INTRODUCTION
In biology, the glycosylation is an important and essential enzymatic process through which glycans are attached to proteins, lipids, or other biological molecules. Evidence showed that the majority of proteins in eukaryotes are posttranslationally glycosylated in the plasma membrane, endoplasmic reticulum, and Golgi apparatus. Such glycosylation plays an important role in the protein stability, cell adhesion, cellular interaction, protein folding, and even in signal transduction. Hence, any defect in the glycosylation process was found to be related to many metabolic, neurodegenerative, and neoplastic disorders [1]. The glycosidic linkages in different glycoproteins are classified according to the functional part of amino acid which conjugates with the sugar. In living organisms, there are 37 types of glycoprotein linkages have been detected, in which thirteen different monosaccharides and eight amino acids are involved. The well-known classes include S-linked, N-linked, P-linked, C-linked, and O-linked glycoproteins. Among them, the O- and N-linked glycoproteins are the most common [2].

The hydroxyl moiety of serine, threonine, hydroxylysine, and hydroxyproline can interact with monosaccharide affording O-linked glycoproteins. Asparagine is the most common amino acid contributed to the formation of N-linked glycoproteins, while P-linked glycoproteins are generated when the phosphorylated serine linked to monosaccharide. Also, cysteine is the major amino acid which participates in the formation of C-linked glycoproteins, while C-linked glycoproteins are generated as tryptophan grafted to monosaccharide. Glypation is another glycoprotein class which occurs when the glycosylphosphatidylinositol (GPI) glycolipid attached to the C-terminus of a protein [3]. In general, glycoproteins are highly found in the cell membrane of eukaryotes. These proteins play important cellular roles due to their participation in the cell-cell recognition and binding to other molecules, beside their role in the intracellular communications [4]. In 1920s, the German scientist Otto Warburg found that the cancerous tissues have a high rate of aerobic glycolysis and their energy is mainly captured from glycolysis rather than mitochondrial oxidative phosphorylation. Hence, these tissues consume a larger amount of glucose compared to the normal tissues. This phenomenon has been commonly known as a Warburg effect and considered as one of the most important indicators of the cancer evolution [5,6]. Laterally, Warburg proposed that the mitochondrial dysfunction is the fundamental cause for enhancing aerobic glycolysis in cancerous tissue. Normally, this type of glycolysis can generate about 17% of the total ATP molecules needed to fulfill the cellular functions. Since cancer cells need more energy for their rapid multiplication [7], over-expression of insulin independent glucose gated channels (GLUT-1) [8] and induction of glycolytic enzymes [9] are the most acceptable mechanisms to satisfy this energy requirement.

The higher glucose consumption of cancer cells was used as a marker for the existence of cancerous tissues and also for the assessment of patient response to the treatment. This marker is verified by identifying the formation of a radiolabeled glucose analogue termed 18F-FDG in cancer tissue using the positron emission tomography [10]. For the healthy individual, this analogue can be uptaken by only tissues with the highest ability of consuming glucose including brain and bladder. For the patient enduring from cancer, this glucose analogue is selectively concentrated in the cancer cells besides its uptaken by the high glucose-consuming tissues. This is mainly attributed to the over expression of GLUT-1 [11].

Regarding the antibacterial activity of glycosyl-conjugates, the attention has been directed in the last three decades toward the antibacterial agents grafted to different types of simple sugars. The main goals of this approach are to reduce the bacterial resistance and to improve the antibacterial activity [12]. Also, the antiradical capacity of many natural and synthetic antioxidants have been document to be improved through their conjugation with different types of simple sugars. This may be due to the improvement of aglycones’ diffusion into intestinal enterocytes and the chemical stability of the resultant conjugates [13].

This review focused on the progress and future directions of exploiting the Warburg effect for selective targeting the antitumor agents to cancer cells. Also the role of...
glycosylation in the improvement of antibacterial and antioxidant activities of their aglycones.

Glycosylation is a new trend for improving the antitumor activity
In the early of 1990s, the idea of grafting mono, di, or polysaccharide to various cytotoxic agents through O-, N-, C- or S-glycosidic linkage has been established. The resultant conjugates showed an improved water solubility, serum stability, and better targeting of their aglycones to the cancerous tissues [14-18].

O-glycosylation for enhancing the aglycone cytotoxic activity
Pohl et al have developed glufosfamide (Figure 1), which is a glycosylated ifosfamide, in an effort to increase the selectivity and reduce the toxicity of the original cytotoxic drug. The results showed an increase in the survival time of test animals received glufosfamide with LD50 greater by 4.5 times for rats and by 2.3 times for mice than those of ifosfamide [19].

In 1997, Briasoulis et al have started the human clinical trials on glufosfamide on twenty patients with tumors of various origins. The maximum tolerated dose was determined to be 190 mg/kg, which is much less than that of ifosfamide (530 mg/kg); consequently, the toxic side effects of glufosfamide was reduced compared with the free aglycone. In this study, ten patients lived with a stable disease, two patients experienced a good response with one of them had complete remission for more than four years, while the remaining eight patients have suffered from a progression of their disease conditions [20].

In order to improve the cytotoxic activity and reduce the toxic side effects of taxoids, Mandai et al were linked paclitaxel at positions 7 and 10 with different monosaccharides including galactose, mannose, glucose, and xylose (Figure 2). The results showed that the resulted four glycosyl- conjugates from the linking at position 10 have more water solubility and better cytotoxic activity than those of non-glycosylated compound [21].

Lin et al have synthesized four novel paclitaxel based glycosyl-conjugates through an ester or ether linkage at the 2' position of paclitaxel. The results revealed that compound shown in Figure 3 with an ester linkage has the best cytotoxic activity and the least side effects [22].
Podophyllotoxin is a natural aryltetralin lignin isolated from the root of the plant Podophyllum peltatum. This natural product possesses good cytotoxic activity versus different cancer cell lines acting on colchicine-binding site on tubulin. The high toxicity and poor water solubility are the main obstacles of podophyllotoxin utilization as an anticancer drug. Many efforts have been made to overcome these issues such as the synthesis of per-butrylated glucoside and 4β-triazoly1-podophyllotoxin glucoside. The compound shown in Figure 4 revealed the highest cytotoxic activity versus five different cancer cell lines with IC50 ranging from 3.27 ± 0.21 to 11.37 ± 0.52 μM [23].

Platinum (IV) prodrugs are used for the treatment of many tumor kinds such as ovarian, head, lung, neck, and colorectal cancers. These prodrugs are usually activated through intracellular reduction followed by the cellular uptake. Depending on Warburg phenomena, Ma et al synthesized a series of glycosylated platinum (IV) prodrugs in order to improve their chemical stability and tumor selectivity. The results exhibited that the compounds shown in Figure 5 have a higher cytotoxic activity for about 166 folds than cisplatin (Figure 6, a), oxaliplatin (Figure 6, b), and satraplatin (Figure 6, c) [24].
Glycosylated Antitumor Ether Lipids (GAELs) are synthetic antitumor agents which possess an inhibitory cytotoxic activity on cancer stem cells responsible for tumor relapse. The 2-amino-2-deoxy-D-glucopyranosyl-based GAELs such as α-GLN (Figure 7, a) and β-GLN (Figure 7, b) showed an improved antitumor activity against epithelial cancer cells in addition to their stem cells. This action, which may be attributed to the presence of amine group in the sugar part, was hypothesized to be due to the generation of acidic vacuoles that release acid and induces cell death. This hypothesis has been augmented by the synthesis of bisamino GAEL compounds (Figure 7, c-e) which possessed 2-3 folds activity against the test cancer cell lines and their stem cells compared to β-GLN [25].
Al-Bujuq et al. have synthesized and investigated the in vitro cytotoxic effect of 4-O-linked-β-D-galactopyranosyl derivatives of phenolic acid esters including methyl ferulate (Figure 8, a) and methyl vanillate (Figure 8, b) on two human cancer cell lines, which are breast cancer cell line (MCF-7) and prostate cancer cell line (PC-3). The results showed these galactosylated compounds have an improved cytotoxic activity compared with their non-galactosylated compounds [26].

Bashir et al. have synthesized and studied the in vitro cytotoxic effect of O-β-glucosylated coumarin-pyrazoline derivatives on two human cancer cell lines, which are breast cancer cell line (MCF-7) and esophageal cancer cell line (SKG). The results showed that the O-β-glycosylated compound (Figure 9) has the best antitumor activity versus the test cancer cell lines compared with their nonglycosylated compounds. These results may indicate the importance of glycosylation for improving the site selectivity of the original compounds [27].
N-glycosylation for enhancing the aglycone cytotoxic activity

Many studies have been carried out to investigate the role of N-linked glycosylation on the cytotoxic activity of their conjugated aglycones [28-30]. Cucciolito et al have synthesized a new class of five-coordinate Pt(II) compounds by their conjugating via N-linked sugar using either pyridine or imidazoline linker. These conjugates have been shown to have an improved cytotoxic activity compared with cisplatin on two cancer cell lines including human breast cancer cell line (MCF-7) and epidermoid carcinoma cell lines (A431). The results acquired from X-ray crystallography showed that N-glycosylation has improved the apoptotic activity through better interaction with DNA and proteins. The compounds shown in Figure 10 have reported to possess the best antitumor activity versus the test cancer cell lines [28].

Alminderej et al have synthesized a new series of compounds containing 1,2,3-triazolyl-1,3,4-thiadiazole-N-glucoside and examined their cytotoxic activity against two cancer cell lines, which are human breast cancer cell line (MCF-7) and human colorectal carcinoma cell line (HCT-16). The results showed that the compounds shown in Figure 11 have the best activity against the test cancer cell lines. These results indicated the significance of N-glycosylation to afford a new triazole scaffold for improving the cytotoxic activity of these heterocyclic compounds [29].
Carreira et al have synthesized and investigated the cytotoxicity of a novel series of styryl pyrazole N-glucoside against human adenocarcinoma cell line (AGS). The results showed an improvement in the cytotoxicity especially for the compound shown in Figure 12 which has an IC₅₀ 73 μM comparing with its non-glycosylated compound having an IC₅₀ more than 100μM. This improvement may indicate the importance of N-glycosylation for enhancing the site selectivity based on Warburg phenomena [30].

C- and S-glycosylation for enhancing the aglycone cytotoxic activity

C-glycosylation is a new strategy which investigated to improve the cytotoxic activity and reduce the toxic side effects of the currently available anticancer agents. Cuccioloito et al have used D-glucose and D-galactose for linking with ether C1 or C6 of platinum complex. The compound 1gal-1 (Figure 13) was found to possess the best cytotoxic activity compared with the other glycosylated platinum complexes with an IC₅₀ 15.2 ± 0.3 μg/ml in murine BALB/c 3T3 cells and 3.9 ± 0.3 μg/ml in fibroblast SVT2 cells using MTT assay next to 48 hours incubation. This improvement in the cytotoxicity may attribute to the enhancement of complex stability in biological media and to increase the selectivity toward tumor cells depending on Warburg effect [31].

Abdelhady and Motaal have extracted and identified the compound 8-C-β-D-(3''-O-acetyl) glucopyranosyl apigenin (Figure 14) from the leaves of the plant Ocimum basilicum. This compound was found to be highly cytotoxic agent against human colon cancer cell line (HCT-116) compared with 80% crude ethanolic extract of the plant. This improved antitumor activity may indicate the significance of C-glycosylation for enhancing the cytotoxic activity [32].
The influence of S-glycosylation on the cytotoxic activity of various antitumor agents has been highly investigated. For instance, Flefel et al. have studied the cytotoxic activity of thioglycoside derivatives of (1,3,4-thiadiazolyl)thiazaspiro[4,5] decane and of thioglycoside derivatives of thiazopyrimidine versus three cancer cell lines, which are HepG-2 (human liver hepatocellular carcinoma), PC-3 (human prostate adenocarcinoma), and HCT-116 (human colorectal carcinoma). The results showed that compounds shown in Figure 15 have an improved cytotoxic activity versus the test cell lines compared with their corresponding aglycones [33].

Yousif et al. have synthesized novel thioglycoside derivatives of imidazolyl indole system and investigated their activities against four cancer cell lines including MCF-7, PC3, HCT-116 and HEPG2. The results showed that the compound shown in Figure 16 has a significant antitumor activity against two cancer cell lines, which are MCF-7 with an IC50 80 ± 7 µM and PC3 with an IC50 15 ± 3 µM. This may indicate the enhanced selectivity of its aglycone because of S-glycosylation [34].

Glycosylation for enhancing the antioxidant activity
Oxidative stress defined as the disturbance in the balance between the production of free radicals and the antioxidant defense mechanisms in a biological system. It was found that many pathological conditions such as inflammation, cardiovascular diseases, and cancer are significantly linked to oxidative stress [35]. There are many endogenous antioxidant defense mechanisms to scavenge the free radicals. These mechanisms can be categorized into enzymatic and non-enzymatic based on their modes of action. Superoxide dismutases, glutathione peroxidase, catalase, and glutathione reductase are examples of the enzymatic antioxidant, while glutathione, thioredoxine, and...
Yasser Fakri Mustafa et al / Antitumor, Antioxidant and Antibacterial Activities of Glycosyl – Conjugated Compounds: A Review

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Yasser Fakri Mustafa et al / Antitumor, Antioxidant and Antibacterial Activities of Glycosyl – Conjugated Compounds: A Review

melatonin are examples of the non-enzymatic endogenous antioxidants [36-40].

To enhance the antioxidant ability of a biological system against free radicals, there are some exogenous antioxidants which are highly effective such as vitamin C, vitamin E, vitamin A, minerals, and polyphenols including flavonoids [41].

Several polysaccharides and glycosyl-conjugates have been recently found to have significant in vitro and in vivo antioxidant potentials. The possible mechanisms of this activity may be attributed to their ability for scavenging the free radicals and chelating the pro-oxidant metals [42].

Many studies concerning the antioxidant activity of glycosyl-conjugates have been performed [43-45]. For instance, Gonzalez-Alfonso et al have glycosylated the (–)-epigallocatechin gallate, which is the predominant catechin in green tea (Camellia Sinensis), through enzymatic cyclodextrin glucotransferase method at different positions, and subsequently examined their scavenging activity toward ABTS free radicals. The results showed that the compound shown in Figure 17 with an α-glycosylation at position 3 of the ring B has a significant increase in the antioxidant activity compared with its original compound. This improvement in the antioxidant activity may be attributed to the enhanced chemical stability of resulted conjugate [43].

Bellia et al have synthesized and examined the antioxidant activity of N- and O-linked β-cyclodextrin of three derivatives of histidine containing dipeptides including carnosine (β-alanine-L-histidine), homocarnosine (γ-amino butyryl-L-histidine), and anserine (β-alanyl-3-methyl-L-histidine). The results of testing their antioxidant activity towards copper-induced human LDL oxidation showed that both N- and O-β-cyclodextrin-anserine conjugate (Figure 18) have the highest antioxidant effect; and that all glycosylated derivatives had more antioxidant activity than their free histidine-containing dipeptides [44].

Zhu et al have isolated and identified fourteen types of flavonoids from Agrimonia pilosa Ledeb such as taxifolin, luteolin, and rutin. This study examined their potential as antioxidant agents using a DPPH radical scavenging assay and vitamin C as a positive control. The results showed that the chemical modification of these flavonoids through...
glycosylation at C6 in the A-ring (Figure 19) has enhanced the antioxidant activity of their original products. This improved antiradical activity may be due to the high uniform distribution of spin density [45].

**Figure 19:** Chemical Structure of the semisynthetic flavonoid prepared by Zhu et al with an enhanced antiradical activity.

**Glycosylation for enhancing the antibacterial activity**

Formerly, it was thought that glycone moieties in glycosidic antibiotics were important to modify their pharmacokinetic features such as aqueous solubility, biodistribution, and bioavailability. Currently, it is believed that these moieties are also important for interacting with the active site of the target. Therefore, the efforts are now focused on the synthesis and modulation of glycone-based antibiotics to create new better tuned antibiotics for better therapeutics [46-49].

Peraman et al have synthesized a novel series of different glycosyl-conjugated quinoxaline in an attempt to improve the anti-tubercular activity of the parent product through enhancement of its intercalating potency. The results showed that quinoxaline conjugated with ribose (Figure 20) has the most promising activity against mycobacteria with minimum inhibitory concentration of 0.65 µM. The in silico docking study of this conjugate revealed that DNA gyrase B subunit is the possible site of action [50].

**Figure 20:** Chemical structure of the glycosylated product synthesized by Peraman et al as an antituberculosis agent.

Sganappa et al have synthesized and investigated the antibacterial activity of a new series of neomycin-sugar conjugates through a domino synthetic process. The final compounds were tested against Klebsiella pneumoniae, methicillin resistant Staphylococcus aureus, and E. coli. The results showed that the compounds shown in Figure 21 have the highest activity against Klebsiella pneumoniae compared with tetracycline as a positive control [51].
Choi et al have investigated the effects of ciprofloxacin (Figure 22, a) conjugation with mono-β-deoxy-β-aminoethylamino-β-cyclodextrin (β-CD) on its aqueous solubility, bioavailability, and antibacterial activity. In chemical synthesis, β-CD takes the oval shape form (Figure 22, b) in which ciprofloxacin is embedded. The results showed that the ciprofloxacin-β-CD conjugate has seven folds increase in the aqueous solubility than the original drug. Furthermore, this conjugate has a significant increase in the antibacterial activity against methicillin resistant Staphylococcus aureus (52).

**CONCLUSION**

Glycosylation of active pharmacological agents represented a new research field for enhancing the biological and pharmacokinetic properties of their aglycones. This review article shows some of the new researches in the fields of improving the antitumor, antioxidant, and antibacterial activities of glycosylated products comparing with their pure aglycones with exploring the possible mechanisms of such change in the activities. The listed results may generate a guideline for the development of new products with improved biological activities based on their conjugation with various sugars.

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**CONFLICT OF INTEREST**

There are no conflicts of interest.

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