supplements, relatively safe, less toxic, and cheaper [15, 19]. The largest conventional therapy is obtained from plant sources, and many of them are nurtured within the neighborhood [20]. As the usual practice in traditional medicine, any substance or medicinal agent of natural origin may be used alone or with other agents as a polyherbal formulation which often requires less expertise [14]. PH is considered to be supplements and so boycotted major laws [21]. Thus, in most cases, measures for dose regulation are lacking in PH preparations as prohibition is difficult to reach [22]. This makes regulating agencies find it difficult to gather and provide the necessary implementation in many countries of the world. Despite the effort of the World Health Organization (WHO) to issue a certification scheme for the regulation of PH, the problem of non-compliance has been on the high side [23]. To this end, important PH like the *Aloe vera*, ginger, *Curcuma longa*, *Moringa oleifera*, *gingko biloba*, kava, milk thistle among others have birth beneficial compounds. Yet, all of these have been documented and updated for known adverse herb reactions [14, 22, 24]. Some variations in formulations among other factors could further influence the final herbal agents and/or products to be marketed. Reports on the adulteration of PH with conventional agents are grossly available and have raised serious concerns [22, 25]. Thus, the need for new regulations for botanicals has long been overdue. Other possibilities of PH being contaminated with undisclosed molecules around have been suggested to influence long-term consumption [26]. In respect, PH combination is a multifactorial antioxidant with the sole aim of scavenging oxidative radicals [16]. Both natural and synthetic antioxidants compounds abound [21, 27]. LPO products also result in deoxyribonucleic acid, cell membrane, and tissue damages in the human body [1, 2]. The global demand for antioxidants is evident and has prompted scientific interests in searching for new and safe antioxidant substances of natural origin. The WHO report shows both relevant authorities of people living in developing and developed countries have approved the use of traditional medicine for their primary healthcare. Plants possess the innate ability to synthesize a wide variety of enzymatic and non-enzymatic antioxidants capable of attenuating ROS-induced oxidative damage [12]. In respect, using appropriate separation techniques, we can now study the presence of phytoconstituents contributing to the antioxidant property of a given plant mixture [11, 16]. These mixtures may serve as a potential

source of exogenous antioxidants to combat the undesirable effects of oxidative stress. Currently, this forms the basis for the PH that we now witnessed.

3. Importance of PH as Ethnomedicines

There are some conditions of inadequate efficacy, side effects, and pharmacokinetic problems of conventional drugs used for disease modulation [14]. Recent studies focused on the pharmacology and feasibility of herbal compounds as a potential strategy for alternative therapy. Now the discovery of novel therapeutic agents with multi-targeted potential is desirable [13, 20, 28]. Protective properties of phytochemicals combat numerous diseases and their vast acceptance and demand in human beings encouraged scientists to assess their effective activities [9, 11]. Artemisinin for instance has been known for decades and forms one of the most commonly prescribed medicinal agents [25]. The same has now emerged into an antimalarial drug used as first-line medication in this regard in addition to its adjuvant anticancer potential [10, 13]. Also, ethnomedicine has risen to provide an alternative to biofilm infections to improve medical treatments by the use of combinatorial treatment of bacterial biofilms as re-potentiators of classical antibiotics [9]. Actions related to anti-growth, anti-biofilm, or anti-quorum-sensing activities, to control bacterial infections have been associated with the use of PH including *P. granatum* or propolis compounds against known bacteria agents [9]. Other important derivatives of ethnomedicines are the phytoestrogens [8, 16]. They are a large family of plant-derived molecules possessing various degrees of estrogen-like activity; they exhibit agonist or antagonist estrogenic properties depending on the tissue. Delay of skin to undergo degenerative changes as it ages is now possible using relevant phytoestrogens knowledge to modulate skin elasticity, reductions in the epidermal thickness and collagen content, elastic fiber degeneration, and increased wrinkling and dryness. In turn, this is a key to senescence control via estrogen modulation [16]. Relevant interventions to target a variety of human diseases ranging from metabolic to brain disorders are now available [3, 12]. Several herbs have been reported to target the main biochemical events that are implicated in mental disorders, mimicking, to some extent, the mechanisms of action of conventional antidepressants and mood stabilizers with a wide margin of tolerability [3, 12]. With both experimental and clinical evidence, they rescue alterations in neurotransmitter and neuroendocrine systems, stimulate neurogenesis and the synthesis of neurotrophic factors, and they counteract oxidative stress, mitochondrial dysfunction, and inflammation e.g. saffron, crocin, etc. play a very significant role as a nutraceutical for cognitive functions affected by body injuries [11]. More so, ethnomedicine encourages nanotechnology development in the preparation of a herbometallic nano-drug [27, 29]. Recent studies on the physicochemical analysis confirmed that specific plant-derived herbometallic nano-drug such as rasa manikya nanoparticle were rich in mineral constituents and showed therapeutic opportunities for combating drug-resistant microbial strains among others [27]. Furthermore, some eminent components of traditional medicinal agents have contributed to cardio-metabolic disease treatment for decades. Phytomedicines such as berberine, lemon balm among others has attracted much interest for their pharmacological actions in managing cardio-metabolic diseases [29]. Recent discoveries of basic, translational and clinical studies have identified many novel molecular targets for phytocompounds, and provided novel evidence supporting the promising therapeutic potentials [16, 23, 26]. Hepatoprotective and renoprotective effects are two major medical challenges worldwide and a wide variety of herbs have been

studied for the management of their related diseases. Bioactive compounds including silymarin, quercetin, curcumin, ginseng, and rutin for instance have long been used in traditional medicine [7]. Both in combating diseases, exerts hypolipidaemic and antioxidant effects, which prevents the fatty acid accumulation in the cells that may result from metabolic imbalances, and which affects multiple processes and signaling pathways [4, 13, 27].

4. Risk factors for PH-induced LPO

There are several setbacks to draw from the recent criticisms of PH applications [14, 25, 30]. This is the reason for the suggestions on standardization developments [15, 24, 28, 31]. The latter may help gain insight into their mechanisms as well as potentials for toxicity.

4.1 Dose

Several PH compounds have been extensively used as a traditional medicine for various therapies [18]. Because of the multifaceted component of PH mixture, several of the published articles showed that manufacturers relied mostly on the documented efficacies of the constituent compounds. There is a lack of specifications for the dose selection of subtherapeutic, therapeutic, and supratherapeutic doses which are used in animal studies, and for animal-to-man dose extrapolations [29]. Till now, there is a lack of formula to relate PH constituents; hence, lack of proper dose extrapolation of a single compound when combined with another may result in the potential mechanistic toxicological effect of the constituent mixture [28, 32]. Several misconceptions about dietary supplements being safe to have increased the number of hospital admissions [14, 17]. Recent studies on the PH dietary supplement popularly known as Cellgevity® (CG) confirmed that this premiere antioxidant supplement formula could act as a pro-oxidant, a substance capable of distorting the antioxidant systems [28]. Studies on the effects of therapeutic and supra-therapeutic doses of CG on reproductive function and biochemical indices in animals demonstrated some detrimental effects. CG is one of the most widely used glutathione supplements that has been considered to be harmless, nevertheless, this general assumption should not be overlooked. It is marketed to salvage for the body glutathione and/or complements its production. D-Ribose and L-Cysteine are the active compounds in CG in addition to the presence of vitamin C, selenium, alpha-lipoic, broccoli seed extract, curcumin, resveratrol, grape seed extract, quercetin, milk thistle seed extract, cordyceps, black pepper, aloe leaf. Some convergent opinions have highlighted the antioxidant activity of spices and their impact on human health, in particular, to increase reduced glutathione (GSH) concentration [12]. Whereas the presence of GSH improves protein function in a perturbed environment, its roles in modulating hypoxic apoptosis or oxidative stress is of great concern. This has put the faith of current supplement antioxidants in doubt, such as protein supplements and others, which now beg for safety evaluation. Previous studies have reported that dietary supplements are now being used to prevent and treat various diseases [7]. We have now understood that any excess of antioxidants could be detrimental and can result in adverse events and even death. Reports over the past few years have implicated the use of herbs and herbal products to generate reactive oxygen and nitrogen species. Mahwangyounpae-tang (MGT) is another but very popular antioxidant PH consisting of about 22 compounds [32]. Reports on MGT showed that MGT extract was safe for use in asthma at the sub-acute repeated

oral dose levels. However, when the doses were increased per daily in rodents, there were correspondingly increased morphological aberrations and organ histoarchitectural changes characterized by hypertrophy of the heart and tubular necrosis of the kidney [32]. Thus, concerns over the doses of antioxidants when using alone, or with other drugs have arisen (**Table 1**).

4.2 Duration of treatment

Evaluations of most of the PH for toxicological profiles are most unlikely not going to be sufficient to determine the endpoint toxicity [24]. For instance, the majority of the acute to chronic studies found in the literature were between 14 and 90 or 180 days. However, only a few studies show reversibility assessments or show species specifications [38]. Over 80–90% of the toxicological studies published showed few or no adverse drug reactions. Also, the parameters for their toxicological endpoint did not capture genotoxicity potential [39]. As with the case of CG, which supposedly should provide a maximum antioxidant function by GSH synthesis rates and concentrations as expressed by the content could act as a pro-oxidant causing oxidative damage in normal humans particularly at high dosages [28]. Such transition in chemical nature could generate pro-oxidant–antioxidant imbalance thereby produce undesirable toxins leading to oxidative stress [22, 25]. Hence, a long-term toxicological profile plus storage history has been recommended to ascertain PH safety.

Polyherbal	Uses	Potential Mechanisms	Potential Target
Mahwangyounpae-tang® [6]	anti-inflammatory and asthma	Immune system, blood, pro-inflammation	Lungs, heart and kidney
Cellgevity® [28]	Antioxidant supplement formula	Immune system, haematotoxicity, pro-inflammation, elevation of serum low density lipoprotein	Blood, liver, testis, kidneys
Hydroxycut [30]	Weight loss supplement	Elevation of hepatic biomarkers, pro-inflammation	Liver
Bon-sante cleanser® [33]	Body hormones booster and energizer	Immune system and body hormones, pro-inflammation	Liver, heart
Bronco-T® [34]	Anti-inflammatory and lung regeneration	Immune system, blood, pro-inflammation, bronchoconstriction	Lungs
Jambadyarista® [35]	Diabetes and its associated complications	Hypolipidic, Nuclear factor-κappa B activation	Pancreas
Shengmai formula® [36]	Cardiovascular diseases	Opening of mitochondrial permeability transition pore, cyclophilin D.	Heart, liver
Hab-e-Kabad Noshadri Hepatic disorders® [37]	Hepatic disorders, abdominal problems.	Elevation of hepatic and renal biomarkers, inflammation	Liver, kidneys and gastrointestinal system

Table 1.
Shows the potential targets of PH-induced lipid peroxidation.

4.3 Lethal dose estimation

The median lethal dose (LD_{50}) is the statistically derived dose following the administration of any PH which is expected to produce death in 50% of the treated population [40]. The toxic effects of chemicals, food substances, pharmaceuticals, etc., have attained great significance in the 21st century [17]. Toxicity tests are mostly used to examine specific adverse events or specific endpoints in disease identifications. Toxicity testing also helps to calculate the No or Low Observed Adverse Effect Level (NOAEL/LOAEL) dose and is helpful for clinical studies [39]. However, the methods of determination of median lethal dose (LD50) may impact negatively on the information for the use of PH. Therefore exaggerated lipid peroxidation levels due to PH might be the results of poor safety methodology [23]. This however can be minimized by using several methods to ascertain the PH LD50 level. Studies have suggested that lack of expertise in this aspect of scientific investigation might influence judgment on the PH formula [24]. Inability to in-cooperate the knowledge of individual median lethal dose might create impurities in the constituent mixture. The five most commonly paraded bitters in Nigeria for type 2 diabetes are Yoyo bitters (YB), Oroki herbal mixture (OB), Ruzu Bitters (RB), Fijk flusher (FB), and Fidson Bitter (FB) respectively. Although, each of this preparation has claimed for several indications, however, scientific investigations for the mixture have reported weight trimming and blood sugar modulations (Kale et al., 2018). Since their constituents are known, their ability to act synergistically and the potential for precipitating adverse herb reaction of LPO have been ascertained [15]. These concerned mixtures have multifaceted constituents most of which have popular applications. Examples include ginseng, *Aloe vera*, *Citrus aurantifolia*, *Sorghum bicolor*, *Mangifera indica*, etc. These have yielded very important phytomedicines or bioactive components that have been confirmed by different studies, although, some exhibit overlapping effects in disease management. *Chenopodium murale* (Chenopodiaceae) has yielded analgesics, anti-inflammatory, anti-fungal, anti-bacterial, anti-oxidant, hypotensive, and hepatoprotective molecules. These PH administrations improved lipid parameters in diabetic rats. While hypercholesterolemia persists in diabetic rats treated with RB and FJB, RB increased LDL in treated rats [15]. Further, RB, FJB, and OB showed tendencies to elevate serum TC while RB increases LDL cholesterol in rats. This indicates the suitability of these products to produce LPO molecules as a risk factor for dyslipidemia in potential users.

4.4 Manufacturer Bias

In contrast to the general belief, medicinal plant preparations have been shown to pose serious health risks in a dose and time-dependent either alone or in combination with other agents [19, 38]. Reports have it that PH contain tightly bound bioactive compounds and have shown the possibilities of an indirect risk that can be independent of the active compound [16]. Countries are now examining national pharmacovigilance data using statistical tools to report population possible risks [24]. This is because, oftentimes, the species specification by manufacturers on PH constituents did not correlate most times with products packaging thereby altering scientific decision on toxicological evaluations [25]. The potential to generate lipid peroxidation by several PH are underestimated [14]. On the other hand, the latter creates a contrast in mind when a single compound has a toxicity level greater than the combined mixture. Additionally, erroneous claims of total cure and or weight supporting supplement some of these PH are marketed with approved consent from regulatory authorities [22]. There are also exaggerations of PH with conventional drugs which have been suggested to contribute in part to the unwanted adverse reaction of some PH [14, 20, 23]. Thus, this issue

associated with production bias has raised concern about quality control, screening methods as well as toxicity scoring which most regulatory authorities however have not been able to properly address it. Bon-santé cleanser® (BS) is a popular PH comprising of anogeissus leiocarpus (DC., family Combretaceae), *Terminalia ivorensis* (A. Chev.), massularia acuminate (G. Don,) Bullock ex Hoyle and macuna pruriens (L.,) DC (fabaceae) respectively which have been formulated into capsule. The proposed claims include androgenic, antipyretic, analgesic, and anti-inflammatory potentials. The pharmacological activities of *M. acuminata*, *T. ivorensis*, A. leiocarpus and *M. pruriens* have been adduced to be due to the presence of glycosides, dimeric antioxidants, phenolics and flavones respectively. Despite the relevance of BS in boosting the body hormones including the follicle-stimulating, luteinizing hormone and testosterone respectively, reports have implicated this BS as a potential hepatotoxicant and a commodity with pro-oxidants status. Increasing the dose of BS was associated with significantly reduced sperm motility, live-dead ratio, testis weight, and cause mild inflammation in the vital organs testis in the animals [40]. There was diminishing return as higher doses did not exert any significantly different change in the level of body hormones which could cause the negative feedback effect on the anterior pituitary [33]. Further, this PH demonstrates the potential to induce LPO of the testicular origin or promote the generation of free radicals in vivo.

5. Molecular mechanisms of PH-induced LPO

Acridocarpus smeathmannii (DC.) is widely used for the treatment of male infertility, anemia, and pains in traditional medicine as well as an herbal mixture. *A. smeathmannii* has gained popularity particularly worldwide for the management of infertility, anemia, pain, and some cutaneous infections [20, 41]. Studies have demonstrated the ethnobotanical relevance of this plant. Thus, recent reports demonstrated the bioactive compounds, sexual behavior, and associated reproductive function via biochemical and pharmacological mechanisms due to the hydroethanolic root extract of *A. smethmannii* (HAS) and subchronic oral toxicity effects of HAS [31]. Also, the organization for economic co-operation and development (Test No. 453) [39] has approved that very long-term toxicological and/or carcinogenicity studies be carried, in particular for PH that are considered not to be potentially toxic at therapeutic and supratherapeutic doses. In this study, the possible systemic toxicological changes following a 180 days administration in Wistar rats of both sexes under approved guidelines for animal use following the procedures as documented by Kilkenny et al. [42] for reporting animal research. Animals (Wistar rats, male: female = 1:1 = 48) received distilled water (10 mL/kg) or HAS (250, 500 or 1000 mg/kg body weight per day) consecutively for 180 days. From the results obtained, HAS (500 and 1000 mg/kg) demonstrates such a tendency to increase oxidative stress parameters via an increase in LPO as malondialdehyde (MDA) levels in vital organs (**Figure 1**) in rats. Although, evidence abounds of the turnover of products of LPO in the body, however, the presence of an adverse reaction may become aggravated time-dependently or produces an interaction with other substances presences [14, 26]. This could impose either a self or even exaggerates the effect of HAS in a given period. Increasing levels of the intracellular antioxidants, GSH levels, could not overcome the LPO products induced by the highest dose of extract in rats, thus, highlighting potential adverse effects of HAS in vivo. This suggests that PH can induce LPO metabolites such as MDA which by this observation could translate into a clinically relevant situation. More so, oestrogen level was reduced in treated female rats as obtained in these results. Also, in both sexes, HAS (500 mg/kg) and HAS (1000 mg/kg) showed elevated serum nitric oxide (NO) levels respectively (**Table 2**).

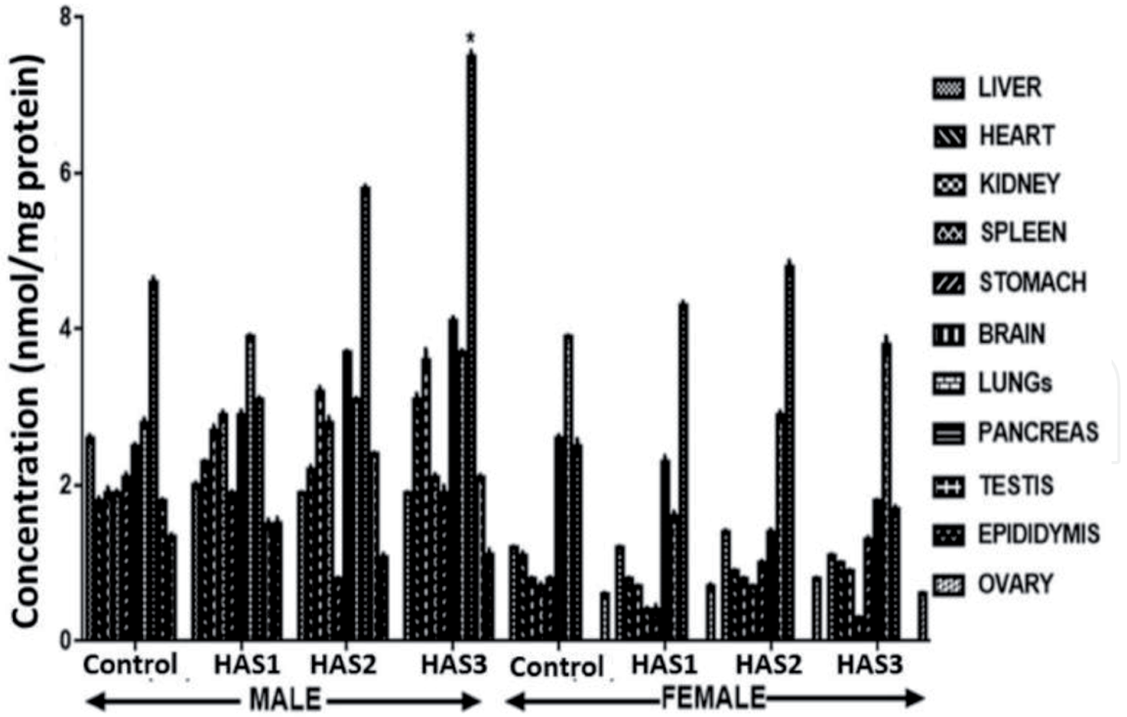

Figure 1.
Effect of HAS on lipid peroxidation in normal Wistar rats. Results are expressed as mean ± S.E.M. n: Total number per group. n = 12. Mortality = HAS (500) (male, 25%), HAS (1000) (male, 33.3%), HAS1 (250 mg/kg) (female, 8.3%), HAS2 (500 mg/kg) (female, 25%) and HAS (1000 mg/kg) (female, 41.7%) respectively. $^{*}p < 0.05$ or $^{**}p < 0.01$ compared with control (distilled water: DW, 10 mL/kg) group. HAS1 (250): 250 mg/kg, HAS2 (500): 500 mg/kg, HAS3 (1000): 1000 mg/kg, HAS: Hydroethanolic extract of Acridocarpus smeathmannii *root.*

	Control	HAS1 (250 mg/kg)	HAS2 (500 mg/kg)	HAS3 (1000 mg/kg)
Testosterone^{x}	3.56 ± 0.09	4.53 ± 0.04*	4.84 ± 0.06*	6.48 ± 0.13*
PSA^{x}	1.83 ± 0.01	1.89 ± 0.01	1.81 ± 0.01	0.69 ± 0.02*
Nitric Oxide^{x}	0.65 ± 0.10	0.85 ± 0.10	1.28 ± 0.10*	1.83 ± 0.20*
TNF-α^{x}	3.19 ± 0.19	2.72 ± 0.19	4.09 ± 0.18	4.46 ± 0.20*
NF-kB^{x}	1.03 ± 0.14	1.64 ± 0.16	2.02 ± 0.13*	2.83 ± 0.12*
Oestrogen^{y}	51.72 ± 1.43	63.81 ± 2.26	51.44 ± 1.38	40.55 ± 2.13*
Progesterone^{y}	43.91 ± 0.39	125.03 ± 0.58*	143.91 ± 0.29**	168.60 ± 0.38**
Nitric Oxide^{y}	1.40 ± 0.01	1.38 ± 0.01	2.50 ± 0.02*	2.40 ± 0.02*
TNF-α^{y}	5.12 ± 0.19	6.90 ± 0.18	6.79 ± 0.19	8.44 ± 0.18*
NF-kB^{y}	1.25 ± 0.11	1.32 ± 0.14	3.23 ± 0.12*	4.11 ± 0.16*

Results are expressed as mean ± S.E.M. n = 12. HAS: hydroethanolic extract of Acridocarpus smeathmannii root. $^{*}p < 0.05$ or $^{**}p < 0.01$ compared with control distilled water group. "x" and "y" in superscript represented "male" and "female" rats respectively. Mortality: HAS (500) (male, 25%), HAS (1000) (male, 33.3%), HAS (250) (female, 8.3%), HAS (500) (female, 25%) and HAS (1000) (female, 41.7%). Oestrogen (pg/mL), Progesterone (ng/mL), Testosterone (ng/mL), Serum Nitric Oxide (nmol/mL), TNF-α: Tumor Necrosis Factor-alpha, PSA: Prostate Specific Antigen (ng/mL). Differences between groups were determined by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, version 20.0) software for windows and Post hoc test for intergroup using the least significant difference, followed by Dunnett's test. Significance was considered at $p < 0.05$. All results were expressed as the mean ± standard error of the mean.

Table 2.
Effect of hydroethanolic root of Acridocarpus smeathmannii *extract on body hormones and molecular biomarkers in serum of normal and treated rats using enzyme-linked immunosorbent assays.*

Nitric oxide plays a very significant role as a signaling molecule in several biological events [43]. Also, the highest dose, HAS (1000 mg/kg) increased serum tumor necrosis factor-alpha (TNF-α) level, a hallmark biomarker of tissue injury, in rats of both sexes (**Table 2**). The presence of TNF-α level could indicate some processes involving the action of nuclear factor kappa-B and a host of other mediators of injury [44]. Even at the lowest dose (250 mg/kg) of HAS administered to rats, serum NF-kB levels increase (**Table 2**). The aforementioned provides such suitability that HAS administration may induce cellular changes ranging from inflammatory reactions to LPO metabolites. An inducible nitric oxide synthase (iNOS) is an importantly upregulated isoform of NOS that synthesis the inducible nitric oxide (iNO) [45]. iNO is a disease-causing agent that can increase iNOS mRNA, protein, and then causes activity that may significantly alter NO turnover [42]. From the study results, administration of HAS after 180 days increases the chances of iNOS expression (**Figure 2**) in rats thereby showing the potential inherent pro-inflammatory and pro-oxidant properties in HAS that can cause tissue damage.

5.1 MDA levels

HAS1 lowered MDA level in the stomach by 32.2% in normal male rats (**Figure 1**). However, HAS2 produces an increased ($p < 0.05$) MDA levels in pancreas, heart, and

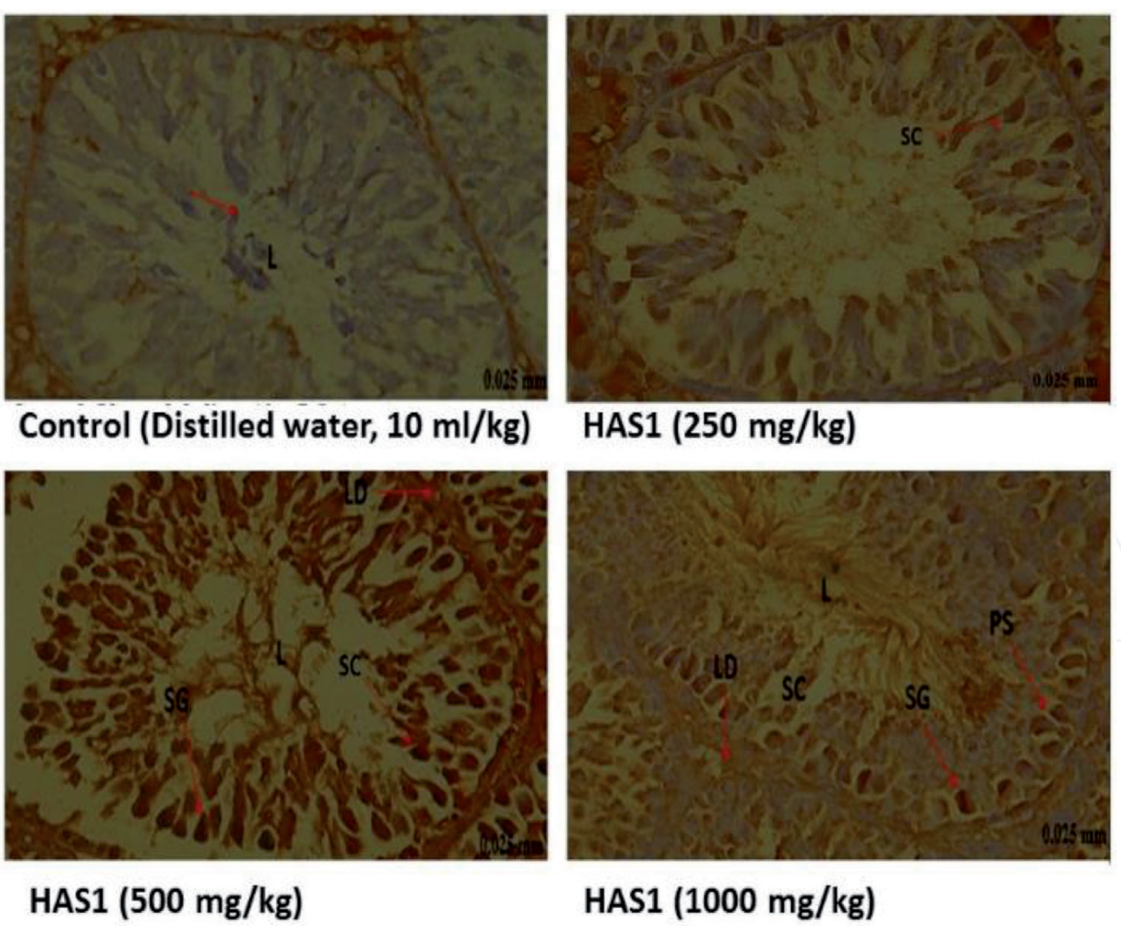

Figure 2.
Immunohistochemical photomicrograph of inducible nitric oxide synthase (iNOS) staining in the testis of male rats. The whole germ layer in control (Normal saline, 10 mL/kg) group show iNOS negative immunoreactivity. The Sertoli cells (arrows) majorly show positive reaction in HAS1 (250 mg/kg) group while the whole germ cells in the HAS2 (500 mg/kg) group have positive reactions. The primary spermatogonia show positive reaction than other cells in the germinal layer in the HAS3 (1000 mg/kg) (arrows). SG, Spermatogonium; SC, Sertoli cell; PS, primary spermatocyte; L, lumen. HAS, Hydroethanolic extract of Acridpcarpus smathmannii *root. (Mag. ×400.)*

brain by 113.64%, 70.37%, and 36% respectively. Additionally, HAS3 elevated MDA levels in testis (79.41%), kidney (52.38%), pancreas (104.55%), heart (88.89%), and brain (96%) respectively in rats. on the other hand, in the female rats, HAS1 caused an increase in MDA level in lung (57.69%) and pancreas (88%) respectively. Similarly, HAS2 produce elevated MDA levels in the lung and pancreas by 80.77% and 44% in the treated rats, whereas, HAS3 further increased ($p < 0.05$) MDA levels in the lung (50%), brain (82.14%), and pancreas (24%) respectively in the treated animals.

5.2 Reproductive hormones and molecular biomarkers

HAS3, HAS2 and HAS1 showed a dose-dependently decrease ($p < 0.05$) testosterone levels by 82.02%, 36.01% and 27.25% respectively (**Table 2**). Additionally, the PSA level was lowered in rats that received HAS3 by 62.44%. On the other hand, in female rats, HAS1 and HAS2 administration elevated serum oestrogen levels by 23.38%, whereas HAS2 and HAS3 lowered oestrogen levels by 0.54% and 21.60% when compared with control. Further, progesterone was increased in all the treated female rats by 283.97% (HAS3), 227.74% (HAS2), and 184.74% (HAS1) respectively. In male rats, serum NO levels were elevated ($p < 0.05$) following HAS2 and HAS3 administrations. Also, HAS3 increased ($p < 0.05$) TNF-α level by 39.99% in rats. In the female rats, however, both HAS2 and HAS3 elevated ($p < 0.05$) serum NO levels by 78.57% and 71.43% respectively. Further, TNF-α and NF-kB were increased in rats serum of HAS3 by 64.84% and 228.8% when compared with control.

6. Conclusions

The evidence that complementary and alternative therapy plays a crucial role in the management of health is indisputable. However, because of the complexity of the phytocompound present in PH, they may act as pro-oxidants as well as antioxidants. Therefore, as pro-oxidants, they generate lipid peroxidation products which are an important risk factors for tissue damage.

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Conflict of interest

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Acronyms and abbreviations

LPO	Lipid Peroxidation
MDA	Malondialdehyde

PH	Polyherbal
TNF-α	Tumor Necrosis Factor-alpha
HAS	Hydroethanolic extract of *Acridocarpus smeathmannii* root
iNOS	Inducible nitric oxide synthase
LD_{50}	Median lethal dose
ROS/RNS	Reactive Oxygen/Nitrogen Species

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