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FUNCTIONAL FOODS (CM WHISNER, SECTION EDITOR)

Recent Evidence of the Beneficial Effects Associated with Glucuronic Acid Contained in Kombucha Beverages

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Abstract

Purpose of the Review Glucuronic acid is contained naturally in kombucha beverages due to the associations between bacteria and yeasts during its fermentation. The purpose of this review is to describe the literature related to the hepatoprotective effect associated with glucuronic acid present in different kombucha beverages.

Recent Findings Although previous research supports beneficial hepatoprotective effects of glucuronic acid consumption from kombucha, these effects are mainly attributed to the tea phytochemicals. However, there are some improvements in methodological deficiencies in some in vivo studies that should be considered. There is no sufficient evidence to generalize the adverse effects of kombucha consumption.

Summary Consumption of kombucha could be considered a safe practice in healthy populations due to its hepatoprotective effects. The content of the beneficial or toxic components is very variable because it depends on its manufacturing process. In persons with side sickness, other conditions such as pregnancy, and hypersensitivity to some kombucha components, a restriction in its consumption must be advisable.

Keywords Kombucha · Glucuronic acid · Glucuronidation · Hepatoprotective effects · Antioxidants

Introduction

Kombucha is considered a fermented beverage with numerous positive health effects. However, its formation involves several processing parameters that change the concentration of the compounds to which those beneficial effects are attributed. Glucuronic acid (GlucUA) is one of the organic acids produced as a result of the fermentation of kombucha, and it is one of its main components [1••]. The GlucUA ($C_6H_{10}O_7$) originates from glucose and has a similar structure, but unlike glucose, GlucUA has a carboxyl group attached to its sixth carbon and is classified as a uronic acid [2]. The production of this compound occurs when there is a glucose-rich medium, such as during the kombucha fermentation [3]. It can be found naturally in plants' gums and mucilage [4].

Furthermore, GlucUA is naturally produced by the liver, and it is considered as the most potent detoxifying natural compound in the organism because of its capability of conjugating itself with toxic metabolites or waste products, forming glucuronides, making them more water soluble, and eliminating them through urine or bile, in a process known as glucuronidation. Also, GlucUA is a vitamin C precursor, and it plays an essential role in the formation of glycosaminoglycans, such as heparin and hyaluronic acid [2].

As a potent detoxifying, compound GlucUA possesses a hepatoprotective effect in the organism. According to this, kombucha consumption has increased, but the specific metabolism and role in the human body of the GlucUA contained in this beverage has not been established by the scientific community. Biochemical markers and histological studies have proved that there is no damage caused by kombucha ingestion, but rather hepatoprotective effects against toxic substances have been reported for in vivo experimental models.

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The necessity of further studies in humans to evaluate this matter is still needed.

Kombucha Beverage Production

Kombucha is produced by fermenting sugared tea with a starter culture, known as SCOBY (symbiotic culture of bacteria and yeast). Usually, black (*Theae nigre folium*) or green tea (*Theae viridis folium*) is used as a principal base beverage, but there have been preparations made with oolong, lemon balm, jasmine, mulberry, and peppermint tea [5]. Other preparations include pomegranate, grape, and sour cherry juice [6–8]. Some investigations have also involved the use of coconut water [9] and coffee [10]. In the standard procedure, approximately 50 g of sucrose are dissolved in 1 L of boiling water to which 5 g of tea leaves are added and allowed to infuse for 5 min. Before inoculating with the starter culture, the tea leaves are filtered out, and the infusion should be allowed to cool down to room temperature at about 20 °C. After that, fermentation with 24 g of the starter culture is typically carried out for about 3 to 14 days at room temperature, in a range of 18 and 26 °C. During this time, a new culture will form and begin to float, while the mother culture will sink to the bottom of the tea broth. The tea will also start to smell like vinegar, and gas bubbles will form due to the carbonic acid produced. Finally, both the mother and daughter cultures are removed, and the liquid is filtered and stored in capped bottles at 4 °C for consumption. Depending on cultural practices, concentrations of tea, sucrose, and starter culture, as well as fermentation periods and temperature may differ [5, 11]. There is evidence that some fermentation periods last up to 60 days [12]. Sanitized utensils should be used in clean work areas during the preparation of kombucha to avoid any contamination [5]. Kombucha contains several acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic, and usnic acids. Most of these acids have the origin of the tea substrate used to prepare kombucha tea. About 0.5–0.6% of dry weight of fresh tea shoot consists of organic acids that are produced by the action of microbes on sugar during fermentation time [13].

Synthesis of Glucuronic Acid (GlucUA)

During the preparation of kombucha tea, an interaction between the base of the tea and the sugar with a culture of bacteria and yeasts takes place. It is important to note that the synthesis of GlucUA is a distinctive property of the natural associations between bacteria and yeast. Therefore, this acid is a key component present in kombucha tea due to its detoxifying activity, helping to protect human health and to prevent chronic non-communicable diseases [2, 14]. The synthesis of GlucUA in kombucha beverages begins when the fermentation process initiates. The invertase of the yeasts hydrolyzes sucrose into two monosaccharides (glucose and fructose). Glucose is transformed into gluconic acid by bacteria metabolism, which is further converted into GlucUA by the acetic acid bacteria. In parallel, fructose is metabolized by bacteria, and ethanol plus CO_2 is produced. Lastly, the acetic acid bacteria (*Acetobacter*) transform the ethanol into acetic acid [15•].

The biologically active form of GlucUA is known as uridine diphosphate (UDP) glucuronic acid. The production of UDP-GlucUA takes place in the liver, where glucose is phosphorylated to glucose-6-phosphate by the enzyme hexokinase. The glucose-6-phosphate isomerizes to glucose-1-phosphate utilizing phosphoglucomutase. Then, glucose-1-phosphate reacts with uridine triphosphate by the UDP-glucose pyrophosphorylase to form UDP-glucose, which oxidizes nicotinamide adenine dinucleotide (NAD+) through the enzyme UDP-glucose-dehydrogenase to form UDP-D-GlucUA [2].

Content of GlucUA in Different Kombucha Beverages

The level of GlucUA in kombucha increases during fermentation time [16••]. However, there have been different results regarding GlucUA's concentration in kombucha (Table 1). Jayabalan et al. [11] reported a maximum concentration of 2.33 g/L of GlucUA after 12 days of fermentation, while Neffe et al. [16••] obtained concentrations of approximately 0.063 g/L after 10 days of fermentation. These different results may be due to differences in the bacterial and yeast cultures, as well as differences in variables such as time, temperature, sucrose concentration, and variety of tea utilized [16••].

Similar values of GlucUA were reported by Xia et al. [17•] in kombucha beverage prepared with soymilk at different processing conditions [18]. GlucUA concentration increased from 1.77 to 2.74 mg/mL at 28 °C. At 37 °C, it increased from 1.77 to 2.00 mg/mL within 36 h and then decreased to 1.23 mg/mL, making evident the differences caused by the microbial behavior due to environmental conditions.

Some attempts to increase the GlucUA biosynthesis in kombucha beverages are reported. As an example, Nguyen et al. [19] evaluated the effects of *Lactobacillus casei* and different fermentation conditions on the GlucUA concentration. Those authors used a combination of *Dekkera bruxellensis* and *Gluconacetobacter* as a model system to simulate the microbial symbiosis between yeast and acetic acid bacteria in kombucha. They concluded that *L. casei* participates as a supporting microorganism to stimulate GlucUA synthesis up to 54.1% greater than the control. Ranges for optimum fermentation conditions to produce the highest amount of GlucUA were 5–

Table 1 Processing parameters and glucuronic acid (GlucUA) concentration in kombucha beverages prepared with different substrates

Substrate type and concentration	Content of sucrose	Fermentation temperature (°C)	Fermentation time (days)	Concentration of GlucUA	Reference
Black tea dried leaves (8 g/L)	80 g/L	27 ± 1	7 14	1.36 ± 0.08 mg/mL 3.23 ± 0.64 mg/mL	[33••]
Green tea dried leaves (8 g/L)	80 g/L	27 ± 1	7 14	1.78 ± 0.12 mg/mL 1.96 ± 0.10 mg/mL	[33••]
Rooibos dried leaves (8 g/L)	80 g/L	27 ± 1	7 14	1.70 ± 0.09 mg/mL 2.87 ± 0.47 mg/mL	[33••]
Raw pu-erh tea (8 g/L)	100 g/L	28	14	1.36*	[34]
Mixture of green tea and black tea leaves (2:1) (2 g/L of green tea, 4 g/L of black tea)	100 g/L	25	10	0.063 ± 4.11 g/L	[16••]
Soymilk (water:dried soybeans ratio, 8:1 w/w) and black tea (1%)	100 g/L	28	3	2.64 ± 0.05 mg/mL	[17•]
			4	2.74 ± 0.01 mg/mL	
		37	3	1.23 ± 0.06 mg/mL	
			4	–	
Pomegranate juice (1 L)	8 g/L	37	14	17.074 g/L	[7]
Black tea leaves (1 g/L) plus *Lactobacillus casei* (8×10^8)	100 g/L	30	5	42.3 mg/L	[35]
Grape juice (1 L)	7 g/L	37	14	178 g/L	[6]
Sour cherry juice (1 L)	8 g/L	37	14	132.5 g/L	[8]
Black tea (1.2%)	10%	24 ± 3	12	2.3 g/L	[36]
Sucrose	–	31	–	44.5 mg/L	[37]

*Relative content (peak area)

15% of sucrose, 3–7 days of fermentation, pH of 5–7, and temperature of 27–37 °C.

The same trend was studied by Nguyen et al. [20] by the obtention of a newly designed symbiosis from the isolated yeast *Dekkera bruxellensis* and the isolated bacteria strain *Gluconacetobacter intermedius.* Those authors achieved the highest concentration of 175.8 ± 7 mg L^{-1} of GlucUA at 7 days of fermentation.

Even when those experiments allowed the optimization of parameters to produce kombucha with high concentration of GlucUA, it is relevant to acknowledge that the SCOBY is a diverse consortium of microorganisms and that the isolation of some specific species cannot be accomplished by the population, since kombucha is a beverage practically produced by an artisanal process.

In an effort to standardize the quality properties of kombucha, Coton et al. [21] used metabarcoding and culture-based methods to compare microbial communities of industrialized black and green kombucha teas. The authors described that species richness decreased over the eighth day of fermentation, and tea type did not influence the yeast community, which is essential for fermentation control.

GlucUA and Liver Detoxification

The process of glucuronidation favoring the elimination of toxic metabolites and/or waste products from the organism is depicted in Fig. 1. The GlucUA is a highly water-soluble compound, and it acts in specific membrane transport systems for excretion. Transport is a crucial process of detoxification, and the liver has an epithelium that actively absorbs an endless number of substances from the blood, metabolizes them, and secretes them into the bile or urine for elimination. These excreted products are known as glucuronides. GlucUA participates in the liver detoxification, favoring the elimination of toxic metabolites and/or waste products from the organism through the process of glucuronidation. Therefore, it plays a supporting role in liver functions [2].

The human being is exposed continuously to foreign substances known as xenobiotics, toxins such as drugs, food colorants, food additives, and tobacco smoke, among others. The body carries out biotransformation processes to eliminate those substances, which, through chemical reactions, modify the components making them more polar and water soluble, thereby facilitating their elimination from the body, either by

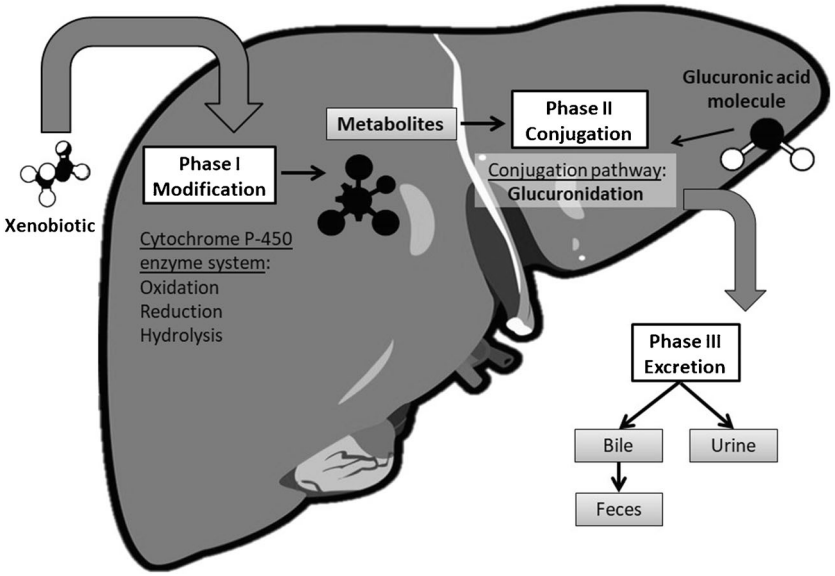

Fig. 1 The process of glucuronidation favoring the elimination of toxic metabolites and/or waste products from the organism

urine or bile. This biotransformation process is carried out mainly in the liver in three phases [2, 22].

First, the liver epithelium absorbs the xenobiotic, and phase I (also called modification) starts. The cytochrome P450 enzyme system carries out oxidation, reduction, or hydrolysis reactions, adding polar groups to the chemical compound to modify its structure and make it more water soluble. Next, the metabolite proceeds to phase II or conjugation. Glucuronidation is the main conjugation pathway, and it consists of the formation of glucuronides by the union of a glucuronic acid molecule to the toxin, utilizing the enzyme glucuronyltransferase (UDPGT). This reaction is the enzymatic addition of a glucuronate ion to organic toxins and other substances [23]. Finally, during phase III, or excretion, the glucuronide, which is already a water-soluble molecule, can be excreted from the body. Those with a higher molecular mass are transported in bile and excreted via feces, while those with a lower molecular mass are excreted via urine [2].

Biological Activity of Kombucha by GlucUA Effect and Liver Detoxification

Glucuronidation reaction occurs in phase II of the metabolism of xenobiotics to be eliminated by the organism not only in the liver but also in the gut tissue. In this step, the hepatic enzyme called UDPGT has critical participation. The conjugation of the phytochemicals (phenolic compounds) with glucuronic acid by this enzyme has the main pathway of detoxification in the mammalian organism. When this conjugation occurs, the functional properties of phytochemicals (bioactivity) are diminished or lost. Nevertheless, several tissues have shown the reverse action caused by the β-glucuronidase activity, which is responsible for cleavage and reactivation of glucuronide conjugates [24].

Several studies have reported the biological activity of kombucha consumption and the beneficial effects on liver detoxification through in vitro and in vivo studies. Nevertheless, controversial findings have recently come out to warn regular consumers about some contraindications of kombucha drinking. This section reviews both scenarios that focus on glucuronic acid and its relation during liver detoxification and cytotoxic effects. Firstly, beneficial claims of kombucha drinking are discussed, followed by the controversial and adverse effects.

Asadbeygi et al. [25] conducted a study evaluating the activity of the UDPGT enzyme associated with kombucha consumption. Albino mice CD1 genus was used as an experimental model. Unfortunately, there is no data about the dose of kombucha that was administered to the different groups of rats. Even when the authors concluded that the beverage induced the synthesis of the UDPGT enzyme, the lack of clarity on the methodology does not allow for comparing their results with further findings.

Some of the first research about the beneficial effects of kombucha against hepatotoxicity was demonstrated in work published by Murugesan et al. [26]. Hepatoprotective effects of kombucha against hepatotoxicity caused by carbon tetrachloride (CCl_4) were addressed in male albino rats used as an experimental model. The authors evaluated the curative properties of the consumption of three beverages, black tea, kombucha, and black tea elaborated with tea fungus enzymes (cellulase and laccase). A twice a week dose of 2.5 mL/kg body weight was administrated orally in three different groups of rats. Each group was also feeding with black tea, kombucha, and black tea added with purified tea fungal cellulase and laccase (3:1 ratio) during tea leaf fermentation, respectively.

The experiment lasted 30 days, and its effectiveness was evidenced by hepatoprotective and curative effects of kombucha via reducing the levels of hepatic enzymes and malondialdehyde in plasma. Moreover, histopathological studies revealed that rats feeding with kombucha showed a more pronounced reduction for the macro- and microvesicular zonal necrosis in preventive and curative treatments. The authors attributed these effects to the high content of polyphenolic compounds and the concentration of the glucuronic acid contained in the beverage.

Recently, Quiao-Won and Teves [27] studied the cytotoxicity of kombucha tea broth from different substrates using brine shrimp lethality assays. Kombucha beverages were prepared to combine the following factors: black or green tea and sweetener brown or white sugar. All tea preparations showed low cytotoxicity against brine shrimp with LC_{50} values (based on the percentage of mortality) ranging from 0.241–0.073 ppm after 6 h (acute) and 0.860–0.101 ppm after 24 h (chronic). Kombucha prepared with black tea and sweetened with brown sugar consistently presented the lowest LC_{50} levels in both acute and chronic effects. Those results, according to the authors, exhibited the safety of kombucha for consumption. It is essential to notice that this study did not attribute specific effects to any kombucha component, which prevents the establishment of some definitive conclusions about it.

Claims about the protective effects of kombucha (produced with black tea) against acetaminophen hepatotoxicity were described by Abshenas et al. [28]. Those authors induced severe hepatotoxicity in a group of female Balb/c mice by oral acetaminophen administration (100 mg per 100 g of body weight). Another group was administrated with acetaminophen plus the ingestion of kombucha tea (0.1 mL per 100 g of body weight) for 7 days. Results showed that activities of liver marker enzymes in serum and the histopathological study were negatively affected in the group of acetaminophen administrated mice. On the contrary, the second group (administrated with kombucha tea) showed better biochemical parameters, and the histopathological assessment demonstrated that severe glycogen storage in hepatocytes, hepatocellular degeneration, and necrosis; mononuclear cell infiltration in the portal area; dilation of the central veins; and capillarization were reduced in this group. They attributed these effects to the capability of reducing the damage induced by the oxidative stress of acetic and glucuronic acids caused by the acetaminophen ingestion. Those acids are capable of conjugating with toxins and facilitating the detoxification process of the body.

The same trend was described by Kabiri et al. [29] in male Wistar rats with induced liver damage by thioacetamide (TAA) administration for 3 weeks. One group was given a TAA dose of 400 mg kg^{-1} of body weight plus 50 mL kg^{-1} of kombucha tea. The other group drank the same kombucha tea amount for 3 weeks, and then the same dose of TAA was administrated. At the end of the study, the results indicated that in both groups of rats, the treatment with kombucha tea promoted a significant reduction in serum hepatic enzymes and bilirubin content attributed to the antioxidant activities of its compounds.

Lately, interesting findings of radiation (electromagnetic field) exposure and kombucha protecting effects on some trace element levels in different organs of rats have been reported [30]. The concentration of copper, iron, and zinc was quantified in the brain, spleen, and intestine. Those trace elements are enzyme system activators and are the link to constituents of organic acids. However, when there are abnormalities in their concentrations, those compounds could generate reactive free radicals, resulting in cellular, lipid bilayer, and DNA damage, as well as changes in enzyme activities resulting in organ toxicity. Those authors studied a group of male Wistar rats exposed to microwaves at 950 MHz and a specific absorption rate of 0.95 W kg^{-1} for 8 weeks (1 h daily) while also being given kombucha (0.1 mL/100 g of body weight) during that period. Another group was also irradiated under the same conditions but given black tea; however, no dose of this beverage is reported. In this investigation, no control group of rats undergoing irradiation, with or without non-tea drink, was assessed, which is an important point to consider. Their results indicated that the group irradiated and administrated with black tea showed significant increases in iron, copper, and copper/zinc ratio, along with a decrease in zinc level in all studied organs. On the contrary, the group irradiated and administrated with kombucha resulted in a successful attenuation of these adverse effects of electromagnetic field exposition, suggesting that kombucha may be beneficial for correcting or preventing negative effects of electromagnetic radiation.

All the studies above contribute to increasing the perception that kombucha consumption is safe. Generally, it can be seen that it is essential to research further the kombucha doses administrated since there was no basis for dose selection. Some studies used 2.5–1 mL per kg of body weight, while other studies did not report the doses evaluated. Further, kombucha tea basis may provide different compounds with varying antioxidant activities depending on the tea used (green, black, or other sources) which, according to the studies evaluated, also have an essential role in the attenuation of the signs and symptoms of some diseases. As a matter of fact, while all the authors agreed about the hepatoprotective effects of the glucuronic acid content, evidence eludes to greater effects of the polyphenolic compounds from each tea source.

All this gives the scientist a new approach to understanding if the health outcomes attributed to kombucha are really given by either phytochemical compounds or glucuronic acid content and the relationship with the enzymatic activation-deactivation-reactivation of the β-glucuronidase. To review, none of the cited investigations have studied this metabolic pathway; specifically, they focus on the liver function evaluated by clinical, morphological, or histopathological assessments. Consequently, to elucidate whether glucuronic acid

and phytochemical compounds from kombucha have health benefits or not, new data must come from clinical trials that have been designed to address these central points.

On the other hand, some cases of liver damage have been reported related to kombucha consumption in patients across a wide range of ages. Nevertheless, some of those adverse effects could be attributed to side sickness (human immunodeficiency virus, acidosis vulnerability, acute kidney failure) or pregnancy that causes hypersensitivity to some kombucha compounds. Contaminants cause other toxicity cases, for example, the vase where kombucha was prepared contained pigments, vase coatings, myogenic substances, or pathogenic bacteria. It was challenging to find recent in vivo studies demonstrating hepatotoxic effects caused by kombucha ingestion. Only one case report was available to discuss this issue.

A case of hepatotoxicity related to kombucha consumption was reported by Gedela et al. [31]. A patient of 58-year-old, non-obese, diabetic female consumed a significant amount of kombucha tea a month before the beginning of the symptoms. Needle biopsy of the liver found a moderate amount of mixed-portal inflammation and inflammatory cells demonstrating bile duct damage and suggesting drug-induced liver injury. The patient was asked for a cessation of tea consumption, in addition to being provided, controlled doses of ursodeoxycholic acid and cholestyramine. After a 2-month follow-up, her liver function test was regular.

In this particular case, the authors did not attribute the adverse effects of compounds naturally found in kombucha. They discussed instead two potential causes: (a) contamination of with spores from more pathogenic yeast or bacteria and (b) processing factors. During fermentation, the pH level is very low (around 1), which could cause leaching of toxic constituents from ceramic or lead containers. It is essential to note that several factors influenced the presence and concentration of beneficial compounds and metabolites in kombucha [13]. First, the substrates per se have different compositions since kombucha is made from several biological sources. For example, the base of the typical production of kombucha beverages is black, green, or oolong tea; nevertheless, several types of research have reported the use of other alternative sources. Secondly, the microbial composition of the SCOBY, the culture used for kombucha fermentation, has a variable microbiological composition according to its origin, the weather, geographical location, and medium used for the fermentation process [1••]. The third factor is the concentration of the ingredients added, such as sugar. Sucrose is used as the primary carbon source at a level of 5–20%, providing the media and nutrients necessary for microorganism development. Cane sugar, molasses, honey, maple, or agave syrups are also used. Furthermore, the fourth factor is the fermentation process, including time, temperature, containers where kombucha is prepared, light conditions, and aeration, among others. Kombucha fermentation is typically known to require a minimum of 3 days to a maximum of 60 days, depending on several cultural practices. The fermentation of kombucha is carried out at room temperature, optimizing fermentation time, but along with other factors combined, it will determine the final concentration of organic substances such as acids, pH level, and polyphenolic compound concentrations [1••]. Taken together, the specific identification of all the ingredients of kombucha is a difficult task because of many differences in substrate availability/selection, method of preparation, and routine of the fermentation process [32].

Conclusions

There is a need for further studies about the hepatoprotective effects associated with glucuronic acid contained in kombucha. Unfortunately, no concrete evidence has been found about how the glucuronic acid acts as the main compound for hepatoprotective effects, in comparison to the phenolic compounds. As long as there is no consistent standardization in the production of this beverage, future research should seek to clarify issues relating to varied practices for kombucha preparation and subsequent variability in metabolic responses among individuals. Therefore, it is crucial to establish good manufacturing practices for kombucha production to understand the benefits of different compounds in kombucha. Public advisory labeling and advertising of some of the potential contraindications of kombucha consumption are also advisable.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no known competing for financial or personal relationships that could have appeared to influence the work reported in this paper.

Human and Animal Rights and Informed Consent Not applicable

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