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A Comprehensive Review on the Physiological Effects of Benzoic Acid and Its Derivatives

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Mousumi Dutta[1]

[1]Goverment General Degree College, Kharagpur-II, Ambegeria, Madpur, Paschim Medinipur- 721149, West Bengal, INDIA

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ABSTRACT

Benzoic acid is an organic chemical. It is metabolised in the liver and is excreted out as hippuric acid. This compound and its derivatives are reported to be used as food, drug preservatives, cosmetic products and pharmaceuticals. Various literature surveys explore various biological properties such as antifungal, antimicrobial, gastrointestinal tract modulator, enhancer of biological metabolism, anti-inflammatory, genotoxic agent etc. The present study will give comprehensive information of the biological activities of this benzoic acid and its derivatives.

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INTRODUCTION

Benzoic acid, bearing molecular formula $C_7H_6O_2$ and the molecular weight is 122.1 g/mol is an aromatic carboxylic acid. Its structural formula is given below (Figure 1A). Benzenecarboxylic acid, phenyl carboxylic acid, carboxybenzene and dracylic acid are the most commonly known synonyms of benzoic acid (ChemID Plus, online). Its texture is white crystalline powder. It is slightly soluble in water (2.9 g/l at 20˚C) and freely soluble in ethanol [1]. Benzoic acid has a synergistic mechanism between nitrogen starvation and benzoic acid, and it is the result of inhibition of macroautophagy by benzoic acid and due this property, benzoic acid used as novel food preservative [2].

Benzoic acid was discovered in the sixteenth century. The dry distillation of gum benzoin was first described by Nostradamus (1556), and then by Alexius Pedemontanus (1560) and Blaise de Vigenère (1596) [3]. Justus von Liebig and Friedrich Wöhler determined the composition of benzoic acid [4]. These latter also investigated how hippuric acid is related to benzoic acid.

Many plant and animal species contains benzoic acid as esters. In most berries, different ripe fruits of several *Vaccinium* species (e.g., cranberry, *V. vitis macrocarpon*; bilberry, *V. myrtillus*) contain a good amount of benzoic acid. Benzoic acid is also formed in apples after infection with the fungus *Nectria galligena*. Among animals, various omnivorous or phytophageous species, e.g., in viscera and muscles of the rock ptarmigan (*Lagopus muta*) as well as in gland secretions of male muskoxen (*Ovibos moschatus*) or Asian bull elephants (*Elephas maximus*) contain identifiable amount of benzoic acid. 20% of benzoic acid and 40% benzoic acid esters are present in gum benzoin [5]. Benzoic acid also can be obtained from the aqueous bark extract of *Terminalia arjuna* [6].

1. Absorption, Distribution, Metabolism and Excretion (ADME) of benzoic acid

Within the acidic atmosphere of stomach the benzoic acid was absorbed by a diffusion process. This study also considered that sodium benzoate, potassium benzoate and calcium benzoate will dissociate into their constituent sodium, potassium or calcium and benzoate ions in the small intestine [7].

2. *Ex vivo* studies

The *ex vivo* study showed that intestinal benzoic acid was absorbed in perfused rat small intestine preparations [8]. Benzoic acid was absorbed rapidly and in an uneven way in the intestinal segmental regions, mainly in the jejunum and slightly in the ileum. The absorption pattern paralleled the distribution of the monocarboxylic acid transporter (Mct1), suggesting that this transporter may play a role in the absorption of benzoic acid [7].

3. *In vivo* studies

In the gastrointestinal tract benzoic acid is being absorbed and liver is the main metabolising site of this compound. Then it was excreted out through urine as hippuric acid, glycine conjugates of benzoic acid [1]. Benzoic acid is being excreted out in an unchanged form when there is a depletion of glycine in the body. The species having capacity of higher rate of glucuronidation of benzoic acid is more susceptible to the benzoic acid toxicity than the other one [9]. Bernhard *et. al.* (1955) showed that benzoic acid and hippuric acid are being generated by the metabolism of phenylalanine and tyrosine [10]. So, phenylalanine is a source of benzoate formation. Benzoate formation may be regulated by intestinal bacteria. Almost complete excretion occurred in the urine within 1–2 days. Benzoic acid may present within the tissue mainly in the form conjugates than the acid itself [7].

4. Various effects of benzoic acid and its derivatives

Nitrogen retention was increased by benzoic acid but it did not significantly affect nutrient digestibility. The pH value or the concentration of ammonia in the gastrointestinal tract was not affected by the supplementation of benzoic acid but, it decreased the number of bacteria in the digesta. Dose-dependently benzoic acid decreased total aerobic, total anaerobic, lactic acid forming and gram-negative bacteria, gram-negative bacteria, acetic acid and total aerobic bacteria respectively in stomach, duodenum, illeum, respectively. So, benzoic acid showed antimicrobial effects in the gastrointestinal tract of piglets and therefore enhances growth performance and nitrogen retention [11].

The dietary intake of benzoic acid decreased the growth and pH of the caecal contents of broiler chickens. On the other hand it increased the dry matter of the digesta. No differences were found in the pH of the crop, ileal, gizzard digesta and rectum content. Increased amount of benzoic acid decreased the contents of lactic acid bacteria and coliform bacteria populations in the caeca [12].

Canola (*Brassica napus* L.) seed germination was analyzed under the action of benzoic acid and their derivatives gallic acid (Figure 1B), vanillic acid (Figure 1C) and salicylic acid (Figure 1D). Seedling length and seedling fresh weight were reduced by benzoic acid. But, root length did not affected by this compound. Gallic, vanillic and salicylic acids reduced all variables, and the most effective was salicylic acid. The effects of salicylic acid showed that both peroxidase (POD) activity in cotyledon and root were increased significantly [13].

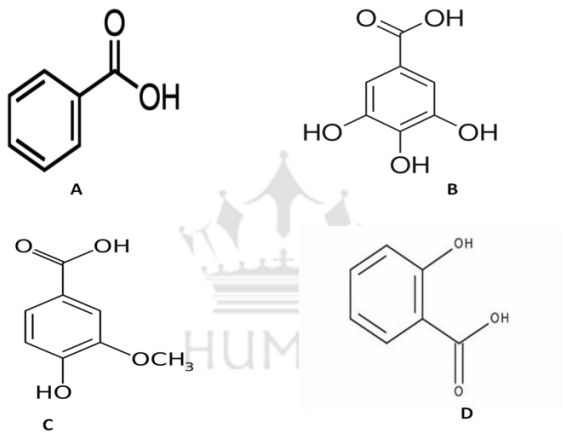

Figure No. 1: Chemical structures of benzoic acid (A), gallic acid (B), vanillic acid (C) and salicylic acid (D).

Lower alkyl esters of *p*-hydroxybenzoic acid (parabens) [Figure 2(A-G)] are widely used as preservatives of pharmaceuticals, cosmetics and allied products. Microbial activity increases with increasing alkyl chain length for the commonly used methyl, ethyl, propyl and butyl parabens, and synergy between parabens has been reported. Experiments stated that combined methyl (M)-, ethyl (E)-, propyl (P)- and butyl (B)-parabens in a matrix analysis showed a promising activity against microbes *in vitro* [14].

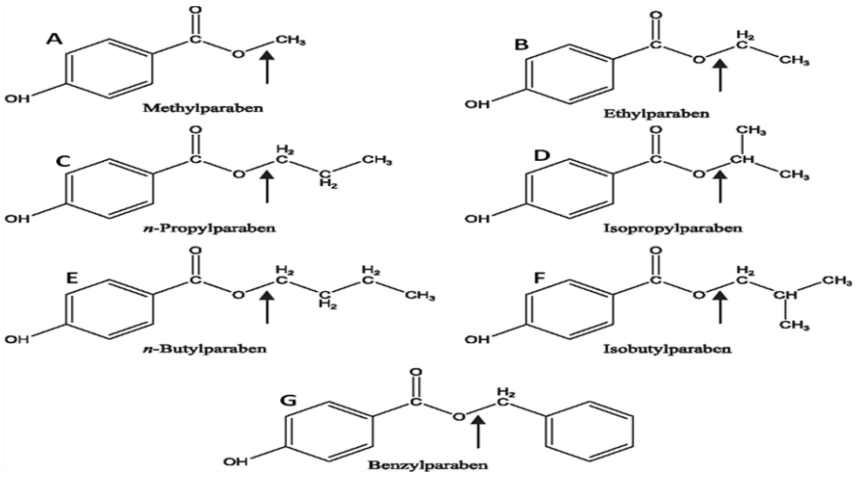

Figure No. 2(A-G): Chemical structures of different parabens.

The neutral form of 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) (Figure 3A) blocked the Ca^{2+} release-activated Ca^{2+} (CRAC) channel. This suggests a direct interaction between NPPB and the CRAC channel, and this is helpful in guiding the development of novel and more selective analogues [15].

2-hydroxy-4-methoxy benzoic acid (Figure 3B) was isolated from methanolic root extract of *Hemidesmus indicus.* This compound showed potent anti-inflammatory, antipyretic and antioxidant properties experimentally. Inflammation and granuloma induced by *Vipera russelli* venom were neutralized and reduced, respectively by this compound. It was established that this compound mainly showed its anti-venom activity via the oxidative stress mediated pathway [16].

Benzoic acid supplementation increased the biosynthesis of PGD2 and PGF2 significantly. But there were no changes in plasma levels of other vasodilating prostaglandins, PGE2 or prostacyclin (PGI2). The increased synthesis of PGD2 was not accompanied by a release of histamine, suggesting that PGD2 was not derived from the mast cell [17].

Benzoate (Figure 3C) metabolism also affects the mitochondrial metabolism both *in vivo* and *in vitro.* Experiment showed that mitochondrial proliferation compensates for the observed decrease in benzoic acid metabolism in isolated mitochondria *in vitro*. This benzoate metabolism was reduced by the Hepatic accumulation of hydrophobic in rat [18].

Experiments with young pigs fed with benzoic acid showed that this compound can improve the performances by improving the nutrient digestion and the jejunal morphology. This

improvement occurs probably *via* increasing the GLP-2 production and the antioxidant capacity in the intestine of young pigs [19]. Actually GLP-2 increases and decreases cell proliferation in intestinal mucosa and apoptosis of epithelial cells, respectively. These mechanisms are useful in promoting the growth and regenerative repair after injury of intestinal mucosa [20-21]. The activities of trypsin, lipase, amylase, maltase, sucrase and lactase in the jejunal digesta of weaned pig were increased by benzoic acid supplementation. [22-23] Benzoic acid improves the morphology of the jejunum by improving the activities of SOD and GSH-PX. However, there have been no other reports relating to the effects of benzoic acid on antioxidant capacity in animals [19].

A cell-permeable superoxide dismutase mimetic and related compound Mn(III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) (Figure 3D), is not a scavenger of nitric oxide but it may inhibit the oxidation of dihydrorhodamine 123. But earlier studies stated zymosan induced non-septic shock can be reduced by MnTBAP by reducing the superoxide formation and, also, by scavenging of peroxynitrire [24].

Benzoic acid is used as preservative in many foods in the form of salt (i.e. Sodium benzoate). Like as benzoic acid, sodium benzoate (Figure 3E) is metabolized within liver to produce hippuric acid. Patients with urea cycle disorders and non-ketotic hyperglycinemia were treated by indirect consumption of sodium benzoate, intravenously [25]. Various physiological disturbances such as carnitine deficiency, hepatic ATP depletion occur due to the usage different dosage of sodium benzoate clinically [26-28]. Acute sodium benzoate exposure did not significantly impact glucose, insulin, or glucagon levels in the experimental model but significant changes in anthranilic acid and acetylgycine were observed [29].

Treatment of urea cycle enzymopathies can be done by using sodium benzoate which can facilitate the alternative pathways of nitrogen excretion [30]. Therapeutic doses are reported to be in the range of 250 to 500 mg/kg-day and are given over several years. Clinical signs of toxicity are reported to be rare at this dose level and in most cases limited to anorexia and vomiting, particularly after bolus intravenous doses [31].

Inhalation of sodium benzoate causes allergies in eyes, skin and also causes asthma and coughing [32]. Toxicities in various organs heart, spleen, kidney, brain, and liver had been observed by the treatment laboratory animals with sodium benzoate. Congenital malformation, vertebral column deformity and even death also may be observed by the

treatment of sodium benzoate [32-34]. Many biological studies concerning sodium benzoate-induced toxicities have been reported. Sodium benzoate toxicity induced a significant increase in DNA content, causes a stimulation of the mitotic process [35]. Sodium benzoate can cause changes in genes expression through releasing free radicals [36]. Growth factors, cell cycle, gene expression and congenital malformations were induced by sodium benzoate in the experimental animals. But the mechanisms of the teratogenic effects of this substance are unknown [37].

Experimental *in vivo* study stated that sodium benzoate showed a concentration-dependent genotoxic effect in liver tissue. The results indicated induction of DNA breaks in rat liver cells under the influence of sodium benzoate intake. There is an alarming signal for the food industry in case of the dosage of this preservative agent [38].

4-hydroxybenzoic acid and trans-4-hydroxy cinnamic acid (Figure 3F) isolated from rice possess antibacterial activity against most of Gram + ve and some of Gram - ve bacteria [39]. This study concluded lipophilicity as an important factor that strongly influences the antimicrobial activity of 4-hydroxybenzoic acid as compare to that of trans-4-hydroxy cinnamic acid [39].

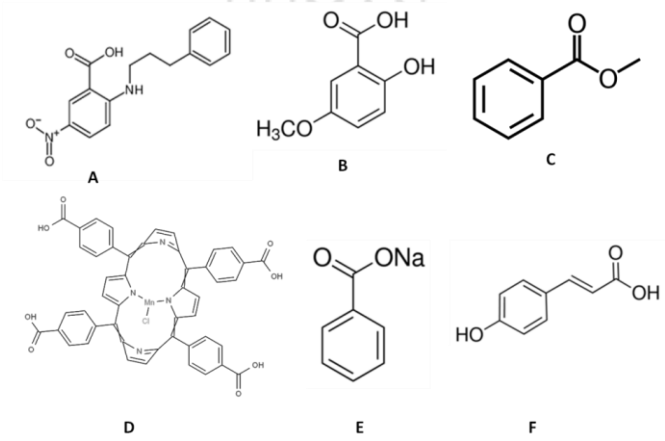

Figure No. 3: Chemical structures of 5-nitro-2-(3-phenylpropylamino)-benzoic acid (A), 2-hydroxy-4-methoxy benzoic acid (B), benzoate (C), Mn(III) tetrakis (4-benzoic acid) porphyrin (D), sodium benzoate (E) and trans-4-hydroxy cinnamic acid (F).

Orsellinic acid (2,4-dihydroxy-6-methyl benzoic acid, Figure 4A), a derivative of 4-hydroxybenzoic acid showed antimicrobial activity against a number of microorganisms.12

A series of phenolic acids like syringic acid (Figure 4B), caffeic acid (Figure 4C) and 4-hydroxybenzoic acid isolated also possess *in vitro* antimicrobial and antifungal activity.13

Polymerization of sickle haemoglobin (Hb) is being inhibited by 3,5-dimethoxy-4-hydroxybenzoic acid (Figure 4D) [40]. It also possesses anti-inflammatory and analgesic activities.

Ellagic acid (Figure 4E), gallic acid, pyrogallic acid (Figure 4F) and (+) catechin (Figure 4G) were isolated from culture of macrophyte *Myriophyllum spicatum* [41]. The growth of algae *Microcystis aeruginosa* was inhibited by these phenolic acids. Growth of various algae such as Cyanobacterium and green algae were inhibited by gallic acid. Trp-P-1 & Glu-P-2 induced mutagenesis was inhibited by caffeic acid. Caffeic acid provides this inhibition activity by inhibiting the formation of mutagenic & carcinogenic N- nitroso compounds *in vitro*. Derivatives of 4-hydroxybenzoic acid (gallic acid, caffeic acid, vanillic acid, gentisic acid [Figure 4H] and syringic acid) possess antimutagenicity against *Salmonella typhimurium*.

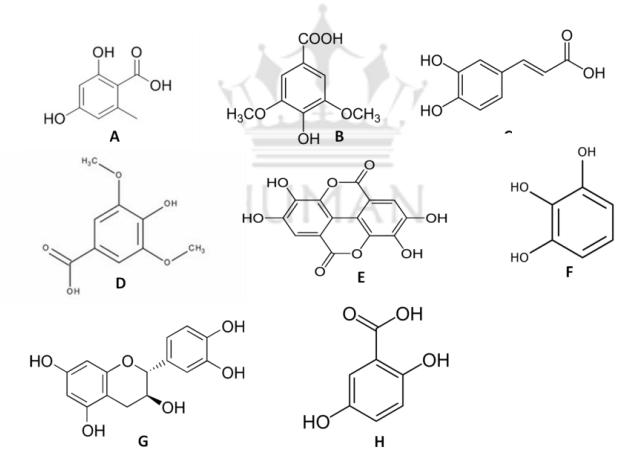

Figure No. 4: Chemical structures of orsellinic acid (A), syringic acid (B), caffeic acid (C), 3,5-dimethoxy-4-hydroxybenzoic acid (D), ellagic acid (E), pyrogallic acid (F), (+) catechin (G) and gentisic acid (H).

Gallic acid possesses antiviral effect against HIVPR. It was testified by using 8-anilino-1 - naphthalene sulfonic acid (ANS). Gallic acid, also, possesses antioxidant, antibacterial, anti-inflammatory, antimutagenic properties [42]. Viral infections as hepatitis B, skin mucosa viral infections can be treated by 3, 4, 5-trimethoxy benzoic acid. Gallic acid ester (3, 4, 5,-trihydroxy benzoic acid ester) can inhibit the synthesis of thromboxane A2 (TXA2). It has a stronger and faster effect against platelets aggregation than aspirin (ASP).

Various phenolic acids such as gentisic acid, ferulic acid (Figure 5A), isoferulic acid (Figure 5B), gallic acid, salicylic acid, sinapic acid (Figure 5C), coumaric acid (Figure 5D), vanillic acid, protocatechuic acid (Figure 5E) and syringic acid possess good antioxidant effects. Among these compounds, gallic acid has highest antioxidant effect [43]. Benzoic acid also has antioxidant property. It showed protection against copper-ascorbate induced oxidative stress in placental mitochondria [6].

Aflatoxin production was inhibited by p-aminobenzoic acid (Figure 5F) and anthranilic acid (Figure 5G) [44] and, also by salicylic acid [45] possibly by reducing the synthesis of pantothenic acid. Previous experiment showed that addition of salicylic acid to media had some connection to the uncoupling of oxidative phosphorylation with a subsequent decrease in adenosine 5'-triphosphate production. Cheshire and Park recently reported that porcine lactate dehydrogenase could be inhibited by salicylate [46].

The performance of turkey poults can be improved by benzoic acid in combination with essential oil compounds. This combination also can improved gut integrity and intestinal microbiota. *In vitro* experiments revealed that the addition of benzoic acid reduced the buffering capacity of the feed offering a significant aim to birds to digest ingested feed [47].

Both, benzoic acid and fumaric acid (Figure 5H) showed bacteriocidal activities against lactic acid, although fumaric acid showed a lesser effect that benzoic acid [48]. These two compounds showed pH-dependent effects on coliform bacteria and lactic acid bacteria in stomach content, and on coliform bacteria in the small-intestine content. The ealier studies showed that benzoic acid provides antibacterial activity in broiler chickens, although lower bacterial counts in gastrointestinal digesta were not reflected in better performance of the birds.

Mitochondria and neutrophils are the main sites of Reactive Oxygen Species (ROS) formation and many others physiological activities. Various phenolic acids had different effects on mitochondria and neutrophils. Both benzoic acid and cinnamic acid provide the pro-oxidant effect on mitochondria and ROS formation are being inhibited in neutrophils. In both mitochondria and neutrophils ROS formation was decreased by phenyl lactate (Figure 5I) and p-hydroxyphenyllactate. But these phenolic acids at high concentration produced local infections and sepsis which may be responsible for lethal outcome. Bifidobacteria and lactobacilli produced *in vitro* considerable amounts of phenyl lactic and p-

hydroxyphenyllactic acids; and benzoic acid was produced by *Serratia marcescens*. The results obtained are particularly urgent in view of the fact of their production by Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, and Acinetobacter baumanii, which are the most frequent causing agents of hospital infectious complications and sepsis [49].

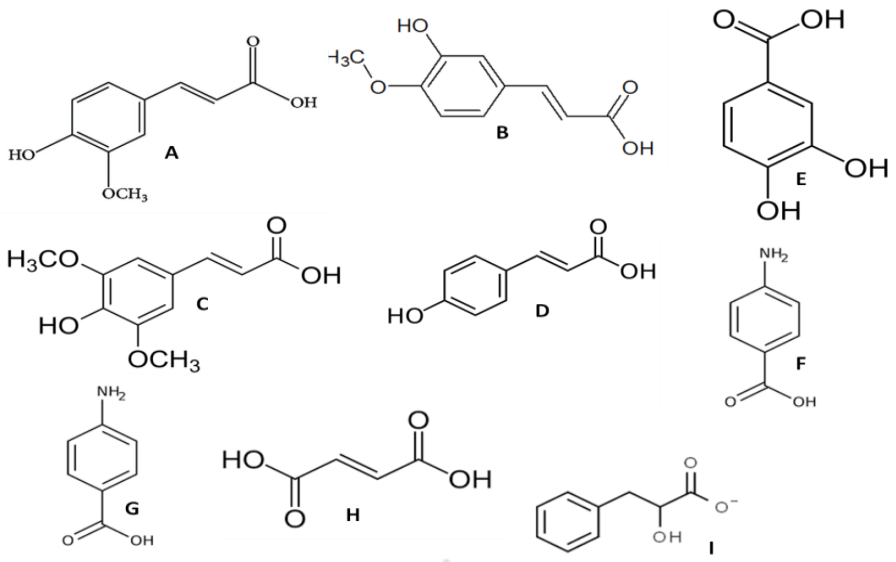

Figure No. 5: Chemical structures of ferulic acid (A), isoferulic acid (B), sinapic acid (C), coumaric acid (D), protocatechuic acid (E), p-aminobenzoic acid (F), anthranilic acid (G), fumaric acid (H) and phenyl lactate (I).

CONCLUSION

Considering the above mentioned versatile uses of benzoic acid and its derivatives, possibilities are still there to explore this molecule for more biological activities by further synthesizing its derivatives. Exploration of this molecule and its derivative will be beneficiary for the physiological system.

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CONFLICT OF INTEREST: None

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DR. Mousumi Dutta
Assistant Professor in Physiology
(West Bengal Education Service)
Government General Degree College,
Kharagpur-II, Ambegeria, Madpur,
Paschim Medinipur- 721149, West Bengal, INDIA