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Review Article

Butylated Hydroxyanisole-Induced Alterations in the Stomach and Kidney of Albino Rat : A Mini Review

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Abstract— Butylated hydroxyanisole or commonly abbreviated as BHA, is mostly employed as a food preservative to prolong the products shelf life by stopping or postponing oxidation processes. The commercial product is usually a combination of 90% 3-BHA and 10% 2-BHA. BHA is generally considered safe when used as a food additive, Since, butylated hydroxyanisole is so widely used, it can be detected in both human tissues and a variety of environmental matrices. Humans are mostly exposed to butylated hydroxyanisole through their diet. One of the primary metabolites that butylated hydroxyanisole can produce under different conditions is tert-butyl hydroquinone (TBHQ). According to a number of studies, butylated hydroxyanisole may harm the thyroid system and result in growth and metabolic problems, neurotoxicity, and cancer. One of the main priorities is to minimize the harmful effects of BHA. Future studies should concentrate on identifying safe, non-toxic, and eco-friendly substitutes for BHA. This review aims to highlight the hazardous and detrimental effects of butylated hydroxyanisole in animal models. We are confident that this assessment will yield important data about the toxicological nature of butylated hydroxyanisole, that will aid in the development of safe usage guidelines.

Keywords— *Butylated hydroxyanisole, stomach, kidney, toxicity*.

1. Introduction

The techniques used to handle and preserve food in order to stop or drastically reduce spoiling—the loss of nutrients, quality, or edibility caused by microorganisms are referred to as food preservation [1]. However, some methods use innocuous bacteria, yeasts, or fungi to give food specific qualities and prolong its shelf life. It also discusses ways to stop aging and discoloration that occur naturally during food preparation [1]. Food, packaging for food, animal feed, personal care products, rubber, petroleum products, and pharmaceuticals all include butylated hydroxyanisole, a common synthetic phenolic antioxidant that prolongs product shelf life by stopping or postponing oxidation events. 2-tert-Butyl-4-hydroxyanisole or 2-BHA and 3-tert-Butyl-4-hydroxyanisole or 3-BHA are two isomers of butylated hydroxyanisole. Typically, the commercial product contains 90% 3-tert-Butyl-4-hydroxyanisole and 10% 2-tert-Butyl-4-hydroxyanisole. Butylated hydroxyanisole is generally considered safe when used as a food additive, according to European Food Safety Authority (EFSA) documentation, as long as the daily consumption of BHA stays within the 0.5 mg kg−1 body weight (BW) maximum suggested acceptable daily intake (ADI) each day [2-9]. However, in practice, butylated hydroxyanisole application, exposure, and consumption are far more complex, and determining the boundaries of butylated hydroxyanisole poisoning can be difficult. Regarding environmental exposure, the use of butylated hydroxyanisole invariably results in widespread environmental contamination. Numerous environmental media, such as litter or dust [10–12], watercourse or streams [13-14], water surface [15], sediment [16-18], emanation or excrement [16-17,19], and cosmetics[20],contain butylated hydroxyanisole. Human exposure to butylated hydroxyanisole is also extremely dangerous and can happen through a variety of ways, including eating certain foods[21-22], breathing in dust[10-12], using cosmetics[20], and more. The crucial necessity of butylated hydroxyanisole safety evaluation is highlighted by the detection of BHA in a wide range of human samples, particularly fatty tissue[23], blood plasma and placenta[24],maternal milk[25],urination[26-27],unguis or thumbnail[28], and serum[29]. Butylated hydroxyanisole offers a wide range of features. Butylated hydroxyanisole is known by several names, including pro-oxidant, estrogenic

disruptor, carcinogen, tumour promoter, and anticancer agent[30]. According to earlier research, butylated hydroxyanisole is linked to growth retardation[31-32], thyroid damage[31,33], metabolic problems[34-36],carcinogenicity[37-41],neurotoxicity[32,42],and reproductive harm[33,43]. Many investigations have examined the exact mechanism of action of butylated hydroxyanisole. Butylated hydroxyanisole may have detrimental effects on energy metabolism[46], endocrine disruption[44], genotoxicity[45], pro-oxidative[32], signalling pathways such as the PI3K/AKT [42-44] and mitogen-activated protein kinase (MAPK) signalling pathways, and an imbalance in calcium homeostasis[42-43].

Butylated hydroxyanisole's toxicological properties and metabolism in animals and humans are briefly examined. There is reason to be concerned about the extensive usage of BHA and its detrimental effects. As a result, a thorough summary and discussion of every facet of BHA are required. This article primarily reviews recent research on BHA, with an emphasis on its toxicity, metabolites, environmental occurrence, and human exposure. Recommendations are offered for future BHA research directions based on the review's findings.

2. Materials and Methods

2.1. Literature survey strategy-

The author followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses, or PRISMA. Both systematic reviews and meta-analyses are frequently published with recommendations. A modest list of suggestions based on evidence is called a recommendation. Google Scholar, PubMed, Research Gate and Science Direct are online resources that the author used to focus on both non-experimental and experimental studies. Research articles from 1980 to 2024 were found using web browser.

2.2. Data Extraction-

The initial studies scanned were those that came up in the search engines. Microsoft Excel was used to import pertinent research, which were then totalled based on their abstract, goal, and findings.

3. Results and Discussion

3.1. Effects of Butylated hydroxyanisole on stomach

In a study conducted by [47] butylated hydroxyanisole was given to male Syrian golden hamsters for one to four weeks. With 3-tert- butylated hydroxyanisole which is an isomer of butylated hydroxyanisole, the dose at fourteen days, the incidence of hyperplasia severity in the forestomach was observed however for hamsters given 2-tert- butylated hydroxyanisole the incidence was nearly at the control level. From this study we conclude that the 3-tert isomer of butylated hydroxyanisole is carcinogenic. In another study conducted by [48] butylated hydroxyanisole was given at the course of 1, 2, and 4 weeks, rats were orally treated with 2% butylated hydroxyanisole in powdered diet.

It was observed that the forestomach mucosa showed modest hyperplasia and hyperkeratosis following a week of epithelial injury. While at weeks 2 and 4, other epithelial abnormalities regressed, hyperplasia and hyperkeratosis exhibited improvement. The epithelial flaws completely disappeared after another 4 weeks of feeding without butylated hydroxyanisole, although the hyperplastic alterations persisted. Accordingly, [49] exposed the antioxidant butylated hydroxyanisole to the diet of F344 rats, carcinogenicity assays revealed that both sexes of hamsters had significant rates of forestomach papilloma and squamous cell carcinoma. After receiving butylated hydroxyanisole for 168 days, male hamsters also developed papilloma that grew downward into the forestomach submucosal region. Therefore, the exposure of the antioxidants alters the chemical carcinogenesis that are two-staged in the liver, kidney, and stomach; although it is also observed that the effects vary according on the organ. A different study using butylated hydroxyanisole in Male Fischer rats (F344) were used to identify and assess the effects of butylated hydroxyanisole. It was observed that the forestomach's epithelial hyperplasia increased at both treatment levels when the rats were administered 0.5% and 2% of butylated hydroxyanisole of their feed for two continuous years. The prevalence of squamous-cell carcinoma and forestomach papilloma rose to 2% [50]. In another study by [51] male Fischer 344 rats were fed butylated hydroxyanisole for at least 9 days, experienced regeneration and necrosis of the epithelial cells of the forestomach. It was also observed that the prefundic area of the forestomach had significantly greater stimulated proliferation and histological alterations than the mid-region. Following 9–27 days of feeding, some more phenols and acids were examined; the majority caused some degree of proliferation of epithelial cells in the rat forestomach, albeit some had a stronger impact on the mid-region than the prefundic region. [52] carried out a study using rats to investigate the impacts of butylated hydroxyanisole, the rats were administered orally for 104 weeks. A powdered meal that included 0, 0.125, 0.25, 0.5, 1, or 2% butylated hydroxyanisole were given to the rats. With the highest dosage, there was a substantial rise in the incidence of forestomach squamous cell carcinoma at 2% butylated hydroxyanisole. The rats fed diets containing 1% and 2% butylated hydroxyanisole, respectively, developed forestomach papilloma. With higher butylated hydroxyanisole dosages, the incidence of forestomach epithelial hyperplasia rose, reaching 100% at the maximum dosage. In a study conducted by [53] in which the rats forestomach lesions caused by butylated hydroxyanisole were tested for reversibility. For 24, 48, or 72 weeks, F344 rats were fed a 2% butylated hydroxyanisole diet; for the remaining 96 weeks of the experiment, they were fed a baseline diet. For 96 weeks, two additional groups of rats were fed either baseline diet or a 2% butylated hydroxyanisole diet. Histopathological comparisons were made between the forestomach lesions at weeks 24, 48, 72, or 96. The findings demonstrated that whereas endophytic proliferation of basal cells (basal cell hyperplasia) continued after butylated hydroxyanisole injection was stopped, exophytic epithelial proliferation (simple hyperplasia or papilloma) generated by butylated

hydroxyanisole was reversible. This implies that butylated hydroxyanisole- induced simple hyperplasia and papilloma of the forestomach are not self-sufficient and require ongoing butylated hydroxyanisole feeding in order to progress. Accordingly in a study conducted by [54] the males of the F344, Lewis, SHR, and Sprague Dawley rat strains were employed to evaluate the forestomach's carcinogenicity following the impact of butylated hydroxyanisole. For 104 weeks, groups of 30 rats were fed a pellet diet that contained 2% butylated hydroxyanisole. Regardless of strain, all rats given butylated hydroxyanisole developed forestomach squamous cell papilloma and hyperplasia; however, the incidents of squamous cell carcinomas varied significantly: 26.7% in F344, 76.7% in SHR, 36.7% in SD and 6.7% in Lewis. The SHR strain also had the most severe cytotoxic effects, as seen by inflammation, which was closely linked to the growth of squamous cell carcinomas. According to the current findings, there are significant strain variations in butylated hydroxyanisole rat forestomach carcinogenesis, and susceptibility to cytotoxicity may be a crucial factor.

Table.1: Studies of butylated hydroxyanisole on stomach in male and female rats

Model animal	Dose of Butylated hydroxyanisole	Duration	Inference	Reference
Male Syrian golden hamsters	-	1-4 weeks	Hyperplasia severity in the forestomach was observed	Ito *et al.,* (1984)
Male rat	1 g BHA/kg BW/day	For 1, 2, 4, 8, 16, or 32 days	Forestomach mucosa showed modest hyperplasia and hyperkeratosis	Altmann *et al.,*(1985)
Male and female Fischer rats(F344)	-	168 days	Papilloma that grew into the forestomach submucosal region.	Ito *et al.,*1985
Male Fischer rats (F344)	-	24 months	Forestomach's epithelial hyperplasia increased at 0.5% and 2% treatment levels	Moch 1986
Male Fischer rats(F344)	-	9 days	Regeneration and necrosis of the epithelial cells of the forestomach along with stimulated proliferation in the prefundic area of the forestomach	Clayson *et al.,*1986
Male rats	0, 0.125, 0.25, 0.5, 1, or 2% butylated hydroxyanisole	104 weeks	Substantial rise in the incidence of forestomach squamous cell carcinoma	Ito *et al.,* 1986
Male Fischer (F344) rats	-	24, 48, or 72 weeks	Endophytic proliferation of basal cells hyperplasia	Masui *et al.,*1987
SHR, Lewis, F344, and Sprague Dawley rat	-	-	Forestomach squamous cell papilloma and hyperplasia; Specifically, SHR strain had the most severe cytotoxic effects, as seen by inflammation, which was closely linked to the growth of squamous cell carcinomas.	Tamano *et al.,*1998

3.2. Effects of butylated hydroxyanisole on kidney

In [55] conducted an experiment in which he used butylated hydroxyanisole and butylated hydroxytoluene, two dietary antioxidants for their impact on renal function. For six days, male rats were gavage 500 mg/kg/day of butylated hydroxyanisole or butylated hydroxytoluene. It was observed that after the second butylated hydroxytoluene treatment, the treated animals' water intake decreased by 35% but their urine volume increased by 78%. For the 6 days of the trial, the osmolality of the urine from the butylated hydroxytoluene-receiving animals decreased, but the butylated hydroxyanisole and control group's osmolality remained unchanged. Animals given either antioxidant had lower levels of potassium and sodium in their urine. Electrolyte excretion decreased on the second day of butylated hydroxytoluene administration, although not proportionately to meal intake. In another study conducted by [56] the rat's electrolyte balance is negatively impacted when the dietary additives butylated hydroxyanisole or butylated hydroxytoluene are administered. For 1, 2, 4, or 6 days, the male rats were given maize oil or butylated hydroxyanisole or butylated hydroxytoluene at the dose of 500 mg/kg body weight (BW). The ability of the slices of renal cortical to augmentation of p-aminohippurate, a proto-type of organic acid and a proto-type of organic base, N-methyl nicotinamide, was assessed after the animals were killed twenty-four hours following the final antioxidant treatment. After the first dose of either antioxidant, organic acid transport decreased. However, it was observed during sixth day of the given treatment, the buildup of p-aminohippurate was similar in the groups treated with butylated hydroxyanisole or butylated hydroxytoluene and the control animals. As per the findings, these phenolic antioxidants specifically inhibit organic transport.[57] revealed that mice fed diets containing 0.75% butylated hydroxyanisole before showed a significantly reduced covalent binding of acetaminophen metabolites to mouse kidney and liver proteins. According to these results, monooxygenases that convert acetaminophen to reactive metabolites are unaffected by butylated hydroxyanisole. The decreased covalent binding may be due to the increased amounts of reduced glutathione (GSH) in the liver and kidney following consumption of butylated hydroxyanisole. According to a study conducted by [58] glutathione S-transferases (GST) have been demonstrated to be induced in the gastrointestinal tract, the kidneys, and the lungs of both male and female BALB/c mice by the anticarcinogen

butylated hydroxyanisole. Butylated hydroxyanisole had minimal impact on pulmonary glutathione S-transferases but induced the highest induction of glutathione S-transferases in the stomach and raised kidney glutathione S-transferases levels. The proximal tubules being the primary location of all renal glutathione S-transferases. In lungs, where the glutathione S-transferases were only present in the bronchi region, dietary butylated hydroxyanisole was found to have the least inductive effects.

Table.2: Studies of butylated hydroxyanisole on kidney in male and female rats

Model organism	Dose of Butylated hydroxyanisole	Duration	Inference	Reference
Male rats	500 mg/kg/day	6 days	Water intake decreased by 35%, but their urine volume increased by 78%.	Ford *et al.,* 1980
Male rats	500 mg/kg BW	1, 2, 4, or 6 days	After the first dose, organic acid transport decreased	Ford et al.,1980
Male rats	-	-	Decreased covalent binding may be due to the increased amounts of reduced glutathione	Miranda *et al.,*1985
Male and female rats	-	-	Minimal impact on pulmonary glutathione S-transferases but induced the highest induction of glutathione S-transferases in the stomach and raised kidney glutathione S-transferases levels	McLellan *et al.,* 1992

4. Conclusion and Future Scope

The available literature suggests that butylated hydroxyanisole alters the biological activities of various organisms. During consumption, butylated hydroxyanisole is inevitably transferred into the surroundings and can be detected in a variety of media. Widely used in a variety of industries, butylated hydroxyanisole is an artificial phenolic antioxidant that effectively extends the shelf life of goods. In rats, it causes forestomach papilloma and squamous cell carcinoma along with possibly resulting in nephrotoxicity, particularly after prolonged exposure to large doses. It may also have an effect on organic ion transport, which may raise oxidative stress and harm the structure of the glomeruli and renal tubules. Among other health risks, exposure to butylated hydroxyanisole may result in growth retardation, thyroid damage, metabolic problems, and carcinogenesis.

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The authors Yamini Makarwar and Varsha Dhurvey conceived the concept and prepared the protocol. Yamini Makarwar conducted the literature search, which was verified by Varsha Dhurvey. Yamini Makarwar wrote the first draft of the manuscript, while Varsha Dhurvey critically corrected it. All of them agreed on the final manuscript.

Conflict of Interest-

There are no conflicts of interest

Data Availability-

The research articles used in this systematic review are available for use. Ethical approval Institutional review board approval is not required.

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